AGROECOSYSTEM LAND MANAGEMENT AND ITS EFFECT ON SOIL ORGANIC CARBON STOCKS AND DYNAMICS IN THE MOLLISOLS OF SOUTHERN WISCONSIN

By

Gregg Rolland Sanford

A dissertation submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

(Agronomy)

At The

University of Wisconsin-Madison

2012

Date of oral examination: 7/31/2012

The dissertation is approved by the following members of the Final Oral Committee:

Christopher J. Kucharik, Associate Professor, Agronomy

Birl Lowery, Professor, Soil Science

Randall D. Jackson, Associate Professor, Agronomy

Joseph G. Lauer, Professor, Agronomy

Maria E. Marin-Spiotta, Assistant Professor, Geography

DEDICATION

This work is dedicated to my friend and advisor Joshua L. Posner who has contributed more to my personal and academic growth and development than any other during my time at the University of Wisconsin – Madison. Thank you for everything that you have done for me, I will miss you.

ACKNOWLEDGEMENTS

There are a number of people who have helped me over the last five years. For their many contributions to my education and general wellbeing I am very grateful. I would first and foremost like to thank my wife Emily and children Hannah, Gretchen and Sylvia who have been unceasingly patient with and supportive of me. My mom and dad Helen and Tim Sanford, my sister Kimberly and brother in law John Frederickson, my Grandparents Naomi Sanford and Ron and Gwenne Heiser, and all of my aunts, uncles, cousins, and nephews (too numerous to count) who have continued to encourage and support me.

A close second to family are the members of my PhD committee who have been phenomenal resources during my studies and have challenged me to dig deeper and go further than I would have done on my own. I would like to express my immense gratitude to Dr. Josh Posner who until his untimely death served as my major advisor. I feel very fortunate to have had the opportunity to know and work closely with Josh over the past 8 years. I have learned a great deal under his tutelage and I will always look back at my graduate studies and remember the great and positive impact he has had on my life. Josh was more of a father figure in my life than an academic advisor or colleague. I would like to thank Dr. Chris Kucharik for his willingness to step in to the position of Major advisor following Josh's passing. Chris has patiently watched as I have done a mediocre job of balancing family, school and work for the past five years. He has been nothing but supportive and reassuring throughout the evolution of my academic journey and for that I am very grateful. Dr. Randy Jackson has been a great boss, advisor, and friend extending back to my first days at the UW when I came in 2004 to work on my MS degree. I was present for the first ever offering of "Grassland Ecology". I loved the class and have had a

I would like to thank both Dr. Ting-Li Lin and Dr. Jon Baldock for their help in analyzing my data in a statistically sound manner. I would also like to thank Dr. Gary Oates, Andy Jakubowski, and David Duncan who have served an unofficial role as data analysis consultants during my PhD. I have enjoyed sitting around with these guys, passing ideas back and forth, arguing about what should or should not be considered a random effect, and determining how best to account for autocorrelation in data. Thanks guys!

Finally I would like to recognize all the wonderful graduate students, technicians, LTEs, and undergraduates in the Kucharik, Jackson, and Posner labs who have either directly or indirectly helped me. In particular I want to thank Brianna Laube, Nikki Caine, and James Tesmer. For all of those I have not mentioned, rest assured that I couldn't have done it without you, if I keep writing though I will have an acknowledgements section that is longer than one of my chapters.

TABLE OF CONTENTS

CHAPTER1: SOIL CARBON LOST FROM MOLLISOLS OF SOUTHERN WISCONSIN WITH 20 YEARS OF AGRICULTURAL BEST MANAGEMENT PRACTICES

CHAPTER 2: SOIL CARBON DYNAMICS ALONG AN AGROECOSYSTEM MANAGEMENT GRADIENT ON MOLLISOLS OF SOUTHERN WISCONSIN

CHAPTER 3: EFFECT OF METHODOLOGICAL CONSIDERATIONS ON SOIL CARBON PARAMETER ESTIMATES OBTAINED VIA THE ACID HYDROLYSIS-INCUBATION METHOD

CHAPTER 4: GENERAL CONCLUSIONS

LIST OF TABLES

ix

LIST OF FIGURES

ABSTRACT

AGROECOSYSTEM LAND MANAGEMENT AND ITS EFFECT ON SOIL ORGANIC CARBON
STOCKS AND DYNAMICS IN THE MOLLISOLS OF SOUTHERN WISCONSIN

Gregg R. Sanford

Under the supervision of Associate Professor Christopher J. Kucharik

At the University of Wisconsin - Madison

Managing agricultural soils to sequester C requires an understanding of how land management practices influence the dynamics of soil organic carbon (SOC). In this study the effect of land management on SOC was assessed using two complementary methods. The first method involved evaluating the influence common Wisconsin cropping systems on total SOC over a 20-year period. The second method consisted of combining long-term soil incubations with acid hydrolysis to estimate the size and turnover rate of three operationally defined SOC pools in southern Wisconsin agroecosystems.

Analysis of total SOC indicated a significant decline of 1.6 g kg^{-1} or 16.6 Mg ha^{-1} across all of the agroecosystems evaluated to a depth of 90 cm. While the pasture system sequestered SOC in the surface 15 cm (4.3 g kg^{-1}) these gains were largely offset by losses at depth (-3.1 g kg^{-1}, 30 to 90 cm). Both no-till (NT) practices and forage crops reduced SOC loss, but neither resulted in C sequestration in the soil profile (0 to 90 cm).

Evaluation of SOC pool sizes and kinetics showed that grassland systems contained the most SOC, with a large proportion of this carbon allocated to mineralizable pools (60%). The relatively high percentage of SOC found in the non-hydrolysable fraction of the production

agricultural systems likely reflected the oxidative loss of labile and accessible C pools in response to historic tillage. Continuously vented incubation chambers were used for this study based on findings from the methodological work conducted.

Results from these studies highlight the importance of land management decisions when seeking to promote SOC sequestration. On soils that are naturally high in SOC, sequestration may be difficult to achieve. Studies of both total carbon and carbon pools are necessary to improve our understanding of how climate and land management change will affect current and future soil C stocks across diverse agricultural landscapes.

PREFACE

Soils are at the very heart of terrestrial ecosystems, making a robust understanding of the soil system crucial to the success and environmental sustainability of any human endeavor on land (Brady and Weil, 2008; Schmidt et al., 2011). Soils provide a number of important ecosystem services to society. They serve as filters for the purification of water resources, they provide a medium for plant growth, supplying essential macro- and micro- nutrients, they serve to recycle nutrients and organic wastes, and they provide habitat a highly diverse soil biota. In addition to these services, soils play a significant role in modifying the atmosphere, particularly as it pertains to climate change and the global carbon cycle.

The importance of soil in the global carbon budget cannot be overstated (Bellamy et al., 2005; Lal, 2008a). The world's soils contain an estimated 2730 Pg of C, almost twice the combined amount of C in the atmosphere and vegetation. This pool is exceeded in size only by our ever diminishing reserves of geologic C (4130 Pg) and the vast amount of C stored in the world's oceans (38,000 Pg C) (Lal, 2008b). The soil organic carbon pool (SOC) makes up 56% of this total, 11% of which is stored in the 1.7 billion hectares of agricultural cropland worldwide (Lal, 2008a; Paustian et al., 2000).

Many agricultural soils have lost a significant amount of the SOC that they contained prior to cultivation (Collins et al., 1999; Lal, 2008a). Globally, between 55 and 78 Gt of carbon has been lost, primarily as CO_2, from agricultural lands (Lal, 2004). Grace et al. (2006) estimated that carbon stocks in Wisconsin in the upper 10 cm of soil have decreased by 67 Mt (14%) since 1850. This is consistent with findings reported by Collins et al. (1999) showing that soil samples (0 to 20cm) taken from continuous corn plots at the University of Wisconsin's Arlington

Research Station (Arlington, WI; 43°17'41"N, 89°23'03"W) had 18% less soil carbon than an adjacent non-cultivated site. These estimates are modest however compared to the estimates of others who suggest that in many cases 30 to 50% of SOC has been lost as a result of agriculture in temperate regions of the world (Lal, 2008a; Ogle et al., 2005).

Agriculture and forestry were the primary source of atmospheric C emissions prior to the early 20th century at which point C emission from fossil fuels outpaced those from land use change (Graedel and Crutzen, 1993; IPCC, 1997). With levels of atmospheric CO_2 rapidly increasing, accompanied by its destabilizing impact on radiative forcing, there is an urgent need for technologies (both low- and high-tech) that can help to moderate atmospheric CO_2.

One potential low-tech solution to this problem is to manage agricultural soils to serve as C sinks; replacing much of the SOC that has been historically lost as a result of cultivation. This approach to atmospheric CO_2 reduction is an attractive option because it is potentially cost effective and can be implemented using current agricultural technologies (Conant et al., 2007; Lal, 2008a). Lal (2004) suggested that the carbon sequestration potential of agricultural soils for the near future (25 to 50 years) was modest (50 to 100 Pg C) but important in that it would be an immediately functional sink for atmospheric CO_2.

Agricultural practices affect SOC mineralization and stabilization by directly altering soil moisture, temperature, aeration, pH, and nutrient availability. Improvements in agricultural genetics and technology over the past 50 years have greatly increased the crop residue contribution to SOC and enabled widespread adoption of soil conserving agricultural practices (Buyanovsky and Wagner, 1998). Cultural practices that are often cited as ways to increase SOC or mitigate its loss include conversion from conventional to NT farming (Huggins et al., 2007;

West and Post, 2002), cultivation of perennial crops (Huggins et al., 1998), use of livestock and green manures (Ogle et al., 2005), increased crop rotation complexity (West and Post, 2002), and the application of fertilizer (Huggins et al., 1998; Nafziger and Dunker, 2011). While there is a majority consensus in the scientific community regarding agricultural practices that favor SOC persistence, discrepancies exist, and the great variability in research findings warrant site-specific SOC assessments.

In addition to site specific assessment of total carbon stocks, a deeper understanding of SOC dynamics is required to develop mechanistic models for SOC stabilization and mineralization. Furthermore, a robust understanding of SOM dynamics and sensitivity to disturbance is of critical importance in our efforts to stabilize current SOC stocks, foster C sequestration, and model SOC behavior under future climate and land-use change scenarios (Schmidt et al., 2011; Schwendenmann and Pendall, 2008).

Historical concepts of SOC stabilization have been challenged by advances in modern SOC elucidation techniques (Kleber and Johnson, 2010; Schmidt et al., 2011). In many cases the chemical complexity of SOC has been shown to impart no added protection from microbial degradation and long term persistence has been equally as likely for proteins and saccharides as for lignin, n-alkanes, long-chain alkanoic acids, and other plant structural tissues (Schmidt et al., 2011). It is clear that SOC stabilization is tightly coupled to temperature, water regime, the quantity and quality of silt and clay minerals upon which C molecules can adsorb, and physical compartmentalization within the soil that can isolate organic compounds from oxygen and microbial decomposers (Kleber and Johnson, 2010; Schmidt et al., 2011; Six et al., 2002).

The complexity of SOC stabilization makes questionable a "one size fits all" approach for climate stabilization via SOC sequestration and greenhouse gas mitigation. While no-till annual grain production might successfully sequester carbon in one locale, C4 grasslands, with their extensive below ground C inputs, may be necessary elsewhere. It must also be conceded that in some instance SOC sequestration may not be practical in a timescale that is meaningful to humans. However, as farmers and land managers work to stabilize and/or augment their soils' SOC stocks, they will benefit from the numerous additional advantages that SOC imparts. Soil organic matter (~60% of which is SOC) is crucial in supplying N and other plant nutrients, it increases a soils water holding capacity, improves soil tilth by encouraging soil aggregation, and reduces the negative impacts of compaction caused by agricultural machinery (Brady and Weil, 2008).

In this study we seek to evaluate the effect of common Midwestern agroecosystems on SOC stocks in the prairie derived soils of southern Wisconsin. In chapter 1, SOC stocks (0 to 90 cm) are quantified and evaluated over a 20 year period in six agricultural systems representative of the upper Midwest. These systems encompass two enterprise types (grain and forage) and include both conventional and alternative agricultural practices. In chapter 2, SOC pools and mineralization rates are evaluated in nine agroecosystems ranging from a high input continuous maize system to a no-input, never-tilled remnant prairie pasture. Long term soil incubations are combined with acid hydrolysis (the "AHI" method) to estimate the size and turnover rate of three operationally defined SOC pools in this study. In addition to chapters 1 and 2 that address the interaction between land management and SOC processes, chapter 3 is concerned with identifying the most appropriate method to employ when using long-term laboratory microcosm soil incubations to estimate SOC pools and kinetics. In this study we evaluate the effects of

continuous versus periodic chamber venting, the importance of mechanical headspace mixing, and the impact of sieved versus intact soil cores on parameter estimates. Because one of the greatest potential utilities of the AHI method is to parameterize global biogeochemical models, methods must be in place to minimize laboratory artifacts.

References

Bellamy P.H., Loveland P.J., Bradley R.I., Lark R.M., and G.J.D. Kirk. 2005. Carbon losses from all soils across England and Wales 1978-2003. Nature 437: 245-248.

Brady N.C., and R.R. Weil. 2008. The nature and properties of soils. 14th edition ed. Prentice Hall, Upper Saddle River, NJ.

Buyanovsky G.A., and G.H. Wagner. 1998. Carbon cycling in cultivated land and its global significance. Global Change Biology 4: 131-141.

Collins H.P., Blevins R.L., Bundy L.G., Christenson D.R., Dick W.A., Huggins D.R., and E.A. Paul. 1999. Soil carbon dynamics in corn-based agroecosystems: Results from carbon-13 natural abundance. Soil Science Society of America Journal 63: 584-591.

Conant R.T., Easter M., Paustian K., Swan A., and S. Williams. 2007. Impacts of periodic tillage on soil C stocks: A synthesis. Soil and Tillage Research 95: 1-10.

Grace P.R., Colunga-Garcia M., Gage S.H., Robertson G.P., and G.R. Safir. 2006. The potential impact of agricultural management and climate change on soil organic carbon of the North Central Region of the United States. Ecosystems 9: 816-827.

Graedel T.E., and P.J. Crutzen. 1993. Atmospheric change - and earch system perspective W.H. Freeman and Company, New York.

Huggins D.R., Allmaras R.R., Clapp C.E., Lamb J.A., and G.W. Randall. 2007. Corn-soybean sequence and tillage effects on soil carbon dynamics and storage. Soil Science Society of America Journal 71: 145-154.

Huggins D.R., Buyanovsky G.A., Wagner G.H., Brown J.R., Darmody R.G., Peck T.R., Lesoing G.W., Vanotti M.B., and L.G. Bundy. 1998. Soil organic C in the tallgrass prairie-derived region of the corn belt: effects of long-term crop management. Soil & Tillage Research 47: 219-234.

IPCC. 1997. Greenhouse gas inventory reference manual, Intergovernmental Panel on Climate Change and International Energy Agency. http://www.ipcc.ch/publications_and_data/publications_and_data_reports.shtml#.UCQK BaHN89U. (accessed, 8 August, 2012).

Kleber M., and M.G. Johnson. 2010. Advances in Understanding the Molecular Structure of Soil Organic Matter: Implications for Interactions in the Environment, Advances in Agronomy, Vol 106, Elsevier Academic Press Inc, San Diego. pp. 77-142.

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

Lal R. 2008a. Carbon Sequestration. Philosophical Transactions of the Royal Society 363: 815-830.

Lal R. 2008b. Sequestration of atmospheric CO2 in global carbon pools. Energy & Environmental Science 1: 86-100.

Nafziger E.D., and R.E. Dunker R.E. 2011. Soil Organic Carbon Trends Over 100 Years in the Morrow Plots. Agronomy Journal 103: 261-267.

Ogle S.M., Breidt F.J., and K. Paustian. 2005. Agricultural management impacts on soil organic carbon storage under moist and dry climatic conditions of temperate and tropical regions. Biogeochemistry 72: 87-121.

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

Schmidt M.W., Torn M.S., Abiven S., Dittmar T., Guggenberger G., Janssens I.A., Kleber M., Kogel-Knabner I., Lehmann J., Manning D.A.C., Nannipieri P., Rasse D.P., Weiner S., and S. Trumbore. 2011. Persistence of soil organic matter as an ecosystem property. Nature 478: 41-56.

Schwendenmann L., and E. Pendall. 2008. Response of soil organic matter dynamics to conversion from tropical forest to grassland as determined by long-term incubation. Biology and Fertility of Soils 44: 1053-1062.

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

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

CHAPTER 1

SOIL CARBON LOST FROM MOLLISOLS OF THE NORTH CENTRAL U.S. WITH 20

YEARS OF AGRICULTURAL BEST MANAGEMENT PRACTICES

1. **Soil carbon lost from Mollisols of southern Wisconsin with 20 years of agricultural best management practices**

1.1 Abstract

Soil organic carbon (SOC) is highly sensitive to agricultural land management, so there is a great deal of interest in managing cultivated soils to sequester atmospheric CO_2. In this study we evaluated the influence of six cropping systems on SOC stocks at the Wisconsin Integrated Cropping System Trial (WICST) over a 20-year period. Analysis of SOC on either a concentration or mass per volume of soil basis indicated a significant decline ($\alpha = 0.01$) across all of the systems at WICST to a depth of 90 cm (-0.08 g kg^{-1} yr^{-1} or -0.83 Mg ha^{-1} yr^{-1}). While the rotationally grazed pasture system sequestered carbon (C) in the surface 15 cm (0.22 g kg^{-1} yr^{-1}), these gains were mostly offset by losses at depth (-0.16 g kg^{-1} yr^{-1}, 0 to 90 cm). Both no-till (NT) practices and inclusion of perennial crops reduced SOC loss, but neither resulted in C sequestration in the soil profile. Results from this study demonstrate the importance of i) comparing current and initial soil samples when evaluating SOC sequestration and ii) evaluating SOC changes throughout the soil profile. The losses of SOC at depths below the plow layer point to either a lack of C input from roots, increased oxidative loss at these depths or a combination of these mechanisms.

1.2 Introduction

The world's soils contain 2500 Pg of C, almost twice the combined amount of C in the atmosphere and vegetation (Batjes, 1996; Lal, 2008). The SOC pool makes up 60% of this total,

170 Pg of which are stored in the 1.7 billion hectares of agricultural cropland worldwide (Lal, 2008; Paustian et al., 2000). Hence, changes in soil C content can have a large effect on the global C budget (Bellamy et al., 2005). There is currently a great deal of interest in managing agricultural soils as C sinks to help offset rising levels of atmospheric CO_2. This approach to atmospheric CO_2 reduction is attractive because it is potentially cost effective and can be implemented using current agricultural technologies (Conant et al., 2007; Lal, 2008). Improvements in agricultural genetics and technology over the past 50 years have greatly increased the crop residue contribution to SOC and enabled widespread adoption of conservation and NT farming practices, both of which are thought to promote C sequestration or slow its loss (Buyanovsky and Wagner, 1998). According to the 2011 Inventory of U.S. Greenhouse Gas Emissions and Sinks compiled by the U.S. EPA, land converted to cropland in 2009 was cited as a source of CO_2 (-1.6 Tg C yr^{-1}), while land continuing in cropland and land converted to grasslands were both considered sinks of atmospheric CO_2 (4.7 and 2.3 Tg C yr^{-1}, respectively)(USEPA, 2011). In addition to the potential mitigation of climate change, increasing SOC levels has many other benefits including; i) improved soil structure, ii) reduced soil erosion, iii) decreased non-point source pollution, iv) increased water holding capacity, v) improved cation exchange capacity, and vi) increased soil fertility for food production (Lal, 2008).

Agricultural practices affect SOC mineralization and stabilization by directly altering soil moisture, temperature, aeration, pH, and nutrient availability. Cultural practices that are often cited as ways to increase SOC or mitigate its loss include conversion from conventional to NT farming (Huggins et al., 2007; West and Post, 2002), cultivation of perennial crops (Huggins et al., 1998), use of livestock and green manures (Ogle et al., 2005), increased crop rotation complexity (West and Post, 2002), and the application of fertilizer (Huggins et al., 1998;

Nafziger and Dunker, 2011). West and Post (2002), in a meta-analysis of 67 long-term experiments, reported that conversion from conventional tillage (CT) to NT resulted in an estimated sequestration rate of 570 ± 140 kg C ha^{-1} yr^{-1} and that by increasing rotation complexity, an additional 200 ± 120 kg C ha^{-1} yr^{-1} could be sequestered. These results are consistent with a global meta-analysis of 167 experiments that showed SOC increased with land set aside, reduced tillage, and increased C inputs through cropping practices (Ogle et al., 2005).

Despite these positive findings, a similarly large body of literature has demonstrated the conditionality of C sequestration or found net losses under what are considered best management scenarios. In an extensive study comparing soil samples from 1978 and 2003, Bellamy et al. (2005) reported C losses across land use and land cover types in the UK. These trends were most pronounced in systems with high initial C content, but were not related to land management, suggesting a link to climate change. Working in southern Minnesota on a clay loam, Huggins et al. (2007) found that under different management practices for annual crops (including NT), C losses of 1.6 to 3.7 Mg C ha^{-1} yr^{-1} occurred over the course of 14 years. They concluded that annual cropping systems had limited potential to restore C levels to those of native sites, and that under the best scenario of continuous corn and NT management, stabilization of initial SOC levels would either require reducing C decomposition rates by over 50% or doubling C inputs. These conclusions were supported by a recent meta-analysis of 69 paired experiments comparing the impacts of CT and NT on SOC sequestration (Luo et al., 2010), who reported that cultivation of native systems for more than 5 years resulted in SOC losses in excess of 20 Mg C ha^{-1} in the surface 60 cm of the soil profile irrespective of cultural practice (CT or NT). When farming practices were switched from CT to NT they found that NT increased SOC accumulation in the surface 30 cm, but that when deeper soils were considered (>40 cm), SOC was stable. Liu et al.

(2011) report that between 1972 and 2007 soils across Iowa lost carbon at a rate of 190 ± 380 kg C ha^{-1} yr^{-1}. They attribute this loss to the combined effect of large scale drainage of previously poorly drained soils and the high baseline SOC content. While both conservation tillage and residue management slowed the loss of SOC these practices were not sufficient to reverse its loss.

Lack of statistical power, the method of carbon assessment (space for time or change over time), and incomplete accounting efforts can all lead to divergent conclusions about SOC stability (Kravchenko and Robertson, 2011; Sanderman and Baldock, 2010; Schmidt et al., 2011; VandenBygaart and Angers, 2006). Sanderman and Baldock (2010) indicated that less than 50% of the studies in major reviews of SOC stock changes have actually followed changes in SOC through time. They asserted that without adequate baseline SOC data, it is impossible to determine whether or not a measured difference between two treatments has resulted in sequestration of atmospheric CO_2. Even when baseline data is available, conclusions of sequestration or loss of SOC can be erroneous if the system in question is not at a state of SOC equilibrium (Sanderman and Baldock, 2010). While most of the changes in SOC associated with agricultural management appear to occur within surface soil horizons (Syswerda et al., 2011), ignoring SOC trends in deeper horizons and/or changes in bulk density (BD) can result in significant errors in the estimation of SOC change. Baker et al. (2007) showed that the differences between NT and CT were primarily stemming from differences in SOC distribution with depth and not SOC accumulation in NT. Lee et al. (2009) evaluated the use of three equivalent soil mass (ESM) correction methods and compared them to the use of a fixed depth method for studying C stock changes. In their study, the comparison of mass based SOC changes at a fixed depth (0 to 15 cm) led to an unrealistic SOC loss of 30% within 6 months

following tillage. They suggested that in instances where appropriate soil BD numbers are unavailable the evaluation of SOC concentration changes is most appropriate.

It is ultimately the interaction of management with climatic and edaphic conditions that drive the stabilization or mineralization of SOC (Schmidt et al., 2011; Six et al., 2002). Historical concepts of SOC stabilization have been challenged by advances in modern SOC elucidation techniques (Kleber and Johnson, 2010; Schmidt et al., 2011). In many cases the chemical complexity of SOC has been shown to impart no added protection from microbial degradation and long term persistence has been just as likely for proteins and saccharides as it is for lignin, n-alkanes, long-chain alkanoic acids, and other plant structural tissues (Schmidt et al., 2011). It is clear that SOC stabilization is tightly coupled to temperature, water regime, the quantity and quality of silt and clay minerals upon which C molecules can adsorb, and physical compartmentalization within the soil that can isolate organic compounds from oxygen and microbial decomposers (Kleber and Johnson, 2010; Schmidt et al., 2011; Six et al., 2002). The complexity of SOC stabilization makes questionable a "one size fits all" approach for climate stabilization via SOC sequestration and greenhouse gas mitigation.

We compared the effects of six cropping systems typical of the North Central U.S.A. (3 grain and 3 forage) on the fate of SOC over 20 years using archived and current soil samples to a depth of 90 cm. Our objectives were to assess the effects of row-crop and perennial agriculture, using best management practices, on SOC and to evaluate the relative importance of tillage, perenniality, and crop residue inputs on SOC. We hypothesized that i) SOC would decrease over time as a result of increased tillage frequency, ii) perennial forage systems - with limited tillage, deep rooted crops, and manure inputs - would sequester greater amounts of SOC (or lose less)

than the annual grain systems, and iii) within enterprise types (grain and forage), systems with greater species diversity would sequester more C (or lose less).

1.3 Materials and methods

1.3.1 Site characteristics and experimental design

This study was conducted at the University of Wisconsin's Agricultural Research Station in Arlington, WI (43°18'N, 89°20'W). The soils at the site are classified as Plano silt loam (fine-silty, mixed, superactive, Mesic Typic Argiudolls). These are relatively deep (>1 m), well drained soils with little relief that were formed under tallgrass prairie vegetation in loess deposits over calcareous glacial till. Native SOC (35 g kg^{-1}), silt (720 g kg^{-1}), and clay (215 g kg^{-1}) contents are high as is crop production potential. Conversion from prairie vegetation to cropland began in the mid-1800s, primarily for the production of wheat. From the 1860s until the middle of the 20th Century, the land was used to produce feed for dairy cattle and from 1960 until the initiation of WICST, the predominant crop rotations were corn (*Zea mays* L.) and alfalfa (*Medicago sativa* L.) with dairy manure serving as the primary source of nutrients (Posner et al., 1995). At the onset of this study, the soils at the site (0 to 15 cm) had an average organic matter concentration of 47 g kg^{-1} (loss on ignition), and an average pH of 6.5 (1:1.3 soil/water), with high levels of soil test P and K (Bray-1 P=108 mg kg^{-1} and exchangeable K=255 mg kg^{-1})(Posner et al., 2008). The mean annual temperature and precipitation at Arlington are 6.9°C and 869 mm, respectively (1981-2010, National Climate Data Center).

The WICST experiment began in 1990 and consists of six cropping system treatments. Three cash-crop and three forage-crop systems were selected for study based on crop diversity

and level of external inputs (Posner et al., 1995; Posner et al., 2008). The grain systems were a high-external input, continuous corn system (CS1); a moderate-external input, NT corn-soybean [*Glycine max* (L.) Merr.] system (CS2), and an organic corn-soybean-winter wheat with interseeded red clover (*Trifolium pratense* L.) system (CS3). Forage systems included a high-input corn-alfalfa system (CS4); an organic oats (*Avena sativa* L.)/alfalfa-corn system (CS5), as well as a rotationally grazed pasture (CS6) seeded to a mixture of red clover, timothy (*Phleum pratense* L.), smooth bromegrass (*Bromus inermis* L.), and orchardgrass (*Dactylis glomerata* L.) (Table 1.1).

In 1989, a corn crop was planted on all 24 ha of WICST to improve the uniformity of crop history and to allow baseline measurements to be made. Some of the baseline variables, yield in particular, were used to block the trial into a four-block randomized complete block design with one replication of the 14 total phases in the six cropping systems placed in each block. Instead of starting all crops in all rotations in the first year, a staggered start was used so that each phase of each rotation was replicated in time as well as space (Loughin, 2006; Posner et al., 1995). After the stagger was completed in 1992, every phase was present every year for all the crop rotations. The 0.3-ha plots were large enough that all field work was done using typical farm equipment. Additional details on the design and implementation of WICST were provided in Posner et al. (2008).

1.3.2 Baseline soil sampling (1989)

Soil samples were collected in 1989 prior to the layout of the WICST plots as part of the baseline descriptive sampling for the trial. Samples were collected on a 27 x 27-m grid that

overlaid the 24-ha field. Samples were taken at four depths (0 to 15, 15 to 30, 30 to 60, and 60 to 90 cm). The first two depths were taken using a 3.2-cm diameter probe and the second two deeper depths were collected using a 1.9-cm diameter probe. Four cores were taken at each sampling point and homogenized by depth. Following initial soil analysis (1989), the remaining soils were dried, ground, and archived. In 2009, approximately 1 g of each dried homogenized soil from the 1989 archive was picked free of any visible plant material prior to analysis of soil C content.

1.3.3 Contemporary soil sampling (2009)

Soil samples were collected from the WICST plots between 22 April and 17 July 2009. Samples were collected using a tractor mounted hydraulic soil sampler fitted with a 3.2-cm diameter soil probe. Each plot was divided into three sections [18 x 52 m: North (N), Center (Cnt.), South (S)] and three soil cores were taken in the middle of each section. Cores were collected in all plots at 19-cm distances from one another (0, 19, and 38 cm). This was done to insure that samples were collected in-row, between-row, and at an intermediate location in all of the corn plots. Samples were then divided into four depth increments (0 to 15, 15 to 30, 30 to 60, and 60 to 90 cm) and composited by depth within each field section. Field moist samples were sieved to 2 mm in the lab and picked free of all visible plant material. Once cleaned of plant material, samples were placed in a 45-°C oven until dry. Approximately 1 g of dried homogenized soil was then place in a ball mill and ground to a fine powder.

1.3.4 Soil carbon determination

Finely ground sub samples of soil taken from the baseline grid (1989) or from each section (N,

Cnt., and S) and depth (0 to 15, 15 to 30, 30 to 60, and 60 to 90) within a WICST plot (2009)

were weighed (8 to 10 mg) and packed into 5 x 9-mm tin capsules. Total C for each encapsulated

sample was then determined by dry combustion using a Flash EA 1112 CN Automatic Elemental

Analyzer (Thermo Finnigan, Milan, Italy). [1] We use total C number interchangeably with SOC in

this study as inorganic carbon in these soils is negligible (<0.05 g kg^{-1}, Paul et al. 2001).

To match the 2009 data set, C values from the 1989 grid were converted to plot-level data

by overlaying the 1990 plot map on the 1989 grid map. The C data for individual plots was then

obtained 1) directly from the 1989 value if the grid and plot map lined up correctly (n=36 plots),

2) by attributing neighboring plots the same initial C content if the 27 x 27-m grid sampling fell

on the plot boundary (n=18), or 3) by averaging the C data from the two neighboring plots if the

1989 grid did not fall on a 1990 plot (n=2). The 1989 sampling provided three sampling

locations (similar to the N, Cnt., and S location in 2009) within a plot, and four depths within a

sampling location.

1.3.5 Bulk density estimation

Soil cores for BD were collected in June 1989 at two sampling depths (0 to 15, 15 to 30 cm) on

the 27 x 27-m baseline sampling grid discussed previously, using a 7.5-cm diameter hammer

core. Bulk densities for individual plots in 1989 were then obtained in the same manner as soil C

estimates for that year. Bulk densities were again estimated in 2007 (3.7-cm hammer core) and

---

[1] Use of equipment name for information purposes only and not an endorsement for the product.

2008 (5.4-cm hydraulic core), but to a depth of 90 cm (0 to 15, 15 to 30, 30 to 60, and 60 to 90 cm). An additional sampling in 2008 was done on a subset of the WICST plots using the same 7.5-mm hammer core used in 1989 to ensure that the two smaller diameter cores produced similar contemporary BD values and were comparable to 1989 values using the large diameter hammer core (data not shown). BD values were averaged across sample locations within a plot for a total of one data point per depth in each plot. The BD samples collected in 2007 and 2008 were so similar to each other that we averaged the sampling times together to provide a more robust estimate of 2009 values. It was assumed that below 30 cm, no significant (or detectable) change in BD was likely to have occurred in the course of the 20-year trial. We believe this was a reasonable assumption given the soil type and equipment history at WICST, i.e. well-structured grassland soils high in organic matter and relatively light agricultural equipment (Hamza and Anderson, 2005; Sanford et al., 2008). Current BD values for 30-60 and 60-90-cm horizons were therefore used for both the 2009 and 1989 sampling times. Equivalent mass corrections were conducted based on the original equivalent soil mass method described by Lee et al. (2009).

1.3.6 Statistical analysis

Linear mixed effects models (PROC MIXED, SAS v9.3) were used to analyze SOC as a function of time (1989 or 2009), system (CS1 – CS6), and depth (0-15, 15-30, 30-60, and 60-90 cm). Time, system, depth and their interactions were treated as fixed effects while block (1-4), plot, and location (N,Cnt.,S) within time and plot were treated as random effects. The resulting linear mixed effects model was

$$y = \mu + \beta_i + Y_j + S_k + Y{*}S_{jk} + \varepsilon_{ijn} + D_l + D{*}Y_{jl} + D{*}S_{kl} + D{*}Y{*}S_{jkl} + \delta_{ijnml}.$$

Where μ = population mean, β = block, Y= year, S=system, and D=depth. The first error term (ε) was used to test the fixed effects of year, system and their interactions, while the second error term (δ) was used to test the fixed effect of depth and its interactions with time and system. The subscripts i, j, k, n, l, and m indicate the block, year, system, plot, depth and location, respectively. This model was used for both SOC concentration (g kg^{-1}) and SOC mass data (Mg C ha^{-1}). The REPEATED statement in PROC MIXED (type = ar[1]) was used to account for spatial auto-correlation between points within a plot location in a given year. Due to the high inherent variability in SOC data we assume significance at α=0.1. The response variable BD was analyzed using a simplified version of the full model, which did not include the random effect of location within plot. An additional approach to evaluating the relative importance of management factors on SOC dynamics across systems was to develop a score for each of four factors that pertained to our original hypotheses (Table 1.2). Scores for the influence of tillage, manure, and residue inputs were developed following the method used by Simonsen et al. (2010). Briefly, for the correlation indices of tillage and manure a -1 (tillage), or +1 (manure) was assigned to every phase in a crop rotation in which tillage or a manure application occurred. The average value over the entire rotation was then used as a corollary with change in SOC. Correlation indices for biomass C inputs were obtained by first selecting the cropping system with the highest input values for above and below ground inputs as estimated by (Jokela et al., 2011). The remaining five cropping systems were then assigned a value relative to the maximum (Table 1.2).

1.3.7 Carbon input estimates

We used C input estimates, based on the approach of Jokela et al. (2011), to facilitate interpretation of system specific effects on SOC. Aboveground biomass inputs were either

measured directly (forage yields, green manure, and residual alfalfa plow-down, wheat grain and straw, and manure applications) or estimated using established harvest indices for corn (0.50) and soybean (0.40) (Bolinder et al., 2007). Below-ground inputs were calculated based on shoot/root ratios and rhizodeposition estimates from Bolinder (2007). In summary, the calculated total C inputs included estimates of the following sources: i) non harvested aboveground biomass, ii) roots, iii) material from root turnover and exudates, and iv) manure.

1.4 Results

1.4.1 SOC lost irrespective of cropping system

We found a significant annual loss of 0.8 Mg C ha^{-1} yr^{-1} (0-90cm, Table 1.3) irrespective of cropping system. The main effect of cropping system was also significant (p=0.03), although the interaction term between year and system was not. This indicated that although the magnitude of change in SOC was not the same among cropping systems, by and large, all lost SOC over the 20 yr period. The greatest loss occurred in the continuous corn system (-41.9 Mg C ha^{-1}, p=0.03), while the rotationally grazed pasture remained stable. The effect of depth was also highly significant (p<0.0001), as would be expected, with SOC values decreasing rapidly below 30 cm. The interaction of system with depth was highly significant (p=0.01) but this was largely due to the fact that SOC appeared to increase slightly in the surface 30 cm of the pasture system, while this was not observed in the other five systems. There were no substantive differences in the general trends observed with concentration or mass data. In both cases it was difficult to detect horizon and system specific significance, likely due to the inherently high variability in the SOC data (Table 1.4) and the loss of power associated with analyzing a subset of the data (i.e. decreasing sample size). Table 1.3 highlights the overall loss of SOC at WICST, irrespective of system, as well as the differences in analysis of concentration and mass data. The only system

to gain carbon over the 20-year period was the rotationally grazed pasture system, but significance in this case was limited to the surface 15 cm (Table 1.3). Contrasts relating to our original hypotheses were used to evaluate management related trends in SOC. This was a particularly useful approach because it provided sufficient power to detect changes in SOC, which were not discernible if systems or horizons within systems were considered independently (Table 1.3). When considering SOC concentration in the 0 to 30 cm horizon, NT systems (CS2, CS6) performed better (lost less) than CT systems (CS1, CS3, CS4, CS5, p=0.06). The effect of enterprise type (grain or forage) on SOC concentration was also highly significant in the surface 30 cm with forage based systems outperforming the grain systems (p=0.01).

Correlation data indicated a negative relationship between SOC stabilization and tillage as well as positive relationships with manure inputs and estimated belowground C inputs when considering the entire soil profile (0 to 90 cm). When analyzed by horizon, significant correlations with SOC change were limited to the surface 15 cm horizon (Table 1.5).

1.4.2 Increased bulk density greatest in surface 15 cm across all cropping systems

Potential changes in BD were analyzed for the top two horizons only (0 to 15 and 15 to 30 cm) due to a lack of deeper BD data from 1989. Across all cropping systems there was a significant (p<0.0001) increase in soil BD of 62 kg m^{-3} in the 20 years since the start of WICST. This trend was most pronounced in the 0-15-cm horizon compared to the 15-30-cm horizon (100 kg m^{-3} vs. 25 kg m^{-3}, respectively) and was also greater in the grain systems than in the forage based systems (87 kg m^{-3} vs. 37 kg m^{-3}, respectively). Of the grain systems, the NT corn and

soybeans underwent the greatest increase in BD of 176 kg m^{-3} (p<0.0001) in the 15 to 30-cm soil horizon.

1.5 Discussion

While NT management, application of manure, and inclusion of perennial crops all served to slow the loss of SOC, none of these practices favored sequestration of SOC over 20 years. The loss of carbon at WICST irrespective of management is consistent with the findings of Bellamy et al. (2005) who showed that SOC was lost across both England and Wales irrespective of land management over a 27-yr period. These observations are also strongly supported by SOC trends observed at a WICST mirror site (decommissioned in 2001) on a poorly drained prairie soil in Walworth county Wisconsin (data not shown). In this trial, C decreased in all systems over the course of 12 years with the exception of the rotationally grazed pasture, which in a similar manner to this study, sequestered C in the 0 to 30 cm horizon. The lack of sequestration observed over the course of 20 years highlights the importance of initial SOC data if conclusions are to be drawn regarding the effects of best management practices on SOC stocks. Had treatment differences at WICST been evaluated based only on 2009 estimates with continuous corn as our control (i.e. space for time analysis), we might have concluded that both the NT corn-soybean and the organic grain system had lost C over the course of the trial while erroneously concluding that the perennial forage systems (CS4-CS6) had sequestered C. Sanderman and Baldock (2010) clearly demonstrated that a gain of SOC following the implementation of improved management may not reflect actual dynamics if soil C is not in a state of equilibrium at

the time of the study. Based on our data, we cannot assume that such a steady state at WICST has yet been reached.

One major contributing factor to the observed loss of SOC at WICST is that current belowground C inputs are likely far smaller than those realized under the native tallgrass prairie. In a review of the relevant literature, DeLuca and Zabinski (2011) reported that under tallgrass prairie, belowground net primary production can range from 8 to 15 Mg ha^{-1} yr^{-1} with an estimated 3 to 5 Mg ha^{-1} yr^{-1} retained in the soil. Over the course of 5,000 to 8,000 years this belowground input led to an estimated 70 to 130 Mg C ha^{-1} in the surface 30 cm of tallgrass prairie soils (DeLuca and Zabinski, 2011). While aboveground productivity in tallgrass prairies may not differ substantially from that of annual cropping systems, belowground productivity is typically far greater than that of annual systems (DeLuca and Zabinski, 2011; Guzman and Al-Kaisi, 2010). The transition at Arlington from tallgrass prairie to forage crops in the 1850s, and later to grain crops (~1960s), resulted in a significant loss of original soil C as well as a reduced contribution of roots to SOC stocks. Without sufficient C inputs to offset the mineralization of organic matter these soils will continue to lose SOC.

The significant interaction of system with depth was likely the result of the rotationally grazed pasture system, which unlike the rest of the systems, sequestered C in the surface 15 cm of the soil profile. Sequestration of SOC in the surface horizon of the pastures was likely the result of both the quantity and quality (morphological characteristics) of belowground net primary production. In perennial grass systems like the rotationally grazed pasture, 80 to 90% of the belowground biomass is concentrated in the surface 30 cm of the soil and is dominated by fine roots (0 to 2 mm) (Jackson et al., 1996; Rasse et al., 2005). Tufekcioglu *et al*. (1998), working in central Iowa, found that live fine-root biomass in perennial cool season pastures

exceeded 6 Mg ha^{-1}, while in both corn and soybean systems (annual grain), fine root biomass

was < 2.3 Mg ha^{-1} in the surface 35 cm. The large surface area associated with fine root biomass

increases the interaction of both fine roots and root hairs with soil micro-pores and micro-

aggregates. Penetration of root hairs into such micro sites, where anoxic conditions prevail and

which are otherwise inaccessible to microbial decomposers, preferentially stabilizes these stocks

of root derived C (Rasse et al., 2005). Such occlusion of root derived C within soil aggregates is

thought to play a key role in the long term stabilization of SOC (Schmidt et al., 2011; Six et al.,

2002; Verchot et al., 2011) and help to explains the observed C sequestration in the 0 to 15 cm

horizon of the rotationally grazed pasture system.

The rotationally grazed pasture treatment exerted a large influence over the statistical

evaluation of tillage, manure inputs, and the degree of system perenniality (grain vs. forage).

When pasture was removed from these comparisons, however, we still found that NT practices,

inclusion of manure, and increased perenniality (i.e. greater proportion of a rotation in perennial

crops) slowed the loss of SOC relative to the more highly tilled or predominantly annual

systems. NT practices are thought to favor SOC accumulation (or slow its loss) by limiting the

amount of mechanically induced oxidation of plant residues and soil organic matter (Lal, 2008).

While some have reported increased SOC associated with the conversion from CT to NT

practices (Conant et al., 2007), others have observed no net sequestration or even loss of SOC

associated with NT (Baker et al., 2007; Huggins et al., 2007; Luo et al., 2010). It should be noted

that crop rotation will influence the observed rate of C turnover in NT systems. A continuous

corn system managed as NT may have led to sequestration of C where the NT corn-soybean

rotation at WICST did not. Inclusion of soybean in the rotation decreases the total quantity of

residue produced (below- and above-ground), decreases the C/N ratio of residue inputs (making

it more favorable for microbial decomposition), and results in a different distribution of the belowground component of that residue (Huggins et al., 2007). The beneficial effects of a perennial system are in part related to the advantages of reduced tillage discussed previously as well as the fact that perennial plants allocate greater C resources to belowground infrastructure than do most annual crops. The influence of belowground biomass on SOC dynamics cannot be understated, playing a greater role in the long term stabilization of soil C than inputs from aboveground biomass (Rasse et al., 2005).

An important and potentially confounding part of the SOC story at WICST may be a Wisconsin climate that has, for the past 57 years, undergone a 1.85°C increase in minimum winter temperatures as well as an increase in growing season precipitation of approximately 50 mm (Kucharik et al., 2010). While the impacts of a warming climate on SOC dynamics are far from being completely understood, there is some evidence to suggest that such trends will lead to a preferential loss of older and more stable C pools (Conant et al., 2008; Conant et al., 2011).

With the exception of the 15 to 30 cm horizon in the two alfalfa rotations (CS4 and CS5), BD increased in all remaining treatments and at both depths over the last 20 years. These changes were most pronounced in the 0 to 15 cm horizon ($+0.1$ Mg m^{-3}) and minimal in the 15 to 30 cm horizon ($+0.03$ Mg m^{-3}). Based on results reported in the soil compaction literature under similar conditions, we assume that there was no detectable change in BD below 30 cm (Hamza and Anderson, 2005; Sanford et al., 2008). Sanford et al. (2008) showed that on Plano silt loams in Southern Wisconsin soil compaction was not detectable below 30 cm when applying dairy slurry using equipment with an 11-Mg axle load. While manure spreading equipment has been the heaviest equipment employed at the site, slurry tankers have only recently been used (2005 onward) and have never exceeded the axle loads reported by Sanford et al. (2008). Furthermore

those plots that receive manure (forage systems CS4 – CS6) have a lower average BD than those that do not receive manure but are more frequently tilled (1.22 Mg m^{-3} forage vs. 1.28 Mg m^{-3} grain). This suggests that increases in soil BD at WICST were less a function of traffic and likely more related to soil consolidation associated with decreased soil aggregation or the well documented phenomenon of surface soil consolidation in NT systems. Data from a recent study of soil quality parameters at WICST supports this theory. Jokela et al. (2011) looking at two different soil depths (0 to 5 and 5 to 20 cm) found that the WICST forage systems (CS4-CS6) had a greater concentration of water stable aggregates than the grain systems (CS1-CS3).

1.6 Conclusions

Observed changes in SOC at the WICST over the past 20 years highlight the importance of accurate C accounting when drawing conclusions about the sequestration potential of best management practices. While NT management strategies, inclusion of perennial crops, and grass pasture all had beneficial effects on the C stocks at WICST, none of the six systems sequester atmospheric C when the entire 90-cm profile was considered. These results are consistent with finding at the WICST mirror site in southern WI, as well as with others who have compared initial and current SOC quantity rather than using space as a surrogate for time (nearby non-cultivated sites or a control treatment). The observed changes at WICST were likely the result of insufficient belowground C inputs to offset loss via SOC oxidation. While a trend in warming night time and winter temperatures as well as an increase in annual precipitation has occurred over the past half century, a connection between regional climate change and SOC dynamics, while potentially important, cannot at this time be clearly established. The trends reported in this

study suggest that the potential to sequester C on prairie-derived soils in the North Central US

may not occur under agricultural best management practices.

1.7 Acknowledgments

1.8 References

Baker J.M., Ochsner T.E., Venterea R.T., and T.J. Griffis. 2007. Tillage and soil carbon sequestration - What do we really know? Agriculture Ecosystems & Environment 118: 1-5.

Batjes N.H. 1996. Total carbon and nitrogen in the soils of the world. European Journal of Soil Science 47: 151-163.

Bellamy P.H., Loveland P.J., Bradley R.I., Lark R.M., and G.J.D. Kirk. 2005. Carbon losses from all soils across England and Wales 1978-2003. Nature 437: 245-248.

Bolinder M.A., Janzen H.H., Gregorich E.G., Angers D.A., and A.J. VandenBygaart. 2007. An approach for estimating net primary productivity and annual carbon inputs to soil for common agricultural crops in Canada. Agriculture Ecosystems & Environment 118: 29-42.

Buyanovsky G.A., and G.H. Wagner. 1998. Carbon cycling in cultivated land and its global significance. Global Change Biology 4: 131-141.

Conant R.T., Easter M., Paustian K., Swan A., and S. Williams. 2007. Impacts of periodic tillage on soil C stocks: A synthesis. Soil and Tillage Research 95: 1-10.

Conant R.T., Steinweg J.M., Haddix M.L., Paul E.A., Plante A.F., and J. Six. 2008. Experimental warming shows that decomposition temperature sensitivity increases with soil organic matter recalcitrance. Ecology 89: 2384-2391.

Conant R.T., Ryan M.G., Ågren G.I., Birge H.E., Davidson E.A., Eliasson P.E., Evans S.E., Frey S.D., Giardina C.P., Hopkins F.M., Hyvönen R., Kirschbaum M.U.F., Lavallee J.M., Leifeld J., Parton W.J., Megan Steinweg J., Wallenstein M.D., Martin Wetterstedt J.Å., and M.A. Bradford. 2011. Temperature and soil organic matter decomposition rates – synthesis of current knowledge and a way forward. Global Change Biology 17: 3392-3404.

DeLuca T.H., and C.A. Zabinski. 2011. Prairie ecosystems and the carbon problem. Frontiers in Ecology and the Environment 9: 407-413.

Guzman J.G., and M.M. Al-Kaisi. 2010. Soil Carbon Dynamics and Carbon Budget of Newly Reconstructed Tall-grass Prairies in South Central Iowa. Journal of Environmental Quality 39: 136-146.

Hamza M.A., and W.K. Anderson. 2005. Soil compaction in cropping systems: A review of the nature, causes and possible solutions. Soil and Tillage Research 82: 121-145.

Huggins D.R., Allmaras R.R., Clapp C.E., Lamb J.A., and G.W. Randall. 2007. Corn-soybean sequence and tillage effects on soil carbon dynamics and storage. Soil Science Society of America Journal 71: 145-154.

Huggins D.R., Buyanovsky G.A., Wagner G.H., Brown J.R., Darmody R.G., Peck T.R., Lesoing G.W., Vanotti M.B., and L.G. Bundy. 1998. Soil organic C in the tallgrass prairie-derived region of the corn belt: effects of long-term crop management. Soil & Tillage Research 47: 219-234.

Jackson R.B., Canadell J., Ehleringer J.R., Mooney H.A., Sala O.E., and E.D. Schulze. 1996. A global analysis of root distributions for terrestrial biomes. Oecologia 108: 389-411.

Jokela W., Posner J., Hedtcke J., Balser T., and H. Read. 2011. Midwest Cropping System Effects on Soil Properties and on a Soil Quality Index. Agronomy Journal 103: 1552-1562.

Kleber M., and M.G. Johnson. 2010. Advances in Understanding the Molecular Structure of Soil Organic Matter: Implications for Interactions in the Environment, Advances in Agronomy, Vol 106, Elsevier Academic Press Inc, San Diego. pp. 77-142.

Kravchenko A.N., and G.P. Robertson. 2011. Whole-Profile Soil Carbon Stocks: The Danger of Assuming Too Much from Analyses of Too Little. Soil Science Society of America Journal 75: 235-240.

Kucharik C.J., Serbin S.P., Vavrus S., Hopkins E.J., and M.M. Motew. 2010. Patterns of climate change across Wisconsin from 1950 to 2006. Physical Geography 31: 1-28.

Lal R. 2008. Carbon Sequestration. Philosophical Transactions of the Royal Society 363: 815-830.

Liu S.G., Tan Z.X., Li Z.P., Zhao S.Q., and W.P. Yuan. 2011. Are soils of Iowa USA currently a carbon sink or source? Simulated changes in SOC stock from 1972 to 2007. Agriculture Ecosystems & Environment 140: 106-112.

Loughin T.M. 2006. Improved experimental design and analysis for long-term experiments. Crop Science 46: 2492-2502.

Luo Z., Wang E., and O.J. Sun. 2010. Can no-tillage stimulate carbon sequestration in agricultural soils? A meta-analysis of paired experiments. Agriculture, Ecosystems & Environment 139: 224-231.

Nafziger E.D., and R.E. Dunker. 2011. Soil Organic Carbon Trends Over 100 Years in the Morrow Plots. Agronomy Journal 103: 261-267.

Ogle S.M., Breidt F.J., and K. Paustian. 2005. Agricultural management impacts on soil organic carbon storage under moist and dry climatic conditions of temperate and tropical regions. Biogeochemistry 72: 87-121.

Paul E.A., Collins H.P., and S.W. Leavitt. 2001. Dynamics of resistant soil carbon of midwestern agricultural soils measured by naturally occurring C-14 abundance. Geoderma 104: 239-256.

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

Posner J.L., Casler M.D., and J.O. Baldock J.O. 1995. The Wisconsin integrated cropping systems trial: Combining agroecology with production agronomy. American Journal of Alternative Agriculture 10: 98-107.

Posner J.L., Baldock J.O., and J.L. Hedtcke. 2008. Organic and conventional production systems in the Wisconsin Integrated Cropping Systems Trials: I. Productivity 1990-2002. Agronomy Journal 100: 253-260.

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

Sanderman J., and J. Baldock. 2010. Accounting for soil carbon sequestration in national inventories: a soil scientist's perspective. Environmental Research Letters 5: 1-6.

Sanford G.R., Posner J.L., Schuler R.T., and J.O. Baldock. 2008. Effect of dairy slurry application on soil compaction and corn Zea mays L.. yield in Southern Wisconsin. Soil & Tillage Research 100: 42-53.

Schmidt M.W., Torn M.S., Abiven S., Dittmar T., Guggenberger G., Janssens I.A., Kleber M., Kogel-Knabner I., Lehmann J., Manning D.A.C., Nannipieri P., Rasse D.P., Weiner S., and S. Trumbore. 2011. Persistence of soil organic matter as an ecosystem property. Nature 478: 41-56.

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

Syswerda S.P., Corbin A.T., Mokma D.L., Kravchenko A.N., and G.P. Robertson. 2011. Agricultural management and soil carbon storage in surface vs. deep layers. Soil Science Society of America Journal 75: 92-101.

Tufekcioglu A., Raich J.W., Isenhart T.M., and R.C. Schultz. 1998. Fine root dynamics, coarse root biomass, root distribution, and soil respiration in a multispecies riparian buffer in Central Iowa, USA. Agroforestry Systems 44: 163-174.

USEPA. 2011. Inventory of US greenhouse gase emissions and sinks: 1990-2009 Executive Summary. http://www.epa.gov/climatechange/Downloads/ghgemissions/US-GHG-Inventory-2011-Executive-Summary.pdf. (accessed, 9 August 2012).

VandenBygaart A.J., and D.A. Angers. 2006. Towards accurate measurements of soil organic carbon stock change in agroecosystems. Canadian Journal of Soil Science 86: 465-471.

Verchot L.V., Dutaur L., Shepherd K.D., and A. Albrecht. 2011. Organi matter stabilization in soil aggregates: Understanding the biogeochemical mechanisms that determine the fate of carbon inputs in soils. Geoderma 161: 182-193.

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

Table 1.1. Cropping system specifics including average yield, primary tillage equipment, first year available nutrients, and estimates for above and below ground C inputs for the six systems at WICST.

Type	Label	System	Crop Phase	Average Yield[a] (Mg ha^{-1})	Primary Tillage Equipment	Annual N-P-K Inputs[b] 1st Yr. Available[d] (kg ha^{-1})	Source[e]	Estimated Annual C Input[c] Above Ground	Below Ground
								------------ (kg ha^{-1}) ------------	
Grain Systems	CS1	continuous corn	corn	10.7	chisel plow	142-5-20	F	**3800**	**2240**
	CS2	corn-soybean	soybean	3.5	no till	0-0-0		*1970*	*1040*
			corn	11.0	no till	136-4-19	L,F	*3910*	*2300*
			system mean					**2940**	**1670**
	CS3	organic grain	soybean	3.2	chisel plow	0-0-0		*1750*	*920*
			winter wheat / green manure[f]	3.7	field cultivator	0-0-0		*1850*	*850*
			corn	8.6	chisel plow	146-0-0	L	*3140*	*1810*
			system mean					**2240**	**1200**
Forage Systems	CS4	conventional forage	alfalfa seeding	6.0	chisel plow	241-23-160	M1	*3390*	*720*
			alfalfa	10.7	none	0-0-0		*800*	*2880*
			alfalfa	9.2	none	0-0-0		*1140*	*9170*
			corn	12.3	chisel plow	240-24-168	L, F, M1	*6890*	*2560*
			system mean					**3050**	**3840**
	CS5	organic forage	oat-alfalfa seeding	8.1	chisel plow	182-17-120	M1	*2830*	*1770*
			alfalfa	11.1	none	0-0-0		*1280*	*8150*
			corn	10.0	chisel plow	176-16-117	L, M1	*5560*	*2110*
			system mean					**3220**	**4010**
	CS6	pasture	mixed pasture[g]	8.4	none	52-5-31	M2	**1590**	**4570**

[a] Forage yields reported at 100% dry matter (DM), corn yields at 84.5% DM, soybean yields at 87% DM, and wheat yields at 86.5% DM.

[b] First-year legume and manure credits included where applicable based on Univ. of Wisconsin Ext. Bulletin A2809 (Laboski 2006).

[c] Soil C inputs were estimated based on harvest index and shoot/root ratios from Bolinder et al., 2007; includes C from post-harvest residue, green manure, dairy cow manure, roots and root exudates.

[d] First year availability accounts only for the nutrients released to a growing crop during the same year it is applied. Manure and other organic forms of nutrients contain more total nutrients than are available to the crop in any given year.

[e] F = commercial fertilizer; L=legume plowdown; M1: Semi-solid manure (155 g kg^{-1} solids) was applied through fall of 2004 and slurry manure (87 g kg^{-1} solids) was applied since, following UW recommendations (Laboski, 2006). Manure applied in the fall prior to corn planting and alfalfa seeding. Total N additions 115 kg total N ha^{-1} yr^{-1} from solid manure and 140 kg total N ha^{-1} yr^{-1} from slurry averaged over the rotation. M2: 65 kg total N ha^{-1}yr^{-1} deposited by five grazing heifers on 1.2 ha at 150 days

[f] Red clover frost seeded or drilled into winter wheat in early spring 1990 to 2005; berseem clover and oats were planted after wheat harvest for improved weed control post-2005.

[g] Pasture mix: Timothy (*Phleum pretense* L.), bromegrass (*Bromus inermis* L.), orchardgrass (*Dactlyis glomerata* L.) and red clover (*Trifolium pretense* L.). Red clover was re-seeded every two or three years with a NT drill

Table 1.2. Correlation indices for each of the six WICST systems used to test management specific hypotheses regarding change in SOC.

Label	System	Tillage[a]	Manure[b]	Estimated Carbon Inputs[c]	
				Above Ground	Below Ground
CS1	continuous corn	1	0	1	0.49
CS2	corn-soybean	0	0	0.77	0.37
CS3	organic grain	1	0	0.59	0.26
CS4	conventional forage	0.5	0.5	0.80	0.84
CS5	organic forage	0.67	0.67	0.85	0.88
CS6	pasture	0	1	0.42	1

[a] The frequency of tillage over the entire rotation where each tilled phase = 1

[b] The frequency of manure application, each manured phase = 1

[c] Estimated C inputs are taken from Table 1: the correlation index for each system is relative to the system contributing the most total biomass: above ground = CS1, below ground = CS6.

Table 1.3. SOC values by year and depth for WICST overall and by system. Values are model estimates rather than mathematical means to account for the imbalance in the experimental design (differing number of plots per system per block) and missing data from the baseline (1989) grid[a]

Type	Label	Depth	g C kg soil^{-1}				Mg C ha^{-1}			
			1989	2009	Δ	pr > |t|[b]	1989	2009	Δ	pr > |t|
WICST	All Systems	0-15 cm	26.4	25.2	**-1.2**	†	46.2	43.9	-2.3	ns
		15-30 cm	21.0	18.3	**-2.7**	**	41.1	36.5	**-4.6**	*
		30-60 cm	10.2	9.0	-1.2	ns	43.6	38.9	**-4.8**	*
		60-90 cm	5.3	4.1	-1.2	ns	23.9	19.0	**-4.9**	*
		average of Δ conc.			**-1.6**	*0.01*	*sum of Δ mass*		**-16.6**	*0.01*
Grain Systems	CS1	0-15 cm	28.6	23.5	-5.1	*	50.3	41.5	-8.8	ns
		15-30 cm	21.8	17.0	-4.8	*	42.9	33.9	-9.0	ns
		30-60 cm	11.5	8.2	-3.3	ns	50.5	35.4	**-15.1**	*
		60-90 cm	5.9	4.3	-1.6	ns	28.3	19.3	-9.0	ns
		average of Δ conc.			**-3.7**	*0.06*	*sum of Δ mass*		**-41.9**	*0.03*
	CS2	0-15 cm	24.2	22.7	-1.5	ns	40.9	38.2	-2.7	ns
		15-30 cm	19.7	16.3	**-3.4**	*	38.6	33.2	-5.4	ns
		30-60 cm	8.6	8.2	-0.4	ns	38.4	37.7	-0.7	ns
		60-90 cm	5.2	3.8	-1.4	ns	22.6	18.5	-4.1	ns
		average of Δ conc.			**-1.7**	*ns*	*sum of Δ mass*		*-12.9*	*ns*
	CS3	0-15 cm	25.8	22.8	**-3.0**	*	46.4	41.0	-5.4	ns
		15-30 cm	19.8	16.3	**-3.5**	**	39.1	33.5	-5.6	ns
		30-60 cm	8.1	7.8	-0.3	ns	37.9	36.5	-1.4	ns
		60-90 cm	4.6	3.4	-1.2	ns	20.6	15.9	-4.7	ns
		average of Δ conc.			**-2.0**	*0.07*	*sum of Δ mass*		*-17.1*	*ns*
Forage Systems	CS4	0-15 cm	27.6	26.8	-0.8	ns	49.0	47.4	-1.6	ns
		15-30 cm	21.8	19.1	**-2.7**	*	42.8	38.1	-4.7	ns
		30-60 cm	10.1	10.2	0.1	ns	43.3	41.6	-1.7	ns
		60-90 cm	5.1	4.4	-0.7	ns	22.6	19.6	-3.0	ns
		average of Δ conc.			**-1.0**	*ns*	*sum of Δ mass*		*-11.0*	*ns*
	CS5	0-15 cm	25.6	24.5	-1.1	ns	46.4	44.0	-2.4	ns
		15-30 cm	19.8	17.7	-2.1	ns	40.5	35.8	-4.7	ns
		30-60 cm	9.0	7.8	-1.2	ns	37.7	33.3	-4.4	ns
		60-90 cm	5.3	4.4	-0.9	ns	24.4	19.6	-4.8	ns
		average of Δ conc.			**-1.3**	*ns*	*sum of Δ mass*		*-16.3*	*ns*
	CS6	0-15 cm	26.8	31.1	**4.3**	†	43.9	51.1	7.2	ns
		15-30 cm	22.9	23.2	0.3	ns	42.8	44.6	1.8	ns
		30-60 cm	13.6	11.8	-1.8	ns	54.0	48.6	-5.4	ns
		60-90 cm	5.8	4.5	-1.3	ns	24.8	20.9	-3.9	ns
		average of Δ conc.			**0.4**	*ns*	*sum of Δ mass*		*-0.3*	*ns*

[a] All values and significance tests were calculated using comparison specific contrasts (ESTIMATE statements within PROC MIXED, SAS v. 9.1.3).

[b] Pr>|t|, ns=not significant at the α=0.1 level, † p<0.1, * p<0.05, ** p<0.01

Table 1.4. SOC concentration and mass at WICST from 1989 and 2009 showing both the rapid decrease in SOC concentration values with depth and the large amount of inherent variability. [a]

SOC Unit	Depth	--------- 1989 ---------			--------- 2009---------		
		n	mean	CV(%)	n	mean	CV(%)
g kg^{-1}	0-15 cm	161	26.3	24.3	167	24.9	24.0
	15-30 cm	164	20.6	34.5	167	18.0	41.6
	30-60 cm	109	10.7	64.4	168	8.9	65.6
	60-90 cm	128	5.3	38.8	168	4.1	48.8
Mg ha^{-1}	0-15 cm	161	46.5	20.4	167	43.9	22.9
	15-30 cm	164	40.9	31.8	166	36.1	35.2
	30-60 cm	109	43.5	61.0	167	38.3	60.1
	60-90 cm	128	22.7	38.9	168	18.7	48.7

[a] All values are arithmetic means (MEANS procedure, SAS v. 9.1.3).

Table 1.5. Correlations (*r*) between SOC change and the four indices outlined in Table 1.2 showing the negative relationship between tillage and SOC stabilization and the positive relationship of organic amendments and below ground productivity on SOC stabilization.

SOC unit	Depth	Tillage	Manure	-------- *Estimated Carbon Inputs*--------	
				Aboveground	Belowground
		--- *r* ---			
g kg-1	0-15 cm	-0.19*[a]	0.26***	-0.09	0.24**
	15-30 cm	-0.09	0.12	-0.05	0.10
	30-60 cm	-0.03	0.02	-0.02	0.02
	60-90 cm	-0.08	0.10	-0.04	0.01
	whole profile	*-0.10**	*0.13***	*-0.05*	*0.11***
Mg ha-1	0-15 cm	-0.20	0.25	-0.09	0.23**
	15-30 cm	-0.11	0.08	-0.08	0.05
	30-60 cm	-0.09	-0.02	-0.05	-0.03
	60-90 cm	-0.12	0.08	-0.07	0.07
	whole profile	*-0.11***	*0.07*	*-0.06*	*0.05*

[a] Probability > |r| under H0: ρ=0, † p<0.1, * p<0.05, ** p<0.01, ***p<0.001

CHAPTER 2

SOIL CARBON DYNAMICS ALONG AN AGROECOSYSTEM LAND-COVER GRADIENT ON

MOLLISOLS OF SOUTHERN WISCONSIN

2. SOIL CARBON DYNAMICS ALONG AN AGROECOSYSTEM LAND-COVER GRADIENT ON

MOLLISOLS OF SOUTHERN WISCONSIN

2.1 Abstract

There is currently a great deal of interest in managing agricultural soils to serve as long-term carbon (C) sinks. To do so requires knowledge of both the size and kinetics of C pools within agroecosystems. In this study, we use a combination of long-term (230-day) soil incubations and 6N HCl acid hydrolysis to evaluate the allocation of soil organic carbon (SOC) between labile and stable pools in nine agroecosystems ranging from continuous, conventionally tilled (CT) maize (*Zea mays* L.) to a remnant prairie in southern Wisconsin on the same soil series (Plano silt loam). While SOC dynamics were similar among divergent production agricultural practices, differences in grassland history and management resulted in significantly different models (p < 0.05) of SOC pools and turnover. In general, the grassland systems had higher SOC concentrations than did the agronomic systems (29 vs. 23 g kg^{-1} soil) with a greater proportion of SOC present in rapidly and slowly mineralizable forms in these systems compared to production agriculture (58 vs. 50% of SOC). The relatively high percentage of SOC found in the non-hydrolysable fraction of the production agricultural systems (50%) likely reflects the oxidative loss of labile and accessible C pools in response to historic tillage. The mean residence times of both the rapidly and slowly mineralized carbon pools were greatest in the remnant prairie system (69 d and 44 yr, respectively) but did not markedly differ among the other grassland and agricultural systems (53 d and 28 yr, respectively). Regional and site specific estimates of C pools and their kinetics are needed for accurate biogeochemical modeling efforts.

Therefore, the AHI method should prove valuable in efforts to better understand how changes in climate and land management (e.g. towards perennial biomass production) will affect current and future soil C stocks across diverse agricultural landscapes.

2.2 Introduction

The soil organic matter pool (SOM) contains more C than the combined amount found in the atmosphere and vegetation globally (Brady and Weil, 2008; Houghton, 2007; Lal, 2008). As a result, changes in this pool, and more specifically the soil organic carbon pool (SOC), can exert a large effect on the global C budget (Bellamy et al., 2005). There is currently a great deal of interest in managing agricultural soils to serve as C sinks to help offset rising levels of atmospheric CO_2. This approach to atmospheric CO_2 reduction is attractive in that it is potentially cost effective and can be implemented using current agricultural technologies (Conant et al., 2007; Lal, 2008). Managing soils to function as long-term C sinks has many other benefits as well such as improving soil structure, reducing erosion and non-point source pollution, increasing water holding capacity and improving the nutrient supplying capacity of a soil for food production (Lal, 2008). A robust understanding of SOM dynamics and sensitivity to disturbance is of critical importance in our efforts to stabilize current SOC stocks, foster C sequestration, and model SOC behavior under future climate and land-use change scenarios (Schmidt et al., 2011; Schwendenmann and Pendall, 2008).

Soil organic matter is a complex continuum of plant, animal, and microbe derived residues that differ in their mineralization rates as well as in their contributions to soil physical and chemical properties (McLauchlan and Hobbie, 2004; Paul et al., 2001a). A number of SOM

fractionation methods have been employed to make sense of this continuum (McLauchlan and Hobbie, 2004; Paul et al., 2006; Poirier et al., 2005; von Lutzow et al., 2007), with the most recent goal of improving our understanding of how land management and climate change will affect terrestrial ecosystem SOC stocks (Conant et al., 2011; Schmidt et al., 2011; Steinweg et al., 2008). Soil organic matter fractionation techniques typically involve the isolation of distinct soil pools based on physical or chemical soil properties (Gulde et al., 2008; Jagadamma and Lal, 2010; Silveira et al., 2008). In addition to the isolation of SOM pools, radiocarbon (^{14}C) measurements and long term in-vitro soil incubations have been used to estimate the turnover rate or mean residence time (MRT) of SOC within these pools (Follett et al., 2007; Paul et al., 2006). Because multiple mechanisms contribute to SOM mineralization and stabilization (Schmidt et al., 2011; Six et al., 2002), it follows that methods for assessing SOM dynamics that incorporate multiple stabilization pathways would be preferred to those that do not (Olk and Gregorich, 2006).

One such method, the "acid hydrolysis-incubation" (AHI) method, combines the use of long term soil incubations and 6N HCl acid hydrolysis to estimate the size and decomposition rate of a rapidly mineralized C pool (C_{rm}), a slowly mineralized C pool (C_{sm}), and an old C pool (C_{nh}) that is considered chemically stable (Collins et al., 2000; Paul et al., 2006; Paul et al., 1999). In this method, long-term (e.g., months to years) monitoring of CO_2 flux rates from soil incubations are used to define the kinetics of rapid and slowly mineralized C as well as the size of the rapidly mineralized C pool. Soil organic carbon studies based on CO_2 flux rates are unique in that soil C pools and kinetics are defined by the microbial community (lability and accessibility of C) rather than by imposed physical or chemical criteria (Fortuna et al., 2003; Paul et al., 2006). Acid hydrolysis is used to isolate a chemically "recalcitrant" pool that is

presumed to represent a stabilized, or older, fraction of SOC; the MRT of this pool is usually obtained via ^{14}C measurements.

The AHI method is not without its potential drawbacks. Bruun and Luxhoi (2006) argued that fitting a two pool [or three pool] model to flux data obtained from soil incubations will only provide meaningful pools of SOM if these distinct pools actually exist. The use of acid-hydrolysis to isolate a stable SOM pool can also be problematic in that; 1) recent plant materials may persist in the non-hydrolysable soil fraction, 2) the size of C_{nh} can, in some instances, respond to land use change on a short timescale, and 3) the acid-hydrolysis technique places an undue emphasis on chemical recalcitrance as the primary mode of SOC stabilization. Schwendenmann and Pendall (2008), Kogel-knabner et al. (1994), Collins et al. (2000), and others have demonstrated that C_{nh} may include recent carbon additions from plant residues. In addition to this discrepancy, Paul et al. (2006) found that C_{nh} is more dynamic than we would expect from its ^{14}C age and can shift in response to changes in land management such as afforestation or cultivation. In a recent literature review, Dungait et al. (2012) demonstrated that SOC preservation is primarily a factor of substrate accessibility, and not chemical recalcitrance, although they assert that some degree of chemical recalcitrance as a C stabilization factor in soils cannot be ruled out.

In spite of these potential complications, the AHI method has proven useful in evaluating SOM dynamics and improving soil biogeochemical models. Paul et al. (2006) conducted a literature review to evaluate the utility of the AHI method and concluded that the method yielded reproducible and sensitive pools of SOC with kinetics that have been validated using ^{13}C and ^{14}C markers. Furthermore, although C_{nh} can be biased by the inclusion of fresh plant and microbial biomass, ^{14}C dating has routinely demonstrated the great age of this residue (Paul et al., 2001b).

Parameter estimates obtained via the AHI method correspond well with the active, slow, and passive C pools used in biogeochemical models such as CENTURY (Parton et al., 1987) and ROTHC (Coleman and Jenkinson, 1996). They can therefore be used to improve regional SOC modeling efforts by tailoring belowground C dynamics to best represent specific regions, soils, and agroecosystems (Collins et al., 2000; Haile-Mariam et al., 2000). Using the System Approach to Land Use Sustainability (SALUS) model Paul et al. (1999) report that parameterization with SOM pool estimates obtained via the AHI method resulted in CO_2-C fluxes that agreed well with field data. They found the greatest agreement between SALUS and field CO_2 flux during periods where no living crop was in the field because root respiration was absent. These results were consistent with those of Paul et al. (2006) who showed that parameterizing the DAYCENT model using estimates of C_{rm}, C_{sm}, and C_{nh}, obtained from the AHI method provided CO_2 evolution rates that were well correlated with field CO_2 measurements.

Paul et al. (1999) evaluated eight cropping systems using the AHI method in the North Central U.S.A on Alfisols of similar soil series. Their aims were to improve upon concepts relating soil characteristics to ecosystem function, and to provide estimates of SOC pools and kinetics that would improve biogeochemical modeling efforts for similar soils and cropping system. Collins et al. (2000) also utilized the AHI method to provide estimates of SOC dynamics that were accurate enough for use in both land management decisions and global change calculations (i.e. modeling efforts). While they worked on both Alfisols and Mollisols of the North Central U.S.A., their work was limited to maize based systems. In the present study we build upon the work of Paul et al. (1999) by looking at a similar range of agroecosystems but on Mollisols of the North Central U.S.A., and expand upon the work of Collins et al. (2000) by

evaluating several non-maize based agroecosystems at a location that is shared between the two studies. The nine agroecosystems evaluated in the current study span a large range of land cover from conventionally tilled (CT) continuous maize to a remnant grazed prairie. The objectives of this study were to: i) study the relative importance of tillage and perenniality on the distribution and kinetics of SOC among the three operationally defined carbon pools between these systems, and ii) provide data on SOM pool size and decomposition rates for future SOC modeling work on Mollisols of the North Central U.S.A. Our initial hypotheses were that: i) total SOC and subsequent rates of CO_2 evolution would be greatest in perennial grass systems as a result of significant fine root biomass and root exudate inputs in these soils, ii) tillage would result in a lower proportion of total carbon in rapid and slowly mineralized pools as a result of oxidative loss with a correspondingly greater proportion in the older non-hydrolyzable C fraction (NHC), and that conversely iii) perennial grass systems would have the highest proportion of total carbon stored in rapidly and slowly mineralized forms.

2.3 Materials and Methods

2.3.1 Agroecosystem selection criteria and characteristics

A total of nine agroecosystems were initially selected for this study. The three criteria for selection of these agroecosystems were, i) that the agroecosystems fall along a land-cover gradient ranging from low-diversity/high-input agricultural systems to high-diversity/low-input native grassland systems, ii) that the agroecosystems share the same soil series, and iii) that the agroecosystems be proximally located.

The nine agroecosystems identified were distributed between four sites within an 11-km radius of the University of Wisconsin's agricultural research station in Arlington, WI (UW-ARL: 43°18'10"N, 89°20'43"W). Two of the four sites were located at the research station. A third site was located at the Madison Audubon Society's Goose Pond Sanctuary (GP: 43°18'58"N, 89°21'54"W) 2 km north of UW-ARL, and the fourth site was on land managed by the Wisconsin Department of Natural Resources (DNR) in Waunakee, WI (WW: 43°13'57"N, 89°26'22"W).

The soils at all four sites were identically classified as Plano silt loam (fine-silty, mixed, superactive, mesic typic argiudolls). These are relatively deep (> 1 m), well drained soils that were formed under tallgrass prairie vegetation in loess deposits over calcareous glacial till. Conversion from prairie vegetation to crop land in the Arlington vicinity began in the mid 1800s, primarily for the production of wheat. From the 1860s until the middle of the 20th century the land was used to produce feed for dairy cattle and from 1960 until restoration (prairie) or trial establishment (agronomic trials) the predominant crop rotations were of maize and alfalfa (*Medicago sativa* L.). The mean annual temperature and precipitation for this area are 6.9°C and 869 mm respectively (1981-2010, National Climate Data Center).

The two sites located at ARL were the Wisconsin Crop Rotation Trial (WCRT) and the Wisconsin Integrated Cropping Systems Trial (WICST). The WCRT was established in 1983. Two agroecosystems from WCRT were selected for the current study – conventionally tilled (CT) and no-till (NT) continuous maize. Further details regarding the Wisconsin Crop Rotation Trial can be found in Pedersen and Lauer (2002).

The main WICST experiment began in 1990. In 1998 a tallgrass prairie planting was nested within the greater WICST experiment. The five agroecosystems selected from WICST include; CT continuous maize, NT maize-soybeans, conventional forage with a year of maize and three years of alfalfa, rotationally grazed pasture, and an 11 year old prairie restoration. For further details on WICST see Posner et al. (2008).

The two sites located outside the research station were chosen to provide a more comprehensive comparison of row-crop agriculture and native grasslands. At GP, a 3.2 – ha tallgrass prairie restoration was established in 1976 on what had previously been row-cropped land. Burning at GP since restoration has taken place every three years on average to reduce weed competition and promote establishment of desirable native species. Dominant prairie species include big bluestem (*Andropogon gerardii* Vitman), indiangrass (*Sorghastrum nutans* [L.] nash), switchgrass (*Panicum virgatum* L.), purple coneflower (*Echinacea purpurea* [L.] Moench), goldenrod (*Solidago spp.* L.), and black-eyed susan (*Rudbeckia hirta* L.). This site has been studied extensively, and more detailed descriptions can be found in (Brye et al., 2002a; Brye et al., 2002b; Kucharik et al., 2001; Wagai et al., 1998).

The WW site is located 10.9 km south of UW-ARL. The area sampled is on a toe slope. The remainder of the prairie remnant is atop a glacial drumlin that was formed during the Wisconsin glaciation (110,000 to 10,000 yr BP). It is this geologic history that has prevented the cultivation of the WW prairie for the production of row crops. Although never tilled, the WW site was used prior to 2000 as grazing land for beef and dairy cattle. In 2000, land management responsibility was taken over by the Wisconsin DNR. Management has since consisted of annual burning and gradual removal of invasive plant species. Current species at the site include non-native C3 pasture grasses such as smooth bromegrass (*Bromus inermis* Leyss.) and Kentucky

bluegrass (*Poa pratensis* L.) as well as native C3 (needlegrass, *Achnatherum spp.* P. Beauv.) and C4 grasses (big bluestem and Indiangrass).

A description of each of the nine agroecosystems and the four sites on which they are located are presented in Table 2.1.

2.3.2 Soil sampling, processing, and analysis

Soil samples from each agroecosystem were collected during the summer of 2010 at a depth of 0 to 15 cm using a 2-cm diameter hand held soil probe. A description of the sampling protocol for each agroecosystem is outlined in Table 2.1. Following collection, soils for each agroecosystem were thoroughly homogenized, sieved to 4 mm, picked free of all visible plant material, and stored in plastic bags at 4°C until further processing.

Aliquots of each soil sample were analyzed for texture, nutrient content, SOC, and NHC. Soil texture was determined on three 50 – g samples from each treatment using a standard hydrometer method (SPAL, 2004). Additional samples from each treatment (100 g) were sent to the University of Wisconsin Soil and Plant Analysis Lab (SPAL) for determination of pH (1:1, soil:water), organic matter (weight loss-on-ignition, 360°C), available P and K (Bray P1 extract), exchangeable Ca and Mg (1N NH_4OAc, pH 7.0), and cation exchange capacity. To determine total soil carbon, four finely ground sub-samples of soil from each agroecosystem were weighed (8-10 mg), packed into a 5 x 9 – mm tin capsule, and analyzed on a Flash EA 1112 CN Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy). We use total C interchangeably with SOC in this study as inorganic carbon in these soils is negligible (<0.05 g kg^{-1}, Paul et al. 2001). Non-hydrolysable carbon was determined for each agroecosystem by refluxing three 2 g

samples of soil from each treatment in 20 ml of 6M HCl at 115°C for 16 hr according to standard published protocols (Paul et al., 2001a; Paul et al., 2006; Sollins et al., 1999).

Samples were prepared for long term (230 d) soil incubations by packing sufficient field moist soils into a 100 – ml specimen cup to reach a desired dry bulk density of 1.27 g cc^{-1} (based on average field values, data not shown). Five specimen cups (replicates) were prepared for each agroecosystem (n=45). Soils were then wetted to 60% water filled pore space (WFPS, Linn and Doran [1984]) based on their packed bulk density (1.27 g cc^{-1}) and an estimated particle density of 2.65 g cc^{-1} (Campbell and Norman, 1998). Packed specimen cups were then placed in 950 – ml glass canning jars, and 20 ml of deionized water was added to the bottom of each jar to maintain internal humidity. Vented metal lids (2 x 7 mm dia. hole, 2% of lid area) were placed on the jars and the soils were allowed to stabilize for 16 hr in the dark at 22.2°C prior to initial CO_2 flux measurements. Room air temperature was held constant at 22.2 ℃ for the duration of the soil incubations.

2.3.3 CO_2 flux measurements

Soil CO_2 flux measurements were made using a LI-820 infrared gas analyzer (IRGA) (LI-COR Biosciences, Lincoln, NE). The LI-820 recorded concentration data in CO_2 parts per million (µl L^{-1}) every 10 s over the course of a 10 – min interval. Data from the initial five minutes of each IRGA reading was discarded to ensure that the system had stabilized prior to estimating the CO_2 flux rate. Flux rates were determined by fitting a simple linear regression model to the output data and then converting from CO_2 concentration change over time (µl L^{-1} s^{-1}) to mass loss of carbon over time (µg C [g soil]$^{-1}$ day^{-1}).

Following each IRGA reading, soil moisture was adjusted to 60% WFPS. Vented lids were then re-attached to the incubation chambers and the jars were placed in the dark until the next IRGA reading. Readings were taken every few days for the first month and then approximately monthly until day 125 at which point readings increased to multiple events per month until the end of the experiment. This resulted in a total of 25 readings over 230 days. Soil moisture content was maintained within 1% of optimum for microbial activity (60% WFPS; Linn and Doran [1984]) throughout the study by adding water weekly to each specimen cup to replace evaporative losses irrespective of IRGA measurements.

2.3.4 Statistical modeling of CO_2 flux data

A three pool constrained model (Eq. 1) with first order kinetics was used to evaluate the size and decomposition rates of three SOC pools (Eq. 1) (Paul et al., 2001a).

Eq. 1
$$C_{t(t)} = C_{rm}e^{-k_{rm}(t)} + C_{sm}e^{-k_{sm}(t)} + C_{nh}e^{-k_{nh}(t)}$$

In this model $C_{t(t)} =$ is total soil organic carbon at time t; C_{rm}, C_{sm}, and C_{nh} represent the C mass in the rapidly mineralized, slowly mineralized, and non-hydrolysable fractions respectively; k_{rm}, k_{sm}, and k_{nh} are the decomposition rates of each fraction. The first derivative of Equation 1 was then used to estimate C_{rm}, k_{rm}, and k_{sm} via curve fitting of CO_2 flux data from each individual incubation chamber (n=5 per treatment) using the NLIN procedure (METHOD = MARQUART) of SAS version 9.3 (Eq. 2).

Eq. 2
$$\frac{dC}{dt} = C_{rm}k_{rm}e^{(-k_{rm}t)} + C_{sm}k_{sm}e^{(-k_{sm}t)} + C_{nh}k_{nh}e^{(-k_{nh}t)}$$

The methodology and modeling technique is consistent with Paul et al. (2001a) and Paul et al. (2006), although the nomenclature is slightly different. Here we replace the subscript *a* (active) with *rm*, *s* (slow) with *sm*, and *r* (resistant) with *nh* to emphasize the fact that these represent operationally defined pools and do not clearly correspond with any one functionally defined component of soil organic matter. The mean residence time (MRT) for each of the three pools is obtained via the inverse of the decomposition rate (1/k) scaled to field time with a Q_{10} of 2.89 (Eq. 3) based on the difference between laboratory temperature (labT = 22 °C) and mean annual temperature (MAT = 6.9 °C).

Eq 3. $$Q_{10} = \left(2^{\left(\frac{labT - MAT}{10} \right)} \right)$$

In Eq. 2, C_{nh} was estimated by 6N HCl acid hydrolysis and k_{nh} was set at 500 yr based on the ^{14}C age of SOC from 0-20 cm for a Plano silt loam at UW-ARL reported by Paul et al. (2001b). Paul et al. (2001b) report a MRT for C_{nh} of 2840 yr (0-20 cm), but use of this value did not improve our modeling effort so the more conservative estimate from SOC was used (data not shown). The slowly mineralized carbon pool (C_{sm}) was estimated by subtracting C_{rm} and C_{nh} from SOC.

2.3.5 Comparing non-linear regression models

Regression model differences were evaluated via F-tests on model reduction. The F-test used for non-linear model comparisons is outlined below in equation 4.

Eq. 4 $$F = \frac{\left(\frac{SSE_{(reduced)} - SSE_{(full)}}{df_{SSE(reduced)} - df_{SSE(full)}} \right)}{\left(\frac{SSE_{(full)}}{df_{SSE(full)}} \right)}$$

Where SSE = sums of squares for error and *df* = degrees of freedom. Numerator degrees of

freedom for the F-test were calculated as the difference between the full and reduced model error

degrees of freedom, and the denominator degrees of freedom for the F-test are taken from the

error degrees of freedom from the full model. A Bonferroni correction was applied to all p-

values to address the issue of multiple statistical comparisons. Asymptotic confidence limits

(95%) provided by the NLIN Procedure in SAS v9.3 were used to compare parameter estimates

for C_{rm}, MRT-C_{rm}, and MRT-C_{sm}.

2.3.6 Statistical analysis of biogeochemical data

The MIXED procedure in SAS version 9.3 was used to analyze soil physical and

chemical data. Each dependent variable was analyzed using a completely randomized design

model structure. The resulting mixed effect model was

Eq 5. $$y = \mu + R_i + T_j + \varepsilon_{ij}$$

Where μ = population mean, R = random effect of the ith replicate (n=3), T = fixed effect of the

j^{th} treatment (n=9), and ε = the error term associated with the interaction of the jth replicate and i^{th}

treatment. Orthogonal contrasts were used to further investigate questions specific to our initial

hypotheses.

2.4 Results

2.4.1 Land cover differences affect CO$_2$ flux dynamics in vitro

Statistical analysis of CO$_2$ flux data (non-linear model comparison) enabled us to condense the nine original agroecosystems into five significantly distinct (p<0.01) land-cover groups (LCGs) (Fig. 2.1a-f). The first LCG consisted solely of the remnant prairie agroecosystem (Rem-P). Rem-P had the highest initial rate of CO$_2$ evolution (16 µg C [g soil]$^{-1}$ day^{-1}) and longest interval (~100 dy) between the initiation of incubation and when the CO$_2$ flux began to stabilize at around 4 µg C (g soil)$^{-1}$ day^{-1}. The 35-year restoration at GP had the most distinct CO$_2$ flux profile and was placed in the second LCG (35y-P). Initial CO$_2$ flux rates were quite high in 35y-P (13 µg C [g soil]$^{-1}$ day^{-1}), followed by a rapid decrease temporarily stabilized at around 6 µg C (g soil)$^{-1}$ day^{-1} between days 50 and 150 and then began to drop once more to around 4 µg C (g soil)$^{-1}$ day^{-1} by day 230. The remaining seven agroecosystems fell into three LCGs. Regression lines for the 11 year prairie restoration at WICST and the NT maize system from WCRT were indistinguishable and were therefore combined into a third LCG representing conservation agricultural practices (CAP). In CAP, CO$_2$ flux rates ranged from 10 to 15 µg C (g soil)$^{-1}$ day^{-1} at day 0 and began to stabilize at around 4 µg C (g soil)$^{-1}$ day^{-1} by day 75. The non-linear model fit for the pasture agroecosystem at WICST, though visually similar, differed significantly (p < 0.01) from CAP and was therefore placed in a fourth LCG (PAST). For PAST, initial CO$_2$ flux rates were 2 to 4 µg C (g soil)$^{-1}$ day^{-1} lower than those in CAP, and stabilization at 4 µg C (g soil)$^{-1}$ day^{-1} occurred by approximately day 50, 25 days earlier than observed for CAP. The remaining four agroecosystems at WCRT (conv. m-m) and WICST (m-m, m-s, and m-a-A-A) were not statistically distinguishable from one another and were therefore placed together in a fifth and final LCG representing conventional agriculture (AG). This LCG had the

greatest total variability throughout the 230 day incubation as well as the lowest average initial

flux rate of 6 µg C (g soil)$^{-1}$ day^{-1} which stabilized by day 50 to 4 µg C (g soil)$^{-1}$ day^{-1} similar to

PAST. Non-linear regression models for the five distinct LCGs (Rem-P, 35y-P, CAP, PAST,

and AG) are presented in Fig 2.1. Although flux rates slowed 30 to 75% in the course of the 230

day incubation, it was unclear if CO_2 flux in all of the systems had reached a long term

equilibrium. This clearly was not the case for 35y-P in which CO_2 flux continued on a negative

trajectory (Fig. 2.1b).

2.4.2 Accessible carbon occupies a greater percentage of SOC in grasslands than conventional production agriculture

By combining SOC and NHC data with estimates obtained via the non-linear regression

modeling, we were able to estimate the size and MRTs of three operationally defined carbon

pools for each of the five LCGs outlined above (Eq. 1 and Table 2.2). Soil organic carbon was

highest in Rem-P and lowest in the AG as we would expect given the history of tillage at these

sites. The remaining three LCGs fell between these two extremes with SOC strongly correlated

with perennial grass cover (PAST > 35y-P > CAP). The size of the rapidly mineralized carbon

pool was small relative to SOC and quite variable, ranging from 0.6% SOC for Rem-P to 0.3%

SOC for 35y-P and PAST. The MRT of C_{rm} was also quite variable but followed the patterns in

the pool size of C_{rm} very closely. Turnover or C_{rm} was slowest in Rem-P and fastest in 35y-P

with the remaining three LCGs averaging 58 days (Table 2.2). In spite of the high parameter

variability associated with C_{rm}, the combined proportion of mineralized carbon (C_{rm} + C_{sm})

relative to SOC followed the trends that we initially hypothesized. The highest proportion of

mineralized carbon was associated with Rem-P at 67.6% SOC followed by 35y-P at 63.8% SOC and CAP at 52.1% SOC. Mineralized C accounted for roughly 50% of SOC in both the PAST and AG LCGs. Implicit in these results is that C_{nh} occupied a greater proportion of SOC in the agricultural systems (CAP, PAST, and AG) than it did in the native prairie systems (Rem-P and 35y-P). In spite of the fact that C_{nh} accounted for a relatively low proportion of SOC in 35y-P, the MRT's at this site for both C_{rm} and C_{sm} were much shorter than those reported from Rem-P and the agricultural systems (Table 2.2). These results confirm that CO_2 flux data observed from the 35y-P soils was not well explained by a three-pool model with first order kinetics, either because of historic management or unexplored edaphic conditions (Fig. 2.1).

2.4.3 Soil physical and chemical properties reflect historic land management

Although each of the five LCGs were from the same soil series, soil physical and chemical parameters differed slightly as a result of historic management and minor edaphic differences (Table 2.3). Soil pH ranged from a high of 6.1 in AG and PAST to a low of 5.5 in Rem-P, while organic matter mirrored the SOC trends and was highest in Rem-P and lowest in AG. Macronutrient levels were similar for the most part with the exception of P and K which were substantially lower in Rem-P than in 35y-P, CAP, PAST, and AG, all of which have had a history of production agriculture. The Rem-P and 35y-P LCGs had significantly lower clay contents than PAST and AG with the CAP LCG intermediate between these extremes. Rem-P is the most distinct texturally with significantly greater sand and significantly less silt than the other four sites. More detailed biogeochemical data for each of the five LCGs can be found in Table 2.3.

2.5 Discussion

2.5.1 Mineralizable SOC pools are greater in grassland than agricultural systems

The rapidly mineralized carbon pool (C_{rm}) is thought to consist of plant residues and non-occluded labile carbon from root and microbial biomass (Collins et al., 2000; Haile-Mariam et al., 2000; Paul et al., 2011). In the current study our estimates of C_{rm} were small relative to SOC (<1%). These estimates are similar to those found by Schwendenmann and Pendall (2008) (0.3 to 0.9 % SOC) in tropical Oxisols and of Haile-Mariam et al. (2000) in California Ultisols (0.7 to 0.9% SOC) but are lower than those reported by Collins et al. (2000) from agricultural Mollisols collected at UW-ARL (~2% SOC). The relatively large C_{rm} in Rem-P likely reflects the long history (> 1000 yrs) of significant C inputs from fine root biomass in this agroecosystem.

The remaining four LCGs (35y-P, CAP, PAST, & AG) had roughly equivalent C_{rm} concentrations although the percent of SOC occupied by C_{rm} ranged from 0.3% in 35y-P and PAST to 0.5% in CAP. The generally lower quantity of C_{rm} in these four LCGs, including the grass systems ranging in age from 11 to 35 yrs, suggests that C dynamics in these systems still largely reflect the agricultural history at these sites (as was evident in CO_2 flux curves). Not surprisingly, based on the non-linear modeling work, the size and MRT of C_{rm} in 35y-P did not fit well within the trends observed in the remaining four LCGs (Fig. 2.1b). If considered in the absence of 35y-P both the size and MRT of C_{rm} tend to increase from AG to Rem-P. The MRT for C_{rm} was longest in Rem-P (69 d) and shortest in 35y-P (37 d). The difference in these two agroecosystems is surprising given the similarities in plant community, soil physical and chemical properties, and management. Brye et al. (2002b), evaluated SOC at the 35y-P site and

compared it to SOC in an adjacent agricultural field. They report lower SOC values in 35y-P than in the adjacent cultivated systems. The atypical behavior of SOC in the 35y-P site is also noted by Kucharik et al. (2001) who state that the 35y-P site did little to sequester SOC between the time of its establishment in 1976 and their analysis (24 yrs later). Furthermore they show that 35y-P contained between 28% and 42% less SOC (surface 1 m) than an adjacent NT maize plot, and 40% to 47% less than it's simulated potential. Our findings combined with those of Brye et al. (2002b), Kucharik et al. (2001) and others suggest that there may be underlying edaphic conditions at the 35y-P site that have not been adequately accounted for by physical or chemical analysis of this soil. Furthermore it suggests that generalization about SOC dynamics within prairie ecosystems, even for systems that are geographically proximal and within the same soil series, may not hold.

The long MRTs associated with C_{rm} and C_{sm} in Rem-P may reflect the influence of fine root morphology on the stabilization of labile plant carbon. The large surface area associated with fine root biomass increases the interaction of both fine roots and root hairs with soil micro-pores and micro-aggregates. Penetration of root hairs into such micro sites, where anoxic conditions prevail and which are otherwise inaccessible to microbial decomposers, preferentially stabilizes these stocks of root derived C (Rasse et al., 2005). While these conditions exist in CAP and PAST they are much younger systems relative to Rem-P. For CAP, PAST, and AG the C_{rm} persisted for 58 days. The MRTs which we report for C_{rm} and C_{sm} are in some instances twice that reported by Collins et al. (2000) from soils collected at UW-ARL (0 to 20 cm).

Between 49 and 60 percent of SOC was present in the slowly mineralized carbon pool, with MRTs ranging from 44 yr in Rem-P to 21 yr in 35y-P (Table 2.2). The greatest pools of C_{sm} were found in the grassland agroecosystems and the lowest in the AG and CAP LCGs. These

results were largely influenced however by the size of the C_{nh} since C_{sm} is calculated as the difference of SOC and the sum of C_{rm} and C_{nh}. While the size and relative percent of C_{rm} was small, the size of C_{nh} was quite large and accounted for 32 to 50 percent of SOC, consistent with the findings of others (Paul et al., 2001b). As we initially hypothesized C_{nh} occupied a significantly greater percentage of SOC in the cultivated AG LCG and in those sites that have recently been converted to grassland or are intensively managed (CAP and PAST) compared to the older grassland systems (Rem-P & 35y-P). These differences may be due in part to the likelihood that labile root and root exudate derived carbon, which is easily hydrolysable, comprised a larger portion of SOC in the Rem-P and 35y-P than in the remaining three LCGs. It may also be the result of tillage induced oxidative loss of labile and easily accessible C at these sites. Jacobs et al. (2010) reported that C_{nh} occupied a greater proportion of SOC in a conventionally tilled agricultural system when compared to a minimally tilled systems, supporting this theory. While recent additions of plant lignin can escape hydrolysis, increasing the size of C_{nh} in agricultural systems (Kogel-knabner et al., 1994; Paul et al., 2006; Schwendenmann and Pendall, 2008), the lack of detectable differences between CAP, PAST, and AG indicate that this was not a major factor influencing the size of C_{nh} in this study.

2.5.2 Differences in belowground C allocation may be more important than specific agricultural management in driving SOC dynamics

Non-linear regression analysis of CO_2 flux data proved highly sensitive to differences in agroecosystems, with five distinct LCGs emerging from the analysis. These LCGs corresponded well with our initial hypothesis that CO_2 flux would be greatest among perennial grass systems

and lowest in the conventional agricultural systems as a result of labile carbon contributions from belowground biomass production and turnover. This hypothesis is supported by the finding of others that perennial grass systems typically foster greater SOC levels than conventional agricultural systems due to increased C inputs from fine-root biomass, root hairs, root exudates, the lack of removal of aboveground vegetation, and the absence of physical soil disturbance (DeLuca and Zabinski, 2011; Guzman and Al-Kaisi, 2010). For example, Jelinski et al. (2009) reported that the annual belowground net primary production of a remnant tallgrass prairie in southern Wisconsin averaged 5.7 Mg ha^{-1} yr^{-1}; more than twice that of an adjacent 11-yr-old prairie restoration (2.8 Mg ha^{-1} yr^{-1}) or a nearby soybean field (2.3 Mg ha^{-1} yr^{-1}). Tufekcioglu et al. (1998), working in central Iowa, found that live fine-root biomass in perennial cool season pastures exceeded 6 Mg ha^{-1}, while in both maize and soybean systems, fine root biomass was < 2.3 Mg ha^{-1} in the surface 35 cm. Cahill et al. (2009) evaluated root production in two 16-year-old grassland systems (one C3 and one C4 system) and an annual grain rotation in southern Wisconsin. They estimated total root biomass (0 to 50 cm) for the C4 and C3 grass systems at 6.6 and 4.8 Mg ha^{-1}, respectively, while total root biomass in the annual system was far lower at 0.7 Mg ha^{-1}. Finally, Kucharik et al. (2006) reported total root biomass (0 to 30 cm) numbers as high as 30.3 and 21.5 Mg ha^{-1} for a remnant and 65 year prairie restoration on poorly drained soils in southern Wisconsin.

In agreement with our study, Yoo et al. (2006) found that mean CO_2 mineralization rates were significantly greater from incubated prairie soil than from cultivated soil (72 vs. 47 µg CO_2 g^{-1} soil d^{-1}, respectively) as part of a 12-day incubation study using soils from adjacent cultivated and native prairie sites in central Illinois. They attributed this to greater C substrate content in the prairie system as was the case in our study. Interestingly, four of the five LCGs in our study

(Rem-P, 35y-P, CAP, and PAST) fell along a continuum of management intensity within perennial grass systems. This suggests that differences in the quantity and quality of below ground carbon inputs may influence SOC dynamics more than aboveground biomass production or agronomic soil disturbance.

We did not detect significant differences between the conventional agricultural systems in spite of their range in management practices (i.e. grain, forage, CT, NT). In a similar incubation study on Alfisols in southern Michigan, Paul et al. (1999) were unable to detect significant differences in carbon mineralization amongst the five agricultural systems they evaluated (ranging from CT maize-soybean to alfalfa). However, they did find that these systems differed from hybrid poplar and two successional plant communities (never tilled and historically tilled) in which cumulative CO_2 flux was much greater than it was in production agricultural systems. In the current study, the agroecosystems within the AG LCG differed substantially from the NT continuous maize, which displayed CO_2 flux dynamics similar to the 11 year prairie restoration, in that they were periodically tilled or returned less crop residue to the soil. These results highlight the sensitivity of this method and suggest that in some situations where independent decomposition models might be expected (as in AG), a single model of soil carbon dynamics may suffice. Alternately, as was the case here, grassland systems of differing age and management intensity may require more system specific consideration.

2.5.3 Methodological considerations

The estimate we report for C_{rm} as a proportion of SOC for the AG LCG is 81% smaller than that reported by Collins et al. (2000) for continuous maize (0.4% vs. 2.2%). We also report

a MRT for C_{rm} that is 30% shorter than that reported by Collins et al. (2000) for the same system (63 d vs. 90 d). These results are surprising given the fact that the soils evaluated in the two studies come from the same soil series on an adjacent site with similar cropping systems and cropping histories. The differences between parameter estimates may be due to a combination of methodological differences: slight difference in temperature (22.2 vs. 25 C), difference in chamber size (950 vs. 160 ml), incubation length (230 vs. 500+ d), and chamber venting. Elevated temperatures have been shown to increase SOC mineralization rates as well as affect its decomposition over time (Conant et al., 2008b), which could partially explain the greater C_{rm} and k_{rm} reported by Collins et al. (2000). A temperature difference of 2.8 °C is relatively small however, and decomposition rates (e.g. k_{rm}) in both studies were scaled based on a Q_{10} which accounts for the lab temperature relative to the field MAT. Differences in chamber size should also be accounted for in CO_2 flux calculations. In the current study, soil incubations lasted 230 days as opposed to 500+ days in Collins et al. (2000). Scharnagl et al. (2010) reported that 900 days of incubation were required to satisfactorily constrain all five carbon pools in the ROTHC model. Others however have reported satisfactory results from incubations ranging in length from 200 to 800 days (Paul et al., 2001a).

In addition to a shorter incubation period, we utilized continuously vented incubation chambers to prevent CO_2 levels within each chamber from deviating significantly from ambient ($\sim$390 μl L^{-1} CO_2) throughout the study. Collins et al. (2000) indicated that incubation chambers were returned to ambient CO_2 levels following headspace measurements that occurred at 10 or 21-day intervals by degassing with compressed air. They do not, however, indicate the CO_2 concentrations reached within the chambers prior to each measurement. While CO_2 concentrations up to 5% or 6% (50,000 to 60,000 mg kg^{-1}) within an incubation chamber are

often deemed acceptable (Paul et al., 2001a; Steinweg et al., 2008), such high levels of CO_2 change the diffusivity of the soil system and can retard CO_2 flux as has been discussed extensively in studies of field CO_2 measurements (Kutzbach et al., 2007; Livingston and Hutchinson, 1995; Livingston et al., 2006). In a controlled laboratory incubation study Bekku et al. (1997) found that it was necessary to maintain the CO_2 concentration within an incubation chamber at that of the ambient air to determine accurate soil flux rates. It is possible that in the current study, continuous chamber venting maintained a high diffusivity gradient between the soil pore space and chamber headspace, permitting respired carbon to exit the soil at a rate that is quicker than would be observed in a parallel experiment that had sealed chambers for extended periods of time. While discrepancies in soil moisture may have also played a role in these differences, we were able to maintain the incubating soils within 1% of their initial levels (60% WFPS), which is well within the 5% error commonly cited in the literature (Conant et al., 2008a; Conant et al., 2008b; Haddix et al., 2011). Based on our finding we suggest further work be directed toward evaluating chamber construction and incubation methods and their effects on parameter estimates and their subsequent modeling utility.

2.5.4 Implications for SOC modeling

The AHI method has been used to compare the relative distribution and kinetics of SOM in agricultural, forest and grassland systems (Collins et al., 2000; Fortuna et al., 2003; Haile-Mariam et al., 2000; Paul et al., 2001a; Paul et al., 2001b). The work of Paul et al. (1999) and Paul et al. (2006) demonstrated the value and applicability of using parameter estimates obtained via the AHI method to improve biogeochemical model output. Others have also demonstrated the

efficacy of utilizing soil mineralization rates and chemical isolates of stabilized carbon to improve biogeochemical modeling. Scharnagl et al. (2010) found that C mineralization rates obtained during soil incubations provided sufficient information to reliably estimate all carbon pools in the ROTHC model. Furthermore, Juston et al. (2010) demonstrated that even rough estimates of an "inert" SOM pool, like those obtained via chemical isolates (e.g. acid hydrolysis), were quite valuable at reducing uncertainties in the Introductory Carbon Balance Model (ICBM). Collins et al. (2000) concluded that sufficient interactive effects with climate, parent material, and soil depth were found that predictive biogeochemical models used for decision making cannot rely on the generalizations about SOM dynamics that are present in most extant models. Rather, they suggested that such models require analytically determined factors, such as those defined by the AHI method, for at least major subdivisions of the soils being studied. Our findings support these conclusions and indicate that even when similar agroecosystems share the commonalities of climate and parent material, site specific model parameters may be required. Additional work to evaluate similar agroecosystems that are geographically proximal and share the same soil characteristics will help to elucidate the degree to which model parameters require finer resolution than that of major soil subdivisions. Further modeling work is also required to evaluate the utility of such site specific parameter estimates.

2.6 Conclusions

In the present study, five LCGs were identified. These LCGs were significantly different with regards to their SOC dynamics, as defined by the size and MRT of three operationally defined SOC pools. Surprisingly, varied production agricultural systems were indistinguishable

from one another while grasslands systems with subtly different land cover and management histories were. The estimates obtained from the constrained three pool model fit to our CO_2 flux data strongly supported our initial hypotheses that tillage would favor an increased proportion of SOC in more stable and presumable older carbon pools (C_{nh}) while perennial grass systems and NT agriculture would increase the proportion of SOC stored in rapidly mineralizable forms. While NT continuous maize demonstrated SOC dynamics similar to an 11 year prairie restoration and other perennial grass systems, it was the remnant prairie system (Rem-P) that supported substantial C allocation to both the rapidly mineralized and slowly mineralized C pool. These results combined with the long MRTs associated with the NG system highlight both the potential sensitivity of this and other grassland systems to future disturbance (management or climate) and the importance of perennial grassland systems in stabilizing below ground additions of labile SOC. Regionally specific estimates of carbon pools and their kinetics from diverse agroecosystems have been shown to improve biogeochemical modeling efforts. Therefore, the AHI method should prove valuable in efforts to better understand how changes in climate and land management (e.g. towards perennial biomass production) will affect current and future soil C stocks across diverse agricultural landscapes.

2.7 Acknowledgements

Madison), Mark Martin (WI-DNR), and Mike and Sue Zauner (Waunakee, WI) who provided

access to key locations for this study.

2.8 References

Bekku Y., Koizumi H., Oikawa T., and H. Iwaki. 1997. Examination of four methods for measuring soil respiration. Applied Soil Ecology 5: 247-254.

Bellamy P.H., Loveland P.J., Bradley R.I., Lark R.M., and G.J.D. Kirk. 2005. Carbon losses from all soils across England and Wales 1978-2003. Nature 437: 245-248.

Brady N.C., and R.R. Weil. 2008. The nature and properties of soils. 14th edition ed. Prentice Hall, Upper Saddle River, NJ.

Bruun S., and J. Luxhoi. 2006. Letter to the editor on "Can incubations be used to measure meaningful pools of soil organic matter?". Soil Science Society of America Journal 70: 2164-2164.

Brye K.R., Norman J.M., and S.T. Gower. 2002a. Assessing the Progress of a Tallgrass Prairie Restoration in Southern Wisconsin. American Midland Naturalist 148: 218-235.

Brye K.R., Gower S.T., Norman J.M., and L.G. Bundy. 2002b. Carbon budgets for a prairie and agroecosystems: Effects of land use and interannual variability. Ecological Applications 12: 962-979.

Cahill K.N., Kucharik C.J., and J.A. Foley. 2009. Prairie restoration and carbon sequestration: difficulties quantifying C sources and sinks using a biometric approach. Ecological Applications 19: 2185-2201.

Campbell G.S., and J.M. Norman. 1998. Heat Flow in the Soil, An Introduction to Environmental Biophysics, Springer, New York. pp. 113-128.

Coleman K., and D.S. Jenkinson. 1996. RothC-26.3 - A model for the turnover of carbon in soil., in: D. S. Powlson, et al. (Eds.), Evaluation of soil organic matter models, Springer-Verlag, Berlin. pp. 237-246.

Collins H.P., Elliott E.T., Paustian K., Bundy L.C., Dick W.A., Huggins D.R., Smucker A.J.M., and E.A. Paul. 2000. Soil carbon pools and fluxes in long-term corn belt agroecosystems. Soil Biology & Biochemistry 32: 157-168.

Conant R.T., Easter M., Paustian K., Swan A., and S. Williams. 2007. Impacts of periodic tillage on soil C stocks: A synthesis. Soil and Tillage Research 95: 1-10.

Conant R.T., Steinweg J.M., Haddix M.L., Paul E.A., Plante A.F., and J. Six. 2008a. experimental warming shows that decomposition temperature sensitivity increases with soil organic matter recalcitrance. Ecology 89: 2384-2391.

Conant R.T., Drijber R.A., Haddix M.L., Parton W.J., Paul E.A., Plante A.F., Six J., and J.M. Steinweg. 2008b. Sensitivity of organic matter decomposition to warming varies with its quality. Global Change Biology 14: 868-877.

Conant R.T., Ryan M.G., Ågren G.I., Birge H.E., Davidson E.A., Eliasson P.E., Evans S.E., Frey S.D., Giardina C.P., Hopkins F.M., Hyvönen R., Kirschbaum M.U.F., Lavallee J.M., Leifeld J., Parton W.J., Megan Steinweg J., Wallenstein M.D., Martin Wetterstedt J.Å., and M.A. Bradford. 2011. Temperature and soil organic matter decomposition rates – synthesis of current knowledge and a way forward. Global Change Biology 17: 3392-3404.

DeLuca T.H., and C.A. Zabinski. 2011. Prairie ecosystems and the carbon problem. Frontiers in Ecology and the Environment 9: 407-413.

Dungait J.A.J., Hopkins D.W., Gregory A.S., and A.P. Whitmore. 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. Global Change Biology 18: 1781-1796.

Follett R.F., Paul E.A., and E.G. Pruessner. 2007. Soil carbon dynamics during a long-term incubation study involving C-13 and C-14 measurements. Soil Science 172: 189-208.

Fortuna A., Harwood R.R., and E.A. Paul. 2003. The effects of compost and crop rotations on carbon turnover and the particulate organic matter fraction. Soil Science 168: 434-444.

Gulde S., Chung H., Amelung W., Chang C., and J. Six. 2008. Soil carbon saturation controls labile and stable carbon pool dynamics. Soil Science Society of America Journal 72: 605-612.

Guzman J.G., and M.M. Al-Kaisi. 2010. Soil Carbon Dynamics and Carbon Budget of Newly Reconstructed Tall-grass Prairies in South Central Iowa. Journal of Environmental Quality 39: 136-146.

Haddix M.L., Plante A.F., Conant R.T., Six J., Steinweg J.M., Magrini-Bair K., Drijber R.A., Morris S.J., and E.A. Paul. 2011. The role of soil characteristics on temperature sensitivity of soil organic matter. Soil Science Society of America Journal 75: 56-68.

Haile-Mariam S., Cheng W., Johnson D.W., Ball J.T., and E.A. Paul. 2000. Use of carbon-13 and carbon-14 to measure the effects of carbon dioxide and nitrogen fertilization on

carbon dynamics in ponderosa pine. Soil Science Society of America Journal 64: 1984-1993.

Houghton R.A. 2007. Balancing the global carbon budget, Annual review of earth and planetary sciences, Annual Reviews, Palo Alto, CA.

Jacobs A., Helfrich M., Hanisch S., Quendt U., Rauber R., and B. Ludwig. 2010. Effect of conventional and minimum tillage on physical and biochemical stabilization of soil organic matter. Biology and Fertility of Soils 46: 671-680.

Jagadamma S., and R. Lal. 2010. Integrating physical and chemical methods for isolating stable soil organic carbon. Geoderma 158: 322-330.

Jelinski N.A., Kucharik C.J., and J.B. Zedler. 2009. A Test of Diversity-Productivity Models in Natural, Degraded, and Restored Wet Prairies. Restoration Ecology 19: 186-193.

Juston J., Andren O., Katterer T., and P.E. Jansson. 2010. Uncertainty analyses for calibrating a soil carbon balance model to agricultural field trial data in Sweden and Kenya. Ecological Modelling 221: 1880-1888.

Kogel-knabner I., Deleeuw J.W., Tegelaar E.W., Hatcher P.G., and H. Kerp. 1994. A lignin-like polymer in the cuticle of spruce needles - implications for the humification of spruce litter. Organic Geochemistry 21: 1219-1228.

Kucharik C.J., Fayram N.J., and K.N. Cahill. 2006. A paired study of prairie carbon stocks, fluxes, and phenology: comparing the world's oldest prairie restoration with an adjacent remnant. Global Change Biology 12: 122-139.

Kucharik C.J., Brye K.R., Norman J.M., Foley J.A., Gower S.T., and L.G. Bundy. 2001. Measurements and modeling of carbon and nitrogen cycling in agroecosystems of southern Wisconsin: Potential for SOC sequestration during the next 50 years. Ecosystems 4: 237-258.

Kutzbach L., Schneider J., Sachs T., Giebels M., Nykanen H., Shurpali N.J., Martikainen P.J., Alm J., and M. Wilmking. 2007. CO2 flux determination by closed-chamber methods can be seriously biased by inappropriate application of linear regression. Biogeosciences 4: 1005-1025.

Lal R. (2008. Carbon Sequestration. Philosophical Transactions of the Royal Society 363: 815-830.

Linn D.M., and J.W. Doran. 1984. Effect of Water-Filled Pore-Space on Carbon-Dioxide and Nitrous-Oxide Production in Tilled and Nontilled Soils. Soil Science Society of America Journal 48: 1267-1272.

Livingston G.P., and G.L. Hutchinson. 1995. Enclosure-based measurement of trace gas exchange: applications and sources of error, in: P. A. Matson and R. C. Harris (Eds.), Biogenic Trace Gases: Measureing Emissions from Soil and Water, Blackwell Sceintific Publications, Oxford. pp. 14-51.

Livingston G.P., Hutchinson G.L., and K. Spartalian. 2006. Trace gas emission in chambers: A non-steady-state diffusion model. Soil Science Society of America Journal 70: 1459-1469.

McLauchlan K.K., and S.E. Hobbie. 2004. Comparison of labile soil organic matter fractionation techniques. Soil Science Society of America Journal 68: 1616-1625.

Olk D.C., and E.G. Gregorich. 2006. Overview of the symposium proceedings, "Meaningful pools in determining soil carbon and nitrogen dynamics". Soil Science Society of America Journal 70: 967-974.

Parton W.J., Schimel D.S., Cole C.V., and D.S. Ojima. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Science Society of America Journal 51: 1173-1179.

Paul E.A., Morris S.J., and S. Bohm. 2001a. The determination of soil C pool sizes and turnover rates: biophysical fractionation and tracers, in: R. Lal, et al. (Eds.), Assessment methods for soil carbon, Lewis Publishers, Boca Raton, FL. pp. 193-206.

Paul E.A., Collins H.P., and S.W. Leavitt. 2001b. Dynamics of resistant soil carbon of midwestern agricultural soils measured by naturally occurring C-14 abundance. Geoderma 104: 239-256.

Paul E.A., Morris S.J., Conant R.T., and A.F. Plante. 2006. Does the acid hydrolysis-incubation method measure meaningful soil organic carbon pools? Soil Science Society of America Journal 70: 1023-1035.

Paul E.A., Follett R.F., Haddix M., and E. Pruessner. 2011. Soil N Dynamics Related to Soil C and Microbial Changes During Long-Term Incubation. Soil Science 176: 527-536.

Paul E.A., Harris D., Collins H.P., Schulthess U., and G.P. Robertson. 1999. Evolution of CO2 and soil carbon dynamics in biologically managed, row-crop agroecosystems. Applied Soil Ecology 11: 53-65.

Pedersen P., and J.G. Lauer. 2002. Influence of rotation sequence on the optimum corn and soybean plant population. Agronomy Journal 94: 968-974.

Poirier N., Sohi S.P., Gaunt J.L., Mahieu N., Randall E.W., Powlson D.S., and R.P. Evershed. 2005. The chemical composition of measurable soil organic matter pools. Organic Geochemistry 36: 1174-1189.

Posner J.L., Baldock J.O., and J.L. Hedtcke. 2008. Organic and conventional production systems in the Wisconsin Integrated Cropping Systems Trials: I. Productivity 1990-2002. Agronomy Journal 100: 253-260.

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

Scharnagl B., Vrugt J.A., Vereecken H., and M. Herbst. 2010. Information content of incubation experiments for inverse estimation of pools in the Rothamsted carbon model: a Bayesian perspective. Biogeosciences 7: 763-776.

Schmidt M.W., Torn M.S., Abiven S., Dittmar T., Guggenberger G., Janssens I.A., Kleber M., Kogel-Knabner I., Lehmann J., Manning D.A.C., Nannipieri P., Rasse D.P., Weiner S., and S. Trumbore. 2011. Persistence of soil organic matter as an ecosystem property. Nature 478: 41-56.

Schwendenmann L., and E. Pendall. 2008. Response of soil organic matter dynamics to conversion from tropical forest to grassland as determined by long-term incubation. Biology and Fertility of Soils 44: 1053-1062.

Silveira M.L., Comerford N.B., Reddy K.R., Cooper W.T., and H. El-Rifai. 2008. Characterization of soil organic carbon pools by acid hydrolysis. Geoderma 144: 405-414.

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

Sollins P., Glassman C., Paul E.A., Swanston C., Lajtha K., Heil J.W., and E.T. Elliott. 1999. Soil Carbon and Nitrogen: Pools and Fraction, in: G. P. Robertson, et al. (Eds.), Standar Soil Methods for Long-Term Ecological Research, Oxford University Press, New York. pp. 89-105.

SPAL. 2004. University of Wisconsin, Soil and Plant Analysis Lab. Particle Size Analysis Hydrometer Method. http://uwlab.soils.wisc.edu/files/procedures/particle_size.pdf (accessed 9 August, 2012).

Steinweg J.M., Plante A.F., Conant R.T., Paul E.A., and D.L. Tanaka. 2008. Patterns of substrate utilization during long-term incubations at different temperatures. Soil Biology & Biochemistry 40: 2722-2728.

Tufekcioglu A., Raich J.W., Isenhart T.M., and R.C. Schultz. 1998. Fine root dynamics, coarse root biomass, root distribution, and soil respiration in a multispecies riparian buffer in Central Iowa, USA. Agroforestry Systems 44: 163-174.

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

Wagai R., Brye K.R., Gower S.T., Norman J.M., and L.G. Bundy. 1998. Land use and environmental factors influencing soil surface CO2 flux and microbial biomass in natural and managed ecosystems in southern Wisconsin. Soil Biology and Biochemistry 30: 1501-1509.

Yoo G., Spomier L.A., and M.M. Wander. 2006. Regulation of carbon mineralization rates by soil structure and water in an agricultural field and a prairie-like soil. Geoderma 135: 16-25.

Table 2.1. Site characteristics and agroecosystem descriptions

Site	Initiated	Agroecosystem[1]	Management	Landscape Position	Slope	Soil Sampling			Primary Tillage Equipment	N Inputs		
						n	Depth cm)	Protocol		1st Yr. Avail. kg ha^{-1})	Timing	Source
WCRT	1983	m-m	conventional	back slope	2-6%	72	0-15	4 field reps, 18 cores per. plot, homogenized	chisel plow	196	annual	F
	1983	m-m	no-till	back slope	2-6%	72	0-15	4 field reps, 18 cores per. plot, homogenized	no-till	196	annual	F
WICST	1990	m-m	conventional	level	0-2%	54	0-15	3 field reps, 18 cores per. plot, homogenized	chisel plow	142	annual	F
	1990	m-s	no-till	level	0-2%	54	0-15	maize phase only: 3 field reps, 18 cores per. plot, homogenized	no-till	136	maize phase	L, F
	1990	m-a-A-A	conventional	level	0-2%	54	0-15	maize phase only: 3 field reps, 18 cores per. plot, homogenized	chisel plow	240	pre and post maize	L, F, M
	1990	Pasture	rotational grazing	level	0-2%	54	0-15	3 field reps, 18 cores per. plot, homogenized	n/a	52	throughout season	M
	1990	11yr. Prairie restoration	Periodic Burns	level	0-2%	54	0-15	3 field reps, 18 cores per. plot, homogenized	n/a	n/a	n/a	n/a
GP	1976	35yr. Prairie restoration	Periodic Burns	back slope	2-6%	48	0-15	12 cores from 4 random areas, homogenized	n/a	n/a	n/a	n/a
WW	n/a	Remnant prairie	Historic Grazing, and Annual Burning	toe slope	2-6%	48	0-15	12 cores from 4 random areas, homogenized	n/a	n/a	n/a	n/a

[1] abbreviations: a – first year alfalfa, A – established alfalfa hay, m – maize, s - soybean

Table 2.2. Parameter estimates for total carbon, carbon pools, and mean residence times of five distinct land-cover groupings (LCGs). Different letters within a given parameter indicate significant at α=0.05.

| | | | | C_{rm}[1] | | | | C_{sm} | | | | C_{nh} | | |
| | | | SOC[2] | Pool | | Field MRT | | Pool[3] | | Field MRT | | Pool[4] | | Field MRT[5] |
LCG	Description	n	g kg^{-1}	g kg^{-1}	% SOC	day	95% CL	g kg^{-1}	% SOC	yr	95% CL	g kg^{-1}	% SOC	yr
Rem-P	Remnant prairie	3	34.2^a	0.21^a	0.6^a	69^a	58-87	22.9^a	67.0^a	44^a	40-49	11.1^b	32.4^b	500
35y-P	35 year prairie restoration	3	26.7^{bc}	0.07^b	0.3^c	37^b	28-55	17.0^b	63.5^a	21^d	20-22	9.7^b	36.2^b	500
CAP	Conservation Ag.[6]	6	24.4^{cd}	0.11^b	0.5^b	58^{ab}	45-80	12.6^{cd}	51.6^b	26^c	24-29	11.7^b	47.9^a	500
PAST	Pasture	3	29.4^b	0.09^b	0.3^c	52^{ab}	40-73	14.5^{bc}	49.4^b	32^b	29-35	14.8^a	50.3^a	500
AG	Conventional Ag.[7]	11	22.8^d	0.08^b	0.4^c	63^{ab}	20-83	11.3^d	49.6^b	31^b	29-33	11.4^b	50.0^a	500

[1] C_{rm} = rapidly mineralized carbon, C_{sm} = slowly mineralized carbon, and C_{nh} = non-hydrolyzable carbon.

[2] Different letters within a given parameter indicate significance at α=0.05.

[3] C_{sm} pool size = SOC-C_{rm}-C_{nh}

[4] Cnh pool estimated via 6N HCl hydrolysis

[5] MRT was set conservatively at 500 yr based on ^{14}C dates for SOC reported in Paul *et al.* 2001a).

[6] CAP: no-till m-m WCRT) and 11yr. Prairie restoration WICST).

[7] AG: conv. m-m WCRT), and conv. m-m, no-till m-s, and m-a-A-A WICST)

Table 2.3. Soil physical and chemical parameters for the five distinct land-cover groupings LCGs)

LCG	Description	n	pH	OM	sand	silt	clay	texture	P	K	Ca	Mg	CEC[1]
				--------------- g kg^{-1}) ---------------					------------ mg kg^{-1}) ------------				cmol kg^{-1})
Rem-P	Remnant prairie	3	5.5	44.0	233.3	580.0	186.7	silt loam	7.7	90.3	1659.0	405.0	11.3
35y-P	35 year prairie restoration	3	5.8	39.7	106.7	706.7	190.0	silt loam	42.3	100.3	1680.0	364.0	10.0
CAP	Conservation Ag.[2]	6	5.8	37.0	61.7	713.3	225.0	silt loam	33.3	139.5	1478.3	411.3	9.8
PAST	Pasture	3	6.1	42.0	70.0	723.3	210.0	silt loam	45.7	120.7	1544.7	492.0	10.7
AG	Conventional Ag.[3]	12	6.1	35.5	75.0	704.2	220.8	silt loam	45.8	121.2	1557.7	453.5	11.1
	Tukey's HSD α=0.05)		*1.2*	*5.2*	*38.9*	*50.1*	*23.9*	*--*	*35.8*	*40.9*	*481.4*	*146.7*	*3.0*

[1]CEC: estimated cation exchange capacity
[2]CAP: no-till m-m WCRT) and 11yr. Prairie restoration WICST).
[3]AG: conv. m-m WCRT), and conv. m-m, no-till m-s, and m-a-A-A WICST)

Figure 2.1. Respiration data and non-linear regression models for the five significantly distinct land-cover groupings (LCGs). a) Rem-P, b) 35y-P, c) CAP, d) PAST, e) AG, and f) the five LCG models displayed together.
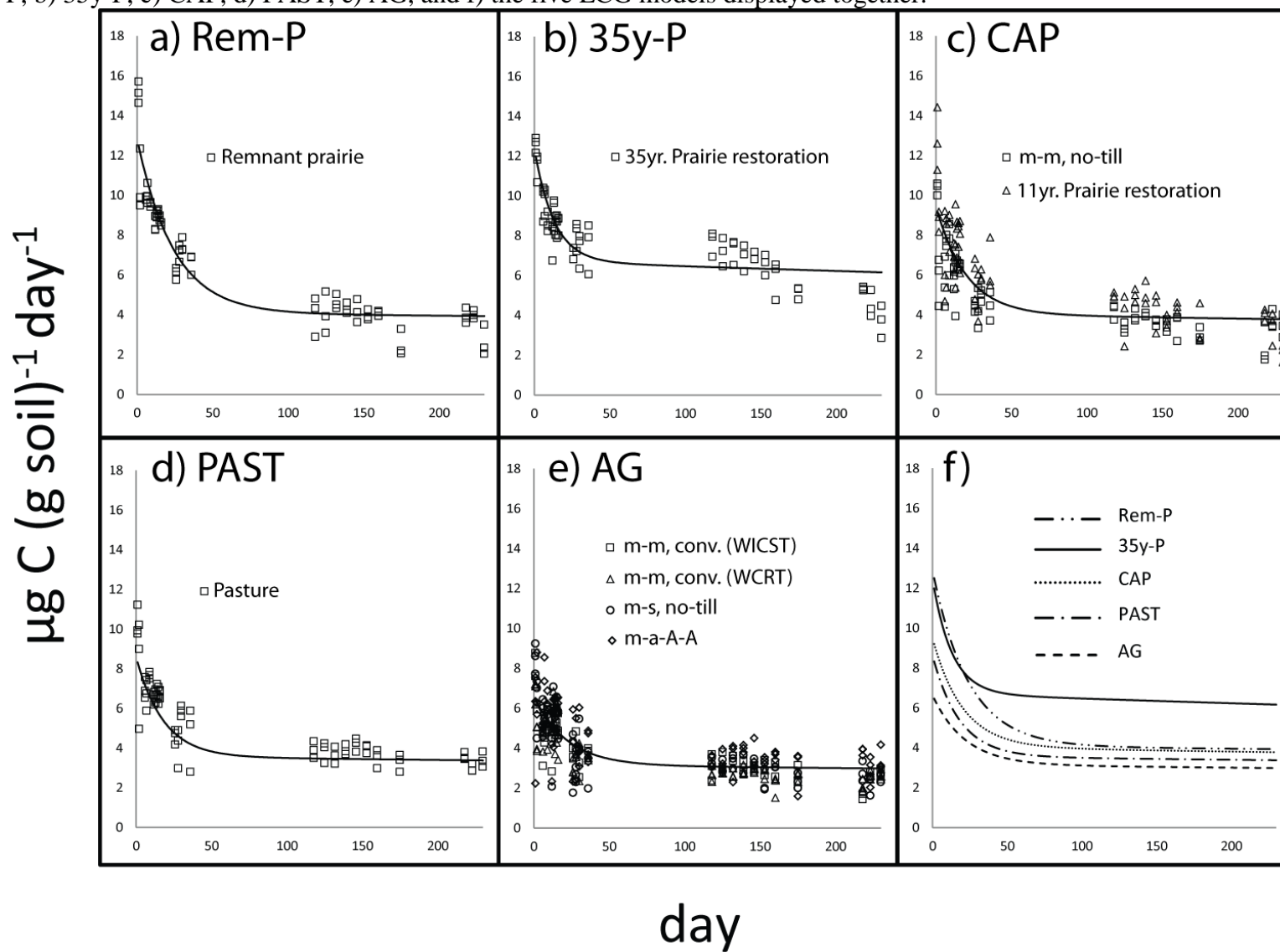
CHAPTER 3

EFFECT OF METHODOLOGICAL CONSIDERATIONS ON SOIL CARBON PARAMETER

ESTIMATES OBTAINED VIA THE ACID HYDROLYSIS-INCUBATION METHOD

3. EFFECT OF METHODOLOGICAL CONSIDERATIONS ON SOIL CARBON PARAMETER ESTIMATES OBTAINED VIA THE ACID HYDROLYSIS-INCUBATION METHOD

3.1 Abstract

Many techniques such as the acid hydrolysis – incubation (AHI) method have been developed with the aim of elucidating and understanding the inherent complexity of soil organic carbon (SOC). While the utility of the AHI method has been demonstrated repeatedly there is no standardized protocol developed for conducting the long-term incubation component of the method. In the current study we evaluated the effects of chamber venting, mechanical headspace mixing, and soil processing on the size and mean residence time of operationally defined pools of SOC obtained via the AHI method. Continuous chamber venting resulted in an estimate of the readily mineralized carbon pool that was 2.3 times larger and turned over 2.9 times slower than the same pool estimated using periodically vented chambers. These differences were primarily attributed to the suppression of CO_2 flux in periodically vented chambers as a result of high internal CO_2 concentrations, and a concomitantly reduced diffusivity gradient. Prior to venting the periodically-vented chambers CO_2 flux rates averaged 2.3 µg C (g soil)$^{-1}$ d^{-1}, while CO_2 flux rates following venting averaged 222.6 µg C (g soil)$^{-1}$ d^{-1}. We did not detect internal stratification of CO_2 suggesting that mechanical headspace mixing is unnecessary in incubation chambers ranging from 1 to 2 liters. Finally, there was no statistical difference between SOC parameter estimates obtained using intact soil cores and sieved soil. Carbon dioxide flux rates from the intact cores were quite variable relative to sieved cores and likely compromised the quality of the parameter estimates obtained from these soils. A standardized protocol is called for that isolates SOC fractions that are; i) repeatable with low errors; 2) amenable to soils with

varying textures and mineralogical characteristics; 3) have a high signal to noise ratio; and 4) are useful in hypothesis testing, while simultaneously seeking to minimize laboratory artifacts.

3.2 Introduction

A wide array of techniques have been employed to fractionate soil organic matter (SOM) into meaningful pools, often with the aim of elucidating ecosystem function and improving biogeochemical modeling efforts (McLauchlan and Hobbie, 2004; Paul et al., 2006; Poirier et al., 2005; von Lutzow et al., 2007). The acid hydrolysis-incubation (AHI) method is one such technique that combines chemical (acid hydrolysis) and biological (long term incubation) fractionation to estimate the size and turnover of three operationally defined SOM pools. In this method, CO_2 respiration rates obtained during soil incubations are used to define the decomposition rates of readily and slowly mineralized carbon (k_{rm} and k_{sm}, respectively) as well as the size of the readily mineralized carbon pool (C_{rm}). Acid hydrolysis is used to define a chemically recalcitrant pool (C_{nh}); the decomposition rate of which (K_{nh}) is usually obtained via ^{14}C measurements. The AHI method has demonstrated its utility in reproducibly measuring meaningful pools of SOM (Collins et al., 2000; Fortuna et al., 2003; Haile-Mariam et al., 2000; Paul et al., 2006). Although the method has been extensively reported upon, it is not without potential drawbacks and interpretational problems (Bruun and Luxhoi, 2006; Paul et al., 2006). Furthermore, while the issues associated with the use of acid hydrolysis have been discussed at length (Collins et al., 2000; Kogel-knabner et al., 1994; Schwendenmann and Pendall, 2008), and a rather consistent protocol is in place (Paul et al., 2001b; Sollins et al., 1999), there are inconsistencies in the literature about the methods used to implement long term in-vitro soil incubations.

Long term soil incubations are typically conducted in the absence of light to discourage the growth of autotrophic organisms, and at a soil moisture that is optimized for microbial growth and respiration (i.e. 60% water filled pore space [WFPS] is common) (Linn and Doran, 1984). The quantity of soil used for incubation studies varies widely from as little as 2.5 g (Risk et al., 2008) to as much as 200 g (Stewart et al., 2009), with 80 to 150 g most commonly reported (Conant et al., 2008a; Plante et al., 2009; Robertson et al., 1999). The size of the incubation chamber varies correspondingly with the quantity of soil used. Discrepancies in soil quantity and chamber size are largely accounted for in calculations of CO_2 efflux per unit mass of soil that are normalized for headspace volume. As such, these considerations may not be of major concern when comparing data between different in-vitro incubation studies. The effects of internal chamber conditions (e.g. CO_2 concentration, distribution) and soil processing (e.g. drying, sieving) however are not well standardized in the incubation literature and may have a marked effect on the outcomes of such studies.

Soil CO_2 flux is strongly governed by the diffusivity gradient generated as a result of concentration differences between the atmosphere and the soil pore space (Healy et al., 1996; Livingston and Hutchinson, 1995). Consequently, if the diffusivity gradient in vitro is drastically different than that encountered in situ, it would be unreasonable to expect realistic soil respiration rates from laboratory incubations. The suppressive effect of non-vented static chambers in field based CO_2 flux measurements has been well documented (Conen and Smith, 2000; Davidson et al., 2002; Kutzbach et al., 2007; Pumpanen et al., 2004). In the field, the reduction in CO_2 flux occurs almost instantaneously and can be attributed in large part to a distortion of the vertical and radial soil gas concentration gradient (Healy et al., 1996). In a controlled laboratory incubation study, Bekku et al. (1997) found that it was necessary to

maintain the CO_2 concentration within an incubation chamber at that of the ambient air to determine accurate soil flux rates. In spite of these concerns, headspace CO_2 concentrations between 50,000 to 60,000 mg kg^{-1} (5% and 6%) are often cited as allowable limits (Conant et al., 2008b; Paul et al., 2001b; Steinweg et al., 2008). These limits are usually accepted to maintain internal humidity and limit soil moisture loss. While such concentrations have been reported within the soil pore space (Glinski and Stepniewski, 1985; Maier et al., 2010), they are not inconsequential, with concentrations in the range of 2.5 to 5% CO_2 documented as inhibitory to further CO_2 production (Santruckova and Simek, 1997).

Proposed solutions to the problems that can arise from an ever decreasing diffusivity gradient, namely inhibition of gaseous flux, have been addressed in the field-based literature by decreasing chamber deployment time (Davidson et al., 2002), using non-linear modeling techniques to account for the non-linear gas flux (Healy et al., 1996; Livingston and Hutchinson, 1995), or including some sort of headspace mixing mechanism such as fans (Christiansen et al., 2011). The benefit of rigorous headspace mixing as occurs with chamber fans however is of questionable benefit (Hutchinson and Livingston, 2002; Pumpanen et al., 2004). Chamber venting has also been discussed at length, but its primary utility has been to equalize pressure between the inside and outside of the chamber and not as a means of maintaining a realistic diffusivity gradient (Livingston and Hutchinson, 1995). While the issues associated with headspace CO_2 accumulation and mixing have been addressed at length as they pertain to in situ gaseous flux measurements, they have not to our knowledge been adequately explored within the in vitro incubation literature. Furthermore, solutions such as decreased chamber deployment time are not practical during long term soil incubations where chambers are necessary to maintain optimal soil moisture levels.

In addition to considerations of chamber headspace concentrations and homogeneity, soil processing can influence the results obtained with the AHI method. Collins et al. (2000) compared the effects of using moist sieved soils versus re-wetted dry sieved soils on the estimation of C_{rm}, k_{rm}, and k_{sm} in corn based agroecosystems across the North Central U.S.A. They report that while estimates of C_{rm} and k_{sm} were not significantly different between soil processing techniques, the MRT of C_{rm} was 2 to 5 times longer for the moist sieved soils. This suggested that the rewetting of previously dried soil may lead to rapid loss of SOC from mineralization of microbial residues, potentially biasing estimates of SOC pool dynamics. Soil structure is also important to consider given the significant role that physical protection is thought to play in SOC stabilization (Dungait et al., 2012; Schmidt et al., 2011; Six et al., 2002). Soils are typically sieved to < 2 mm or < 4 mm and then thoroughly homogenized prior to incubation (Collins et al., 2000; Paul et al., 1999). Although infrequently reported, intact soils have also been used for in vitro incubation studies. Plante et al. (2009), in a short duration incubation study (15 d), evaluated CO_2 flux from intact soils and compared it to soils that were either sieved to < 2 mm or crushed to < 75 µm. They report that the three physical disruption treatments differed significantly, with sieved and crushed soils respiring 190% more CO_2 on average than the intact core at 25 ℃. They also reported an interactive effect with temperature, showing an apparent decrease in the effect of soil disruption as incubation temperature climbed from 15 to 35 ℃.

The greatest potential utility of the AHI method lies in the provision of SOC parameter estimates that can be used as inputs for models such as CENTURY and ROTHC. The work of Paul et al. (1999) and Paul et al. (2006) demonstrated the value and applicability of using such parameters to improve biogeochemical model output. Others have also demonstrated the efficacy

of utilizing soil mineralization rates and chemical isolates of stabilized carbon (e.g. C_{nh}) to improve biogeochemical modeling (Juston et al., 2010; Scharnagl et al., 2010). The potential benefits of this method may be compromised, however, by known issues with chamber based CO_2 flux measurements and lack of consistency in the application of long-term incubations. Progress toward understanding the impact of such variables, particularly as they pertain to parameter estimation in the AHI method, will aid in the development of protocols that minimize laboratory artifacts and ultimately improve the utility of such techniques for biogeochemical simulation models.

We evaluated the effects of chamber venting and mixing as well as soil processing on the parameter estimates obtained via the AHI method in three independent laboratory experiments. Our initial hypotheses were that i) sealed incubation chambers would result in suppressed CO_2 flux relative to continuously vented chambers, ultimately affecting SOC parameter estimates, ii) mechanical headspace mixing would homogenize CO_2 concentrations gradients within the incubation chamber, and iii) sieved soil samples would generate greater CO_2 fluxes compared to intact soil cores as a result of the disruption of physically protected labile SOC.

3.3 Materials and Methods

3.3.1 Experimental overview

Three independent laboratory experiments were conducted between June 2009 and March 2012 to evaluate the effects of continuous chamber venting (VENT), headspace mixing (MIX), and sieving (SIEVE) on soil carbon pool size and carbon pool decomposition estimates as defined by the AHI method (Paul et al., 2006).

3.3.2 Soil characteristics, sampling, and processing

Soils for the three laboratory experiments were collected at the University of Wisconsin's Arlington Agricultural Research Station (UW-ARL: 43°18'10"N, 89°20'43"W). The soils were sampled from sites currently in agricultural row-crop production and were classified as Plano silt loam (fine-silty, mixed, superactive, mesic typic argiudolls). These are relatively deep (> 1 m), well-drained soils that were formed under tallgrass prairie vegetation in loess deposits over calcareous glacial till. Soils for each of the three experiments (VENT, MIX, and SIEVE) were sampled from adjacent sites at UW-ARL during the summers of 2009, 2010, and 2011, and are representative of the Ap horizon (~ 0 to 25 cm) of these soils.

Aliquots of homogenized soils from each experiment were analyzed for texture, nutrient content, SOC, and non-hydrolyzable C (NHC). Soil texture was determined on three 50 g samples from each treatment using a standard hydrometer method (SPAL, 2004). Additional samples from each treatment (100 g) were sent to the University of Wisconsin Soil and Plant Analysis Lab (SPAL) for determination of pH (1:1, soil:water), organic matter (weight loss-on-ignition, 360 °C), available P and K (Bray P1 extract), exchangeable Ca and Mg (1N NH_4OAc, pH 7.0), and cation exchange capacity. To determine total soil carbon, finely ground sub-samples of soil were weighed (8-10 mg), packed into a 5 x 9-mm tin capsule, and analyzed on a Flash EA 1112 CN Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy). We used total C values interchangeably with SOC for these studies, as inorganic carbon in these soils is negligible (< 0.05 g kg^{-1}, Paul et al. 2001). NHC was determined for each experiment by refluxing soil samples (2 g) in 6M HCl (20 ml) at 116°C for 16 hr according to standard

published protocols (Paul et al., 2001b; Paul et al., 2006; Sollins et al., 1999). Physical and

biogeochemical details for each of the experimental soils are presented in (Table 3.1).

3.3.3 Instantaneous CO_2 flux rate measurements

Instantaneous soil CO_2 flux measurements were made using a LI-820 infrared gas

analyzer (IRGA) (LI-COR Biosciences, Lincoln, NE) that was directly connected in a continuous

loop to the incubation chamber. A one liter min^{-1} inline pump (Brailsford TD-3LS[11]) provided

gas flow through the measurement system. The LI-820 recorded CO_2 concentration data ($\mu l\ L^{-1}$)

every 10 s over the course of a 25-min interval. Data from the initial five minutes of each time

series of IRGA readings were discarded to ensure that the system had stabilized prior to

estimating CO_2 flux rate. Flux rates were determined by fitting a simple linear regression model

to the output data and then converting from CO_2 concentration change over time ($\mu l\ L^{-1}\ s^{-1}$) to

mass loss of carbon over time ($\mu g\ C\ [g\ soil]^{-1}\ day^{-1}$).

3.3.4 VENT: experimental specifics

VENT was setup as a completely randomized design with two treatments each replicated

5 times. The two treatments were: continuously vented chambers (CV), and periodically vented

chambers (PV). Soils for VENT were moist sieved to 2 mm, picked free of all visible plant

material, dried and stored until incubations could be initiated. Dry samples were thoroughly

mixed to minimize errors due to soil heterogeneity and facilitate comparison of laboratory

methods.   Samples were prepared for long term soil incubations by packing sufficient soil into a

100 ml specimen cup to reach a desired dry bulk density of 1.27 Mg m^{-3} (based on average field

values, data not shown). Five specimen cups (replicates) were prepared for each treatment

(N=10). Soils were then wetted to 60% water filled pore space (WFPS, Linn and Doran [1984]) based on their pressed bulk density (1.27 Mg m^{-3}) and an estimated particle density of 2.65 Mg m^{-3} (Campbell and Norman, 1998). Packed specimen cups were placed in 950 ml glass canning jars, and 20 ml of deionized water was added to the bottom of each jar to maintain internal humidity. Vented, metal lids (1 x 8 mm dia. hole, 1% of lid area) were placed on the jars and the soils were allowed to stabilize for 72 hr in the dark at 22°C prior to initial CO_2 respiration measurements. Following the stabilization phase, vented metal lids (2 x 8 mm dia. hole, 2% of lid area) were placed on the CV chambers and non-vented lids with two air-tight "quick connects" were placed on the PV chambers for the long term incubations. The first CO_2 flux measurements were taken immediately following initial lid placement. At the time of CO_2 readings, chamber lids were either directly connected to the LI-820 (PV), or replaced with quick-connect fitted lids (CV) and connected to the LI-820. Soil water content was monitored and maintained within 5% of ideal water filled pore space (IWFPS = 60%) by moistening the soils at the end of each CO_2 reading for the CV soils. Moisture in the PV soils was only checked during periodic venting. PV chambers were vented before the internal CO_2 concentration approached 3.5% (35,000 µl L^{-1} CO_2). At this point soils were removed from their incubation chamber, adjusted for any moisture loss, and returned to their sealed chambers following a thorough chamber flushing with dry pressurized air (340 ± 0.7 µl L^{-1} CO_2). Immediately following chamber flushing and moisture adjustment, the PV chambers were analyzed for CO_2 flux using the LI-820 to determine the effect of a sudden change in ambient CO_2 concentration on gaseous diffusion from the soil.

Soils were incubated at 22°C in the absence of light for 325 days. At the onset of the incubations, CO_2 readings were taken at two day intervals. This continued for a month when

CO_2 readings decreased to once a week. After three months, CO_2 readings were taken on average at 24 day intervals until the end of the experiment.

3.3.5 MIX: experimental specifics

MIX was set up as a 2 x 2 factorial in a completely randomized design with the factorial treatments being applied to the incubation chamber. The two factors were: 1) fans: with or without, and 2) continuous venting: with or without. Each factorial combination was replicated four times. The four combinations were: - fan / - vent (C), + fan / - vent (F), - fan / + vent (V), and + fan / + vent (FV).

Soils for MIX were moist sieved to 4 mm, picked free of all visible plant material, dried and stored until incubations could be initiated. Dry samples were thoroughly homogenized to minimize errors due to soil heterogeneity and facilitate comparison of laboratory methods. Samples were prepared for long term soil incubations by packing sufficient field moist soils into a 100 ml specimen cup to reach a desired dry bulk density of 1.27 Mg m^{-3} (based on average field values, data not shown). Four specimen cups (replicates) were prepared for each treatment (N=16). Soils were then wetted to 60% water filled pore space (WFPS, Linn and Doran [1984]) based on their pressed bulk density (1.27 Mg m^{-3}) and an estimated particle density of 2.65 Mg m^{-3} (Campbell and Norman, 1998). Following re-wetting, soils were allowed 24 hours to pre-incubate before collecting CO_2 efflux data. Packed specimen cups were then placed in 2200 ml polyvinyl chloride bottles. For the F and FV factor combinations, a 0.08 cm^3 min^{-1} 12V axial fan was located inside the chamber. Fans were kept running at all times while the soils were in the incubation chambers. For the V and FV factor combinations two 8mm holes located in the chamber lids allowed gaseous exchange between the chamber headspace and the lab

environment. In addition to fans and vent holes, each chamber was fitted with three septa located on the side of the chamber at heights of 1, 6, and 10cm from the soil surface (L, M, and H respectively) to monitor potential CO_2 stratification within each chamber between CO_2 efflux measurements. Just prior to measuring CO_2 evolution rates, 30 ml of headspace was simultaneously taken with a syringe from each of the three septa (L, M, and H) located along the side of the incubation chambers. Syringe needles were positioned directly above the soil sample in the center air column of the incubation chambers headspace. Headspace samples were stored in pre-evacuated 30 ml vials until they could be analyzed for CO_2 concentration on a Shimadzu GC-14B gas chromatograph. At the time of CO_2 reading, soils were removed from their incubation chambers and placed into a 1.9 L glass chamber that was connected to the LI-820. Soil water content was monitored and maintained within 5% of IWFPS by moistening the soils at the end of each CO_2 reading. After checking and correcting soil water content, soils were returned to their incubation chambers.

Soils were incubated at 22°C in the absence of light for 283 days. At the onset of incubations CO_2 readings were taken at two day intervals. This continued for three weeks at which point CO_2 readings were limited to once a week for the remainder of the experiment.

3.3.6 SIEVE: experimental specifics

SIEVE was set up as a completely randomized design with three treatments. The three treatments were: intact soil (I), sieved soil with root biomass (SR), and sieved soil with no root biomass (SNR). Each treatment was replicated four times.

Soils for SIEVE were collected using a slide-hammer soil core fitted with an aluminum sleeve (76mm H x 76mm D) to permit removal of undisturbed soil samples. Four randomly

selected cores were kept intact and refrigerated at 4°C until the start of incubations. Of the

remaining 8 cores, 4 randomly selected cores were moist sieved to 2 mm, removing all visible

plant material, and re-packed into aluminum sleeves, while 4 randomly selected cores were moist

sieved to 2 mm and re-packed into aluminum sleeves without removing any plant material. Soils

were repacked to a bulk density of 1.29 g cm^{-3} based on the average soil bulk density from the

field location in which the soils were sampled. Soils were then wetted to 60% water filled pore

space (WFPS, Linn and Doran [1984]) based on their pressed bulk density (1.29 Mg m^{-3}) and

an estimated particle density of 2.65 Mg m^{-3} (Campbell and Norman, 1998). Following re-

wetting, soils were allowed 24 hours to pre-incubate before collecting CO_2 efflux data. Soil

samples were placed in 2200 ml polyvinyl chloride bottles for incubation. Two 8 mm holes

located in the chamber lids allowed gaseous exchange between the chamber headspace and the

lab environment. At the time of CO_2 reading soils were removed from their incubation chambers

and placed into a 1.9 L glass chamber that was connected to the LI-820. Soil water content was

monitored and maintained within 5% of IWFPS by moistening the soils at the end of each CO_2

reading. After checking and correcting soil water content soils were returned to their incubation

chambers.

Soils were incubated at 22°C in the absence of light for 184 days. At the onset of

incubations CO_2 readings were taken at two day intervals. This continued for three weeks at

which point CO_2 readings were limited to once a week for the remainder of the experiment.

3.3.7 Statistical Modeling of CO_2 Flux Data

A three pool constrained model (Eq. 1) with first order kinetics was used to evaluate the

size and decomposition rates of three SOC pools (Eq. 1) (Paul et al., 2001b).

Eq. 1
$$C_{t(t)} = C_{rm}e^{-k_{rm}(t)} + C_{sm}e^{-k_{sm}(t)} + C_{nh}e^{-k_{nh}(t)}$$

In this model $C_{t(t)} =$ is total soil organic carbon at time t; C_{rm}, C_{sm}, and C_{nh} represent the C mass

in the readily mineralized, slowly mineralized, and non-hydrolysable fractions respectively; k_{rm},

k_{sm}, and k_{nh} are the decomposition rates of each fraction. The first derivative of Equation 1was

then used to estimate C_{rm}, k_{rm}, and k_{sm} via curve fitting of CO_2 respiration data from each

individual incubation chamber using the NLIN procedure (METHOD = MARQUART) of SAS

version 9.3 (Eq. 2).

Eq. 2
$$\frac{dC}{dt} = C_{rm}k_{rm}e^{(-k_{rm}t)} + C_{sm}k_{sm}e^{(-k_{sm}t)} + C_{nh}k_{nh}e^{(-k_{nh}t)}$$

The methodology and modeling technique is consistent with Paul et al. (2001b) and Paul et al.

(2006), although the nomenclature is slightly different. Here we replace the subscript a (active)

with rm, s (slow) with sm, and r (resistant) with nh to emphasize the fact that these represent

operationally defined pools and do not clearly correspond with any one functionally defined

component of soil organic matter. The mean residence time (MRT) for each of the three pools is

obtained via the inverse of the decomposition rate (1/k) scaled to field time with a Q_{10} of 2.89

(Eq. 3).

Eq 3.
$$Q_{10} = \left(2^{\left(\frac{labT - MAT}{10}\right)} \right)$$

In this model C_{nh} is estimated by 6N HCl acid hydrolysis and k_{nh} was set at 2840 yr based on the

[14]C age of C_{nh} from 0-20 cm for a Plano silt loam at UW-ARL reported by Paul et al. (2001a).

The slowly mineralized carbon pool (Csm) is estimated as SOC-Crm-Cnh.

3.3.8 Comparing non-linear regression models

Regression model differences were evaluated via F-tests on model reduction. The F-test used for non-linear model comparisons is outlined below in equation 4.

$$\text{Eq. 4} \qquad F = \frac{\left(\frac{SSE_{(reduced)} - SSE_{(full)}}{df_{SSE(reduced)} - df_{SSE(full)}}\right)}{\left(\frac{SSE_{(full)}}{df_{SSE(full)}}\right)}$$

Where SSE = sums of squares for error and *df* = degrees of freedom. Numerator degrees of freedom for the F-test were calculated as the difference between the full and reduced model error degrees of freedom, and the denominator degrees of freedom for the F-test are taken from the error degrees of freedom from the full model. A Bonferroni correction was applied to all p-values obtained in experiments with multiple comparisons.

3.3.9 Statistical Analysis of parameter estimates and CO_2 chamber concentrations

Treatment specific comparisons of CO_2 chamber concentrations and SOC pool estimates within each experiment were conducted using standard mixed effects models in SAS v 9.3. The model to compare SOC parameter estimates and decomposition rates for all three experiments was

$$\text{Eq 5.} \qquad y_{ij} = \mu + R_i + T_j + \varepsilon_{ij}$$

Where μ = population mean, R = random effect of the ith replicate, T = fixed effect of the jth treatment, and ε = the error term associated with the interaction of the jth replicate and i^{th} treatment.

The model used for the comparison of CO_2 concentration pre- and post-venting in the VENT experiment was

Eq 6. $$y_{ijk} = \mu + R_i + D_j + \varepsilon_{ij} + T_k + D * T_{jk} + \delta_{ijk}$$

Where μ = population mean, R = random effect of the ith replicate, D = fixed effect of the jth day (0 to 325), and ε = the error term associated with the interaction of the jth replicate and i^{th} day, T = fixed effect of kth time (pre- or post-venting), D*T = the interaction of the jth day with the kth time, and δ = error term for each individual measurement and the correlation structure between times (pre- or post-venting) within a given day, assuming that they are not independent. In this case the compound symmetric variance-covariance structure (type = CS) was used is SAS PROC MIXED.

The model used for the comparison of CO_2 stratification in the MIX experiment was

Eq 7. $$y_{ijk} = \mu + R_i + T_j + \varepsilon_{ij} + H_k + T * H_{jk} + \delta_{ijk}$$

Where μ = population mean, R = random effect of the ith replicate, T = fixed effect of the jth treatment (C, F, or V), and ε = the error term associated with the interaction of the jth replicate and i^{th} treatment, H = fixed effect of kth height (1, 6, or 10 cm above the soil), T*H = the interaction of the jth treatment with the kth height, and δ = error term for each individual measurement and the correlation structure between heights (1, 6, or 10 cm above the soil) within a given incubation chamber , assuming that they are not independent. In this case the first order autoregressive variance-covariance structure (type = ar[1]) was used is SAS PROC Mixed.

3.4 Results

3.4.1 VENT

Concentrations of CO_2 within the CV chambers were on average 29 times lower than those in the PV chambers throughout the length of the 325 day incubation (452 ± 10 and $13,245\pm1290$ µl L^{-1} CO_2, respectively). As a result of such high concentrations (Fig. 3.1a), the use of instantaneous IRGA measurements (CO_2 flux over 20 min period) to determine CO_2 flux rates in the PV treatment proved unreliable (Fig. 3.1b). As CO_2 accumulated in the PV chambers it became increasingly difficult to both estimate a positive flux with the IRGA and constrain measurement error. In order to deal with this measurement constraint, CO_2 flux rates in PV were determined by taking the difference in average CO_2 concentrations between two measurement periods and dividing by the elapsed time. This estimation method provided flux rates much closer to those obtained with instantaneous readings in CV (Fig. 3.1b) and was therefore used for SOM parameter estimation.

Non-linear regression analysis of CO_2 flux data showed that CV and PV were significantly different ($p < 0.0001$), justifying the use of treatment specific regression models for the estimation of SOC pools and kinetics (Fig. 3.2). In PV, initial CO_2 flux diminished rapidly between days 1 and 12 after which it stabilized at between 3 and 4 µg C (g soil)$^{-1}$ day^{-1}. In general, the CO_2 flux curve for CV was more gradual than PV for the first 40 days of incubation after which it too began to stabilize between 3 and 4 µg C (g soil)$^{-1}$ day^{-1}.

Parameter estimates for each treatment reflect the differences observed in CO_2 flux data (Table 3.2). The most notable differences were observed in C_{rm} and its MRT. From this analysis the C_{rm} pool size was estimated to be 50% smaller in PV than in CV ($p = 0.0005$) with a MRT of

just 35% that estimated in CV (p = 0.0025). The difference observed in C_{sm}, while statistically significant, is not large enough to be of any practical biological significance. It should be noted that since C_{sm} is estimated based on SOC, C_{rm}, and C_{nh}, it is not independent of any of these other parameters. As was obvious from the non-linear regression models (Fig. 3.2), there was no significant difference between the MRT's of C_{sm} for CV or PV.

As chamber CO_2 concentrations within the PV treatment accumulated there was a concomitant reduction in CO_2 flux from the soil as evidenced by the respiration data shown in Fig. 3.2. Carbon dioxide flux rates were analyzed prior to, and immediately following chamber venting in PV to determine whether or not this decrease in respiration was primarily the result of storage within the soil system (concentration gradient driven reduction in flux), or as a result of some effect on the microbial community (CO_2 toxicity or O_2 limitation). The later would likely require greater post-venting time to elapse before CO_2 flux rates rebounded. The analysis of time of measurement (time = pre- or post-venting) and day of incubation (day = 12, 24, 45, 95, 121, 325) showed that time, day, and their interaction were highly significant (p < 0.0001 for all three). Pre-vented flux rates in PV averaged 2.3 μg C (g soil)$^{-1}$ day^{-1} while post-venting flux rates averaged 222.6 μg C (g soil)$^{-1}$ day^{-1}. The significant interaction of time and day was due to the fact that post-venting flux rates were greatest at the initiation of the experiment and decreased over time, becoming statistically indistinguishable by day 121 (Fig. 3.3).

3.4.2 MIX

Of the initial four factorial combinations, significant and sustained water loss from VF resulted in our abandoning this treatment. Of the three remaining treatments, non-linear

regression analysis of CO_2 flux data showed that C, F and V were all significantly different from one another ($p < 0.0001$). As was the case in the VENT experiment, treatment specific regression models (n = 3) were used to estimate SOC pools and kinetics (Fig. 3.4). In this experiment, CO_2 flux in treatment V diminished the most rapidly between days 1 and 18 followed by a very gradual decrease in flux from 8 µg C (g soil)$^{-1}$ day^{-1} on day 28 to 5 µg C (g soil)$^{-1}$ day^{-1} on day 283. In spite of their statistical difference, treatments F and C had CO_2 flux curves that roughly paralleled each other throughout the experiment.

Parameter estimates for the MIX experiment (Table 3.3) reflect the sustained higher respiration rates in C and V as well as the more gradual draw down. The size of C_{rm} and its mean residence time were roughly two times greater in C than in V. The effect of these three treatments on initial CO_2 flux had an inverse effect on estimations of C_{sm} and its mean residence time. Estimates of slowly mineralized carbon in this case were largest in treatment V and smallest in treatment C.

Analysis of headspace samples taken from three heights within each chamber showed a significant effect of treatment ($p = 0.0006$) but no height effect ($p = 0.79$) or interactive effect with height and treatment ($p = 0.37$). In this analysis, average internal CO_2 concentrations within treatments C, F, and V were 894, 764, and 450 µl L^{-1} CO_2 respectively. Within a given treatment there was very little variability in CO_2 concentration with height (Fig. 3.5).

3.4.3 SIEVE

Non-linear regression analysis of CO_2 flux data showed that I, SR, and SNR were significantly different ($p < 0.0001$); therefore an independent model was used to estimate SOC

pools and kinetics for each treatment (Fig. 3.6). Treatment I had the most variable CO_2 flux rates throughout the length of the experiment with an average coefficient of variation (CV) of 32% compared to 15% in SR and just 10% in SNR. Flux rates for treatment I also began lower (approximately 16 µg C [g soil]$^{-1}$ day^{-1}) and decreased more gradually than they did in either SR or SNR, finally stabilizing at around 7 µg C (g soil)$^{-1}$ day^{-1}. Treatment SR had the highest initial flux rate of 27 µg C (g soil)$^{-1}$ day^{-1} on average which stabilized at around 10 µg C (g soil)$^{-1}$ day^{-1}. The third treatment SNR had a slightly lower initial respiration, which stabilized between treatments I and SN at around 9 µg C (g soil)$^{-1}$ day^{-1}.

In spite of the significant difference between non-linear regression models for I, SR, and SNR, with the exception of the MRT of C_{rm} (p = 0.0146), there were no detectable parameter differences between treatments. The C_{rm} pool made up just 0.3% of SOC with a mean residence time of approximately 10 (SR and SNR) to 30 days (I). The size of the C_{sm} pool accounted for over half of SOC with C_{nh} accounting for just over 40% SOC (Table 3.4).

3.4.4 Soil Moisture

Soil moisture was well maintained within the 5% loss cutoff cited as acceptable in the AHI literature for the duration of all three experiments. Nevertheless, measurement of water loss relative to IWFPS indicated that vented systems lost more moisture on average than did non-vented systems. In the VENT experiment, where PV chambers are sealed for the longest time period, change in IWFPS ranged from an average loss of 1.2% in CV to an average gain of 0.3% in PV (p < 0.0001). Venting in the MIX experiment was intermediate between VENT and SIEVE in that sealed chambers (C and F) were vented at the time of CO_2 flux reading. In this

experiment, changes in IWFPS, ranged from -0.3% in C to -2.1% in V (C<F≤V, p<0.0001). There were no detectable differences in SIEVE will an average loss of -0.6% relative to IWFPS in all treatments.

3.5 Discussion

3.5.1 Chamber CO_2 concentrations, flux suppression, and parameter estimates

In the VENT experiment the PV chambers led to a suppression of CO_2, most significantly in the first 30 days, relative to the CV chambers. This suppression of CO_2 flux manifested itself in both a reduced size and MRT of C_{rm} when compared to the CV treatment (Table 3.2). Although we are not aware of a body of literature documenting such an effect for the AHI method, the phenomenon of suppressed CO_2 flux in sealed chambers as they are deployed for field research is well documented (Healy et al., 1996; Livingston and Hutchinson, 1995; Nay et al., 1994). Pumpanen et al. (2004) evaluated the effect of 20 different chamber configurations for measuring soil CO_2 flux and compared their accuracy against known CO_2 fluxes ranging from 0.32 to 10.01 µmol CO_2 m^{-2} s^{-1}. They report that most of the non-steady-state systems, analogous to the incubation chambers in the current study, underestimated CO_2 fluxes by 10% on average. Furthermore they showed that fluxes calculated over a 30 minute time period were about 15% lower than those calculated over a 10 min period. This difference was attributed to a reduction in the diffusivity gradient as a result of increased internal CO_2 concentration. It is clear from the analysis of pre- and post-venting CO_2 flux rates that as time passed in the VENT experiment the amount of CO_2 being stored in the system decreased to the point where CO_2 flux rates post-venting became indistinguishable from those pre-venting (Fig. 3.3).

Although not directly comparable to the VENT experiment, the MIX experiment highlights the same suppressive effect of sealed chambers on soil CO_2 flux. In this experiment the chambers that were sealed between readings (C and F) had much higher flux rates throughout the experiment resulting in significantly greater estimates of the size and MRT of C_{rm} (Table 3.3). In the VENT experiment these same parameters were significantly smaller in the sealed chambers. This apparent discrepancy arises in the VENT experiment because the CO_2 flux was measured over the sealed phase of the measurement cycle whereas in the MIX experiment the CO_2 flux was measured immediately after opening the chamber to connect it to the IRGA. Flux rates obtained from treatments C and F in the MIX experiment therefore likely represent the mass efflux of gas stored within the soil pore space following a significant increase in the diffusivity gradient upon venting the chambers. This observation in the MIX experiment contradicts the findings of Thuries et al. (2000). They evaluated CO_2 flux using alkali traps from sealed incubation chambers as well as from soils that were stored in holding chambers prior to moving to a single chamber for flux measurements. Although they report lower CO_2 flux in the sealed chamber than in either of the two scenarios utilizing holding chambers (76 ± 24 vs. 80 ± 18 or 84 ± 18 mg CO_2-C kg^{-1} soil), these differences were not significant, likely due to the high variability within each treatment. Another potential explanation for this seeming lack of difference may be that alkali traps, which have been repeatedly shown to overestimate CO_2 flux were used. Bekku et al. (1997) evaluated the use of alkali traps, gas chromatography, and IRGA for determining CO_2 flux. They found the internal chamber CO_2 concentrations were so reduced relative to ambient levels (20 to 250 $\mu l \ L^{-1}$) that CO_2 flux rates were enhanced as a result of an artificially high diffusivity gradient by 30%. It is possible in the case of Thuries et al. (2000) that

CO_2 flux was overestimated in all three treatments as a result of the alkali traps, masking any detectible signal of post-venting flux differences.

3.5.2 Mechanical headspace mixing had a negative effect on incubated soils

Our findings from the MIX experiment suggest that stratification of internal CO_2 is of minimal concern relative to the profound impact that internal concentration gradients (or lack thereof) can have on the CO_2 storage in, and flux from the soil pore space. While fans did not impart a detectable benefit relative to CO_2 homogeneity, they did negatively affect the water balance of both treatments in which they were used. For the FV treatment water loss was significantly higher than the 5% threshold (-13%, CV = 37%) and the treatment was therefore abandoned. Moisture loss was minimal in treatment F but greater on average than in treatment C supporting the general idea that fans do not impart a clearly defined benefit and may be detrimental in maintaining ideal soil conditions. The use of fans within CO_2 flux chambers has been discouraged by a number of authors either because they have been deemed unnecessary (Healy et al., 1996; Hutchinson and Livingston, 2002), or because they have been implicated in creating pressure differentials that alter gaseous diffusion (Fang and Moncrieff, 1998; Pumpanen et al., 2004). In their evaluation of 20 different chamber configurations, Pumpanen et al. (2004) found that fan induced headspace mixing can be a major source of error. The concluded that excessive turbulence inside the chamber led to mass flow of CO_2 from the soil, thus artificially elevating flux readings.

3.5.3 Soil sieving decreased CO_2 flux measurement error without significantly impacting SOC parameter estimates.

Results from the SIEVE experiment were somewhat ambiguous. While intact soil cores produced a more gradual CO_2 flux curve (Fig. 3.6), which resulted in a significantly longer MRT for C_{rm}, the flux data was extremely variable. It is therefore difficult to determine whether or not the MRT of the readily mineralized carbon pool reflects its "true" MRT or an artifact of the laboratory technique. The size of C_{rm} and C_{sm}, as well as the MRT of C_{sm} were indistinguishable between treatments. Plante et al. (2009) reported that two physical disruption treatments (< 2 mm sieved and < 75 μm sieved) differed significantly from intact soil cores in a 15 d incubation study ($p < 0.001$). In their study the sieved and crushed soils respired 190% more CO_2 on average than did the intact core at 25ºC. They also reported an interactive effect with temperature ($p = 0.005$), showing an apparent decrease in the effect of soil disruption as incubation temperature climbed from 15 to 35ºC. Given the high variability associated with CO_2 flux readings from intact soil cores, and the relative lack of difference between sieved and intact cores, the standard practice of sieving soils to 4 mm or 2 mm will likely yield the most repeatable flux rates.

3.5.4 Soil moisture can be adequately maintained in continuously vented chambers

In the current study, soil moisture varied from a loss of 2.1% to a gain of 0.3% relative to IWFPS. Both are well within the accepted range of 5% cited in the literature (Conant et al., 2008a; Haddix et al., 2011). While sealed chambers retarded or stopped moisture loss they tended to lead to soil moistures above 60% WFPS. It is likely that a deviation in either direction

would have an impact on microbial respiration and ultimately affect soil CO_2 flux although the range in soil moistures we report may not have a detectible impact. While differences between sealed and vented chamber are somewhat confounded by minimal differences in soil moisture, the CO_2 flux measurements made pre- and post-venting strongly point to the diffusivity gradient and not soil moisture as the principle driving factor between observed treatment differences.

3.5.5 The importance of methodological considerations largely depends on experimental objectives

Results from each of the three experiments conducted here highlight the importance of methodological considerations when using the AHI method. Because SOC parameter estimates are obtained by fitting non-linear regression models to CO_2 flux data, the method is very sensitive to minor methodologically induced changes to gaseous diffusion. In the current study, with the exception of the VF treatment in the MIX experiment, the AHI method yielded repeatable estimates of SOC pool sizes and their decomposition rates. As such, either continuously vented or periodically vented chambers would be appropriate if the primary research objective was to compare land management or treatment effects within a study. If the goal is to estimate SOC parameter estimates that will be used in modeling work, care must be taken to minimize the influence of laboratory artifacts on measured CO_2 flux rates. This is particularly important given the artificial nature of laboratory incubation work. The work of Paul et al. (1999) and Paul et al. (2006) demonstrated the value and applicability of using parameter estimates obtained via the AHI method to improve biogeochemical model output. Others have also demonstrated the efficacy of utilizing soil mineralization rates and chemical isolates of

stabilized carbon to improve biogeochemical modeling (Juston et al., 2010; Scharnagl et al., 2010). In spite of the strong correlation between modeled and measured CO_2 flux demonstrated by Paul et al. (1999) and Paul et al. (2006) using AHI derived parameters, modeled flux was approximately one third lower than that measured by field sampling. They attributed these differences to lack of root respiration in the laboratory, and the delay between residue additions to the soil and their subsequent breakdown and mineralization. It is not unreasonable, however, to assume that this discrepancy (the underestimation of CO_2 flux by one third) may have arisen in part from techniques that suppress, and as a result underestimate the CO_2 flux, from incubated soils in the lab. Further research is warranted to determine if parameter estimates obtained via periodically vented or continuously vented chambers provide greater predictive modeling power.

3.6 Conclusions

Use of both continuous vented and periodically vented chambers maintained soil moisture at an acceptable level and produced SOC parameter estimates that were easily repeatable, had a high signal to noise ratio, and were valuable in hypothesis testing. Nevertheless, the high CO_2 concentrations that developed in periodically vented chambers did suppress CO_2 flux, supporting our first hypothesis. This was demonstrated by, i) reduced pre-venting flux rates in the VENT experiment relative to the continuously vented chamber, and ii) exaggerated post-venting flux rates in the VENT and MIX experiments, again relative to the continuously vented chamber. While these differences may not be important for treatment comparison and hypothesis testing in a given study, they do have potentially large implications for comparison between studies or when using AHI derived parameter estimates in

biogeochemical models. A standardized protocol is called for that meets the criteria outlined by Paul et al. (2006), while simultaneously seeking minimizing laboratory artifacts. Further work is needed to investigate biogeochemical model performance when parameters obtained from both continuously vented and periodically vented chambers are used. Contrary to our final two hypotheses, we were not able to detect internal chamber CO_2 stratification, and while intact soil cores did differ from sieved soils in their flux rates, it was primarily due the high variability associated with the former. In light of these findings, we recommend sieving and homogenizing soils to minimize variability but do not recommend the use of fans in AHI incubation chambers.

3.7 Acknowledgements

3.8 References

Baker J.M., Ochsner T.E., Venterea R.T., and T. J. Griffis. 2007. Tillage and soil carbon sequestration - What do we really know? Agriculture Ecosystems & Environment 118: 1-5.

Batjes N.H. 1996. Total carbon and nitrogen in the soils of the world. European Journal of Soil Science 47: 151-163.

Bekku Y., Koizumi H., Oikawa T., and H. Iwaki. 1997. Examination of four methods for measuring soil respiration. Applied Soil Ecology 5: 247-254.

Bellamy P.H., Loveland P.J., Bradley R.I., Lark R.M., and G.J.D. Kirk. 2005. Carbon losses from all soils across England and Wales 1978-2003. Nature 437: 245-248.

Bolinder M.A., Janzen H.H., Gregorich E.G., Angers D.A., and A.J. VandenBygaart. 2007. An approach for estimating net primary productivity and annual carbon inputs to soil for common agricultural crops in Canada. Agriculture Ecosystems & Environment 118: 29-42.

Brady N.C., and R.R. Weil. 2008. The nature and properties of soils. 14th edition ed. Prentice Hall, Upper Saddle River, NJ.

Bruun S., and J. Luxhoi. 2006. Letter to the editor on "Can incubations be used to measure meaningful pools of soil organic matter?". Soil Science Society of America Journal 70: 2164-2164.

Brye K.R., Norman J.M., and S.T. Gower. 2002a. Assessing the Progress of a Tallgrass Prairie Restoration in Southern Wisconsin. American Midland Naturalist 148: 218-235.

Brye K.R., Gower S.T., Norman J.M., and L.G. Bundy. 2002b. Carbon budgets for a prairie and agroecosystems: Effects of land use and interannual variability. Ecological Applications 12: 962-979.

Buyanovsky G.A., and G.H. Wagner. 1998. Carbon cycling in cultivated land and its global significance. Global Change Biology 4: 131-141.

Cahill K.N., Kucharik C.J., and J.A. Foley. 2009. Prairie restoration and carbon sequestration: difficulties quantifying C sources and sinks using a biometric approach. Ecological Applications 19: 2185-2201.

Campbell G.S., and J.M. Norman. 1998. Heat Flow in the Soil, An Introduction to Environmental Biophysics, Springer, New York. pp. 113-128.

Christiansen J.R., Korhonen J.F.J., Juszczak R., Giebels M., and M. Pihlatie. 2011. Assessing the effects of chamber placement, manual sampling and headspace mixing on CH4 fluxes in a laboratory experiment. Plant and Soil 343: 171-185.

Coleman K., and D.S. Jenkinson. 1996. RothC-26.3 - A model for the turnover of carbon in soil., in: D. S. Powlson, et al. (Eds.), Evaluation of soil organic matter models, Springer-Verlag, Berlin. pp. 237-246.

Collins H.P., Blevins R.L., Bundy L.G., Christenson D.R., Dick W.A., Huggins D.R., and E.A. Paul. 1999. Soil carbon dynamics in corn-based agroecosystems: Results from carbon-13 natural abundance. Soil Science Society of America Journal 63: 584-591.

Collins H.P., Elliott E.T., Paustian K., Bundy L.C., Dick W.A., Huggins D.R., Smucker A.J.M., and E.A. Paul. 2000. Soil carbon pools and fluxes in long-term corn belt agroecosystems. Soil Biology & Biochemistry 32: 157-168.

Conant R.T., Easter M., Paustian K., Swan A., and S. Williams. 2007. Impacts of periodic tillage on soil C stocks: A synthesis. Soil and Tillage Research 95: 1-10.

Conant R.T., Steinweg J.M., Haddix M.L., Paul E.A., Plante A.F., and J. Six. 2008a. Experimental warming shows that decomposition temperature sensitivity increases with soil organic matter recalcitrance. Ecology 89: 2384-2391.

Conant R.T., Drijber R.A., Haddix M.L., Parton W.J., Paul E.A., Plante A.F., Six J., and J.M. Steinweg. 2008b. Sensitivity of organic matter decomposition to warming varies with its quality. Global Change Biology 14: 868-877.

Conant R.T., Ryan M.G., Ågren G.I., Birge H.E., Davidson E.A., Eliasson P.E., Evans S.E., Frey S.D., Giardina C.P., Hopkins F.M., Hyvönen R., Kirschbaum M.U.F., Lavallee J.M., Leifeld J., Parton W.J., Megan Steinweg J., Wallenstein M.D., Martin Wetterstedt J.Å., and M.A. Bradford. 2011. Temperature and soil organic matter decomposition rates – synthesis of current knowledge and a way forward. Global Change Biology 17: 3392-3404.

Conen F., and K.A. Smith. 2000. An explanation of linear increases in gas concentration under closed chambers used to measure gas exchange between soil and the atmosphere. European Journal of Soil Science 51: 111-117.

Davidson E.A., Savage K., Verchot L.V., and R. Navarro. 2002. Minimizing artifacts and biases in chamber-based measurements of soil respiration. Agriculture and Forest Meteorology 113: 21-37.

DeLuca T.H., and C.A. Zabinski. 2011. Prairie ecosystems and the carbon problem. Frontiers in Ecology and the Environment 9: 407-413.

Dungait J.A.J., Hopkins D.W., Gregory A.S., and A.P. Whitmore. 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. Global Change Biology 18: 1781-1796.

Fang C., and J.B. Moncrieff. 1998. An open-top chamber for measuring soil respiration and the influence of pressure difference on CO_2 efflux measurement. Functional Ecology 12: 319-325.

Follett R.F., Paul E.A., and E.G. Pruessner. 2007. Soil carbon dynamics during a long-term incubation study involving C-13 and C-14 measurements. Soil Science 172: 189-208.

Fortuna A., Harwood R.R., and E.A. Paul. 2003. The effects of compost and crop rotations on carbon turnover and the particulate organic matter fraction. Soil Science 168: 434-444.

Glinski J., and W. Stepniewski. 1985. Soil aeration and its role for plants CRC Press, Boca Raton.

Grace P.R., Colunga-Garcia M., Gage S.H., Robertson G.P., and G.R. Safir. 2006. The potential impact of agricultural management and climate change on soil organic carbon of the North Central Region of the United States. Ecosystems 9: 816-827.

Graedel T.E., and P.J. Crutzen. 1993. Atmospheric change - and earch system perspective W.H. Freeman and Company, New York.

Gulde S., Chung H., Amelung W., Chang C., and J. Six. 2008. Soil carbon saturation controls labile and stable carbon pool dynamics. Soil Science Society of America Journal 72: 605-612.

Guzman J.G., and M.M. Al-Kaisi. 2010. Soil Carbon Dynamics and Carbon Budget of Newly Reconstructed Tall-grass Prairies in South Central Iowa. Journal of Environmental Quality 39: 136-146.

Haddix M.L., Plante A.F., Conant R.T., Six J., Steinweg J.M., Magrini-Bair K., Drijber R.A., Morris S.J., and E.A. Paul. 2011. The role of soil characteristics on temperature sensitivity of soil organic matter. Soil Science Society of America Journal 75: 56-68.

Haile-Mariam S., Cheng W., Johnson D.W., Ball J.T., and E.A. Paul. 2000. Use of carbon-13 and carbon-14 to measure the effects of carbon dioxide and nitrogen fertilization on carbon dynamics in ponderosa pine. Soil Science Society of America Journal 64: 1984-1993.

Hamza M.A., and W.K. Anderson. 2005. Soil compaction in cropping systems: A review of the nature, causes and possible solutions. Soil and Tillage Research 82: 121-145.

Healy R.W., Striegl R.G., Russell T.F., Hutchinson G.L., and G.P. Livingston. 1996. Numerical evaluation of static-chamber measurements of soil-atmosphere gas exchange: Identification of physical processes. Soil Science Society of America Journal 60: 740-747.

Huggins D.R., Allmaras R.R., Clapp C.E., Lamb J.A., and G.W. Randall. 2007. Corn-soybean sequence and tillage effects on soil carbon dynamics and storage. Soil Science Society of America Journal 71: 145-154.

Huggins D.R., Buyanovsky G.A., Wagner G.H., Brown J.R., Darmody R.G., Peck T.R., Lesoing G.W., Vanotti M.B., and L.G. Bundy. 1998. Soil organic C in the tallgrass prairie-derived region of the corn belt: effects of long-term crop management. Soil & Tillage Research 47: 219-234.

Hutchinson G.L., and G.P. Livingston. 2002. Soil-Atmosphere Gas Exchange, in: J. H. Dane and G. C. Topp (Eds.), Methods of Soil Analysis: Part 1, Physical Methods, Soil Science Society of America, Madison, WI. pp. 1159-1182.

IPCC. 1997. Greenhouse gas inventory reference manual, Intergovernmental Panel on Climate Change and International Energy Agency. http://www.ipcc.ch/publications_and_data/publications_and_data_reports.shtml#.UCQK BaHN89U. (accessed, 8 August, 2012).

Jackson R.B., Canadell J., Ehleringer J.R., Mooney H.A., Sala O.E., and E.D. Schulze. 1996. A global analysis of root distributions for terrestrial biomes. Oecologia 108: 389-411.

Jacobs A., Helfrich M., Hanisch S., Quendt U., Rauber R., and B. Ludwig. 2010. Effect of conventional and minimum tillage on physical and biochemical stabilization of soil organic matter. Biology and Fertility of Soils 46: 671-680.

Jagadamma S., and R. Lal. 2010. Integrating physical and chemical methods for isolating stable soil organic carbon. Geoderma 158: 322-330.

Jelinski N.A., Kucharik C.J., and J.B. Zedler. 2009. A Test of Diversity-Productivity Models in Natural, Degraded, and Restored Wet Prairies. Restoration Ecology 19: 186-193.

Jokela W., Posner J., Hedtcke J., Balser T., and H. Read. 2011. Midwest Cropping System Effects on Soil Properties and on a Soil Quality Index. Agronomy Journal 103: 1552-1562.

Juston J., Andren O., Katterer T., and P.E. Jansson. 2010. Uncertainty analyses for calibrating a soil carbon balance model to agricultural field trial data in Sweden and Kenya. Ecological Modelling 221: 1880-1888.

Kleber M., and M.G. Johnson. 2010. Advances in Understanding the Molecular Structure of Soil Organic Matter: Implications for Interactions in the Environment, Advances in Agronomy, Vol 106, Elsevier Academic Press Inc, San Diego. pp. 77-142.

Kogel-knabner I., Deleeuw J.W., Tegelaar E.W., Hatcher P.G., and H. Kerp. 1994. A lignin-like polymer in the cuticle of spruce needles - implications for the humification of spruce litter. Organic Geochemistry 21: 1219-1228.

Kravchenko A.N., and G.P. Robertson. 2011. Whole-Profile Soil Carbon Stocks: The Danger of Assuming Too Much from Analyses of Too Little. Soil Science Society of America Journal 75: 235-240.

Kucharik C.J., Fayram N.J., and K.N. Cahill. 2006. A paired study of prairie carbon stocks, fluxes, and phenology: comparing the world's oldest prairie restoration with an adjacent remnant. Global Change Biology 12: 122-139.

Kucharik C.J., Serbin S.P., Vavrus S., Hopkins E.J., and M.M. Motew. 2010. Patterns of climate change across Wisconsin from 1950 to 2006. Physical Geography 31: 1-28.

Kucharik C.J., Brye K.R., Norman J.M., Foley J.A., Gower S.T., and L.G. Bundy. 2001. Measurements and modeling of carbon and nitrogen cycling in agroecosystems of southern Wisconsin: Potential for SOC sequestration during the next 50 years. Ecosystems 4: 237-258.

Kutzbach L., Schneider J., Sachs T., Giebels M., Nykanen H., Shurpali N.J., Martikainen P.J., Alm J., and M. Wilmking. 2007. CO2 flux determination by closed-chamber methods can be seriously biased by inappropriate application of linear regression. Biogeosciences 4: 1005-1025.

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

Lal R. 2008a. Carbon Sequestration. Philosophical Transactions of the Royal Society 363: 815-830.

Lal R. 2008b. Sequestration of atmospheric CO2 in global carbon pools. Energy & Environmental Science 1: 86-100.

Linn D.M., and J.W. Doran. 1984. Effect of Water-Filled Pore-Space on Carbon-Dioxide and Nitrous-Oxide Production in Tilled and Nontilled Soils. Soil Science Society of America Journal 48: 1267-1272.

Liu S.G., Tan Z.X., Li Z.P., Zhao S.Q., and W.P. Yuan. 2011. Are soils of Iowa USA currently a carbon sink or source? Simulated changes in SOC stock from 1972 to 2007. Agriculture Ecosystems & Environment 140: 106-112.

Livingston G.P., and G.L. Hutchinson. 1995. Enclosure-based measurement of trace gas exchange: applications and sources of error, in: P. A. Matson and R. C. Harris (Eds.), Biogenic Trace Gases: Measureing Emissions from Soil and Water, Blackwell Sceintific Publications, Oxford. pp. 14-51.

Livingston G.P., Hutchinson G.L., and K. Spartalian. 2006. Trace gas emission in chambers: A non-steady-state diffusion model. Soil Science Society of America Journal 70: 1459-1469.
Loughin T.M. 2006. Improved experimental design and analysis for long-term experiments. Crop Science 46: 2492-2502.

Luo Z., Wang E., and O.J. Sun. 2010. Can no-tillage stimulate carbon sequestration in agricultural soils? A meta-analysis of paired experiments. Agriculture, Ecosystems & Environment 139: 224-231.

Maier M., Schack-Kirchner H., Hildebrand E.E., and J. Holst. 2010. Pore-space CO2 dynamics in a deep, well-aerated soil. European Journal of Soil Science 61: 877-887.

McLauchlan K.K., and S.E. Hobbie. 2004. Comparison of labile soil organic matter fractionation techniques. Soil Science Society of America Journal 68: 1616-1625.

Meese B.G., Carter P.R., Oplinger E.S., and J.W. Pendleton. 1991. Corn/Soybean Rotation Effects as Influenced by Tillage, Nitrogen, and Hybrid/Cultivar. Journal of Production Agriculture 4:7.

Nafziger E.D., and R.E. Dunker. 2011. Soil Organic Carbon Trends Over 100 Years in the Morrow Plots. Agronomy Journal 103: 261-267.

Nay S.M., Mattson K.G., and B.T. Bormann. 1994. Biases of chamber methods for measuring soil co2 efflux demonstrated with a laboratory apparatus. Ecology 75: 2460-2463.

Ogle S.M., Breidt F.J., and K. Paustian. 2005. Agricultural management impacts on soil organic carbon storage under moist and dry climatic conditions of temperate and tropical regions. Biogeochemistry 72: 87-121.

Olk D.C., and E.G. Gregorich. 2006. Overview of the symposium proceedings, "Meaningful pools in determining soil carbon and nitrogen dynamics". Soil Science Society of America Journal 70: 967-974.

Parton W.J., Schimel D.S., Cole C.V., and D.S. Ojima. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Science Society of America Journal 51: 1173-1179.

Paul E.A., Collins H.P., and S.W. Leavitt. 2001a. Dynamics of resistant soil carbon of midwestern agricultural soils measured by naturally occurring C-14 abundance. Geoderma 104: 239-256.

Paul E.A., Morris S.J., and S. Bohm. 2001b. The Determination of Soil C Pool Sizes and Turnover Rates: Biophysical Fractionation and Tracers, in: R. Lal, et al. (Eds.), Assessment Methods for Soil Carbon, Lewis Publishers, New York. pp. 193-206.

Paul E.A., Morris S.J., Conant R.T., and A.F. Plante. 2006. Does the acid hydrolysis-incubation method measure meaningful soil organic carbon pools? Soil Science Society of America Journal 70: 1023-1035.

Paul E.A., Follett R.F., Haddix M., and E. Pruessner. 2011. Soil N Dynamics Related to Soil C and Microbial Changes During Long-Term Incubation. Soil Science 176: 527-536.

Paul E.A., Harris D., Collins H.P., Schulthess U., and G.P. Robertson. 1999. Evolution of CO2 and soil carbon dynamics in biologically managed, row-crop agroecosystems. Applied Soil Ecology 11: 53-65.

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

Pedersen P., and J.G. Lauer. 2002. Influence of rotation sequence on the optimum corn and soybean plant population. Agronomy Journal 94: 968-974.

Plante A.F., Six J., Paul E.A., and R.T. Conant. 2009. Does Physical Protection of Soil Organic Matter Attenuate Temperature Sensitivity? Soil Science Society of America Journal 73: 1168-1172.

Poirier N., Sohi S.P., Gaunt J.L., Mahieu N., Randall E.W., Powlson D.S., and R.P. Evershed. 2005. The chemical composition of measurable soil organic matter pools. Organic Geochemistry 36: 1174-1189.

Posner J.L., Casler M.D., and J.O. Baldock. 1995. The Wisconsin integrated cropping systems trial: Combining agroecology with production agronomy. American Journal of Alternative Agriculture 10: 98-107.

Posner J.L., Baldock J.O., and J.L. Hedtcke. 2008. Organic and conventional production systems in the Wisconsin Integrated Cropping Systems Trials: I. Productivity 1990-2002. Agronomy Journal 100: 253-260.

Pumpanen J., Kolari P., Ilvesniemi H., Minkkinen K., Vesala T., Niinisto S., Lohila A., Larmola T., Morero M., Pihlatie M., Janssens I., Yuste J.C., Grunzweig J.M., Reth S., Subke J.A., Savage K., Kutsch W., Ostreng G., Ziegler W., Anthoni P., Lindroth A., and P. Hari. 2004. Comparison of different chamber techniques for measuring soil CO2 efflux. Agricultural and Forest Meteorology 123: 159-176.

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

Risk D., Kellman L., Beltrami H., and A. Diochon. 2008. In situ incubations highlight the environmental constraints on soil organic carbon decomposition. Environmental Research Letters 3.

Robertson G.P., Wedin D., Groffman P.M., Blair J.M., Holland E.A., Nadelhoffer K.J., and D. Harris. 1999. Soil carbon and nitrogen availability: nitrogen mineralization, nitrification, and soil respiration potentials, in: G. P. Robertson, et al. (Eds.), Standard soil methods for long-term ecological research, Oxford University Press, New York. pp. 258-271.

Sanderman J., and J. Baldock. 2010. Accounting for soil carbon sequestration in national inventories: a soil scientist's perspective. Environmental Research Letters 5: 1-6.

Sanford G.R., Posner J.L., Schuler R.T., and J.O. Baldock. 2008. Effect of dairy slurry application on soil compaction and corn Zea mays L.. yield in Southern Wisconsin. Soil & Tillage Research 100: 42-53.

Santruckova H., and M. Simek. 1997. Effect of soil CO2 concentration on microbial biomass. Biology and Fertility of Soils 25: 269-273.

Scharnagl B., Vrugt J.A., Vereecken H., and M. Herbst. 2010. Information content of incubation experiments for inverse estimation of pools in the Rothamsted carbon model: a Bayesian perspective. Biogeosciences 7: 763-776.

Schmidt M.W., Torn M.S., Abiven S., Dittmar T., Guggenberger G., Janssens I.A., Kleber M., Kogel-Knabner I., Lehmann J., Manning D.A.C., Nannipieri P., Rasse D.P., Weiner S., and S. Trumbore. 2011. Persistence of soil organic matter as an ecosystem property. Nature 478: 41-56.

Schwendenmann L., and E. Pendall. 2008. Response of soil organic matter dynamics to conversion from tropical forest to grassland as determined by long-term incubation. Biology and Fertility of Soils 44: 1053-1062.

Silveira M.L., Comerford N.B., Reddy K.R., Cooper W.T., and H. El-Rifai. 2008. Characterization of soil organic carbon pools by acid hydrolysis. Geoderma 144: 405-414.

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

Sollins P., Glassman C., Paul E.A., Swanston C., Lajtha K., Heil J.W., and E.T. Elliott. 1999. Soil Carbon and Nitrogen: Pools and Fraction, in: G. P. Robertson, et al. (Eds.), Standar Soil Methods for Long-Term Ecological Research, Oxford University Press, New York. pp. 89-105.

SPAL. 2004. University of Wisconsin, Soil and Plant Analysis Lab. Particle Size Analysis Hydrometer Method. http://uwlab.soils.wisc.edu/files/procedures/particle_size.pdf (accessed 9 August, 2012).

Steinweg J.M., Plante A.F., Conant R.T., Paul E.A., and D.L. Tanaka. 2008. Patterns of substrate utilization during long-term incubations at different temperatures. Soil Biology & Biochemistry 40: 2722-2728.

Stewart C.E., Paustian K., Conant R.T., Plante A.F., and J. Six. 2009. Soil carbon saturation: Implications for measurable carbon pool dynamics in long-term incubations. Soil Biology & Biochemistry 41: 357-366.

Syswerda S.P., Corbin A.T., Mokma D.L., Kravchenko A.N., and G.P. Robertson. 2011. Agricultural management and soil carbon storage in surface vs. deep layers. Soil Science Society of America Journal 75: 92-101.

Thuries L., Larre-Larrouy M.C., and M. Pansu. 2000. Evaluation of three incubation designs for mineralization kinetics of organic materials in soil. Communications in Soil Science and Plant Analysis 31: 289-304.

Tufekcioglu A., Raich J.W., Isenhart T.M., and R.C. Schultz. 1998. Fine root dynamics, coarse root biomass, root distribution, and soil respiration in a multispecies riparian buffer in Central Iowa, USA. Agroforestry Systems 44: 163-174.

USEPA. 2011. Inventory of US greenhouse gase emissions and sinks: 1990-2009 Executive Summary. http://www.epa.gov/climatechange/Downloads/ghgemissions/US-GHG-Inventory-2011-Executive-Summary.pdf. (accessed, 9 August 2012).

VandenBygaart A.J., and D.A. Angers. 2006. Towards accurate measurements of soil organic carbon stock change in agroecosystems. Canadian Journal of Soil Science 86: 465-471.

Verchot L.V., Dutaur L., Shepherd K.D., and A. Albrecht. 2011. Organi matter stabilization in soil aggregates: Understanding the biogeochemical mechanisms that determine the fate of carbon inputs in soils. Geoderma 161: 182-193.

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

Wagai R., Brye K.R., Gower S.T., Norman J.M., and L.G. Bundy. 1998. Land use and environmental factors influencing soil surface CO2 flux and microbial biomass in natural and managed ecosystems in southern Wisconsin. Soil Biology and Biochemistry 30: 1501-1509.

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

Yoo G., Spomier L.A., and M.M. Wander. 2006. Regulation of carbon mineralization rates by soil structure and water in an agricultural field and a prairie-like soil. Geoderma 135: 16-25.

Table 3.1. Soil physical and chemical parameters for the three independent incubation experiments: VENT, MIX, SIEVE. Numbers in parentheses represent one standard error of the mean.

Exp.	Trt.	Description	n	pH	SOM[1]	sand	silt	clay	texture	P	K	Ca	Mg	CEC
					--------------- (g kg^{-1}) ---------------					----------------------- (mg kg^{-1}) -----------------------				(cmol kg^{-1})
VENT	CV:	continuously vented	3	7.5 (0.0)	29 (0.0)	234	560	206	silt loam	95 (0.6)	184 (0.7)	2281 (31.8)	563 (15.0)	16 (0.6)
	PV:	periodically vented												
MIX	C:	- fan / - vent	3	6.4 (0.0)	37 (0.0)	90	650	250	silt loam	63 (0.7)	165 (3.1)	1731 (174.0)	507 (20.6)	13 (1.2)
	F:	+ fan / - vent												
	V:	- fan / + vent												
	FV:	+ fan / + vent												
SIEVE	I:	intact soil core	3	5.6 (0.1)	37 (0.0)	110	660	230	silt loam	177 (1.0)	185 (1.5)	1835 (20.2)	525 (22.3)	14 (0.0)
	SR:	2mm sieved + roots												
	SNR:	2mm sieved - roots												

[1]SOM = soil organic matter determined by weight loss on ignition

Table 3.2. VENT parameter estimates for the continuously vented (CV) and periodically vented (PV) incubation chambers. Numbers in parenthesis represent one standard error of the mean.

				-------------- C_{rm}[1] --------------			-------------- C_{sm} --------------			---------- C_{nh} ----------		
			SOC	Pool		Field MRT	Pool[2]		Field MRT	Pool[3]		Field MRT[4]
					%			%			%	
Experiment	Treatment	n	g kg⁻¹	g kg⁻¹	SOC	day	g kg⁻¹	SOC	yr	g kg⁻¹	SOC	yr
VENT	CV	5	34.7	0.14 (0.01)	0.4	23 (3)	22.68 (0.01)	65.4	66 (6)	11.87	34.2	2840
	PV	5	34.7	0.06 (0.01)	0.2	8 (1)	22.75 (0.01)	65.6	70 (12)	11.87	34.2	2840
	p-value			*0.0005*	*--*	*0.0025*	*0.0005*	*--*	*0.7675*	*--*	*--*	*--*

[1] C_{rm} = readily mineralized carbon, C_{sm} = slowly mineralized carbon, and C_{nh} = non-hydrolyzable carbon.
[2] C_{sm} pool size = SOC-C_{rm}-C_{nh}
[3] C_{nh} pool estimated via 6N HCl hydrolysis
[4] MRT was set at 2840 yr based on ^{14}C dates for SOC reported in Paul *et al.* (2001a).

Table 3.3. MIX parameter estimates for the -fan/-vent (C), +fan/-vent (F), and –fan/+vent (V) chambers. Numbers in parenthesis represent one standard error of the mean.

				-------------- C_{rm}[1] --------------			--------------- C_{sm} ---------------			---------- C_{nh} ----------		
			SOC	Pool		Field MRT	Pool[2]		Field MRT	Pool[3]		Field MRT[4]
Experiment	Treatment	n	g kg^{-1}	g kg^{-1}	% SOC	day	g kg^{-1}	% SOC	yr	g kg^{-1}	% SOC	yr
MIX	C	4	19.8	0.74 (0.02)	3.7	24	8.89 (0.02)	44.9	5.1	10.17	51.4	2840
	F	4	19.8	0.63 (0.06)	3.2	21	9.00 (0.06)	45.5	6.7	10.17	51.4	2840
	V	4	19.8	0.35 (0.02)	1.8	10	9.28 (0.02)	46.9	8.8	10.17	51.4	2840
	p-value			0.0004	--	<0.0001	0.0004	--	<0.0001	--	--	--
	LSD (α=0.05)			0.112	--	4	0.112	--	0.7	--	--	--

[1] C_{rm} = readily mineralized carbon, C_{sm} = slowly mineralized carbon, and C_{nh} = non-hydrolyzable carbon.
[2] C_{sm} pool size = SOC-C_{rm}-C_{nh}
[3] C_{nh} pool estimated via 6N HCl hydrolysis
[4] MRT was set at 2840 yr based on ^{14}C dates for SOC reported in Paul *et al.* (2001a).

Table 3.4. SIEVE parameter estimates for the Intact (I), sieved with roots (SR), and sieved with no roots (SNR) chambers. Numbers in parenthesis represent one standard error of the mean.

Experiment	Treatment	n	SOC $g\ kg^{-1}$	C_{rm}[1] Pool $g\ kg^{-1}$	C_{rm} Pool % SOC	C_{rm} Field MRT day	C_{sm} Pool[2] $g\ kg^{-1}$	C_{sm} Pool % SOC	C_{sm} Field MRT yr	C_{nh} Pool[3] $g\ kg^{-1}$	C_{nh} Pool % SOC	C_{nh} Field MRT[4] yr
	I	5	23.5	0.07 (0.01)	0.3	30	13.25 (0.01)	56.4	13	10.18	43.3	2840
MIX	**SR**	5	23.5	0.06 (0.01)	0.3	11	13.27 (0.01)	56.4	10	10.18	43.3	2840
	SNR	5	23.5	0.07 (0.01)	0.3	10	13.26 (0.01)	56.4	8	10.18	43.3	2840
	p-value			*0.63*	*--*	*0.0146*	*0.64*	*--*	*0.11*	*--*	*--*	*--*
	LSD (α=0.05)			*0.03*	*--*	*14*	*0.03*	*--*	*5*	*--*	*--*	*--*

[1] C_{rm} = readily mineralized carbon, C_{sm} = slowly mineralized carbon, and C_{nh} = non-hydrolyzable carbon.
[2] C_{sm} pool size = SOC-C_{rm}-C_{nh}
[3] C_{nh} pool estimated via 6N HCl hydrolysis
[4] MRT was set at 2840 yr based on ^{14}C dates for SOC reported in Paul *et al.* (2001a).

Figure 3.1. A) Internal CO_2 concentration in the VENT experiment during long term soil incubations for the continuously vented (CV) and periodically vented (PV) chambers, and B) estimation of CO_2 flux over time from both direct IRGA readings (direct) and for CO_2 accumulation between time points (by difference). Error bars represent one standard error of the mean (n=5 per day and treatment).
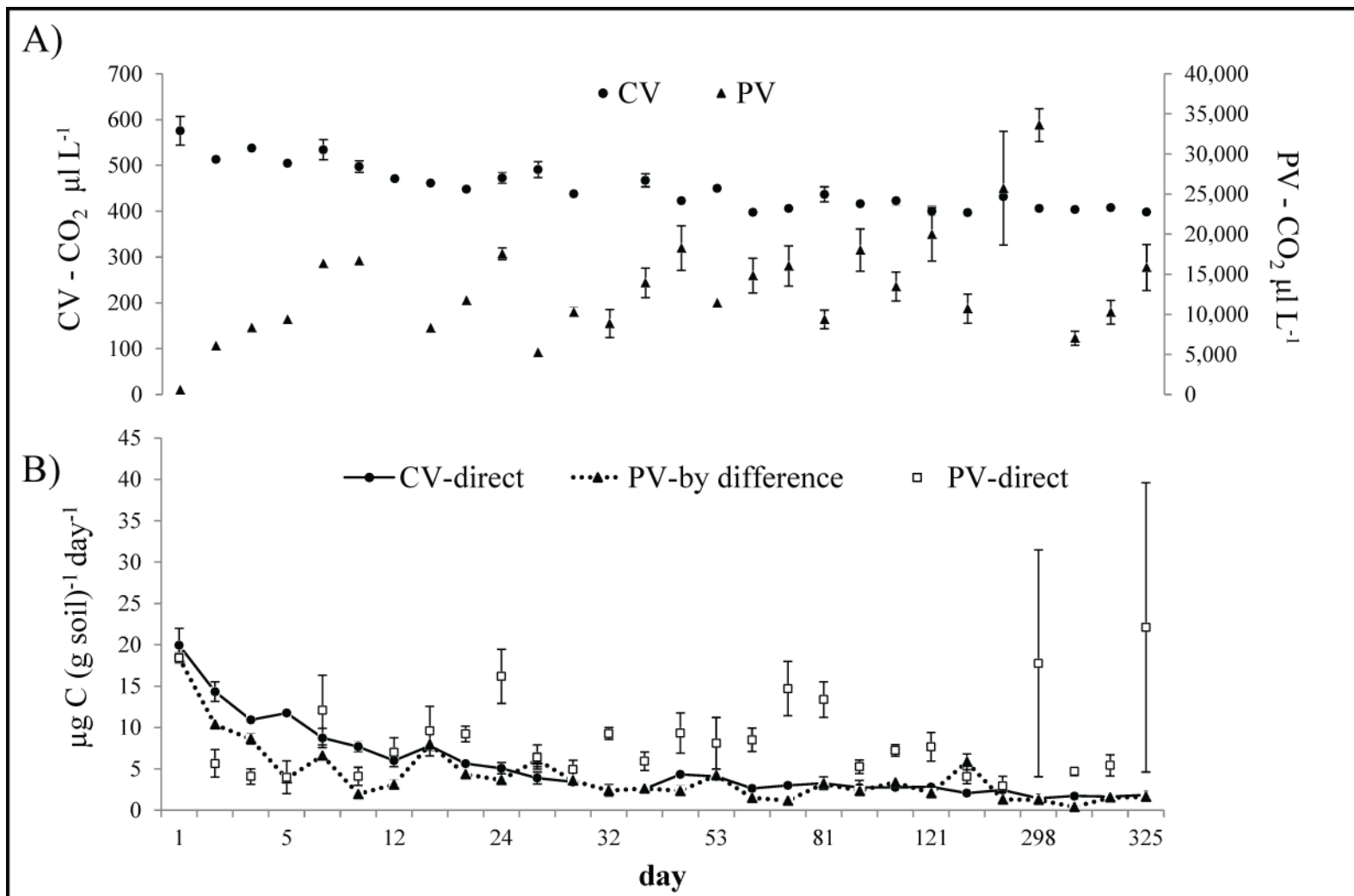
Figure 3.2. CO_2 respiration data (VENT) for the continuously vented (CV) and periodically vented (PV) incubation chambers. Solid and dashed lines represent significantly different ($p < 0.0001$) non-linear regression models.

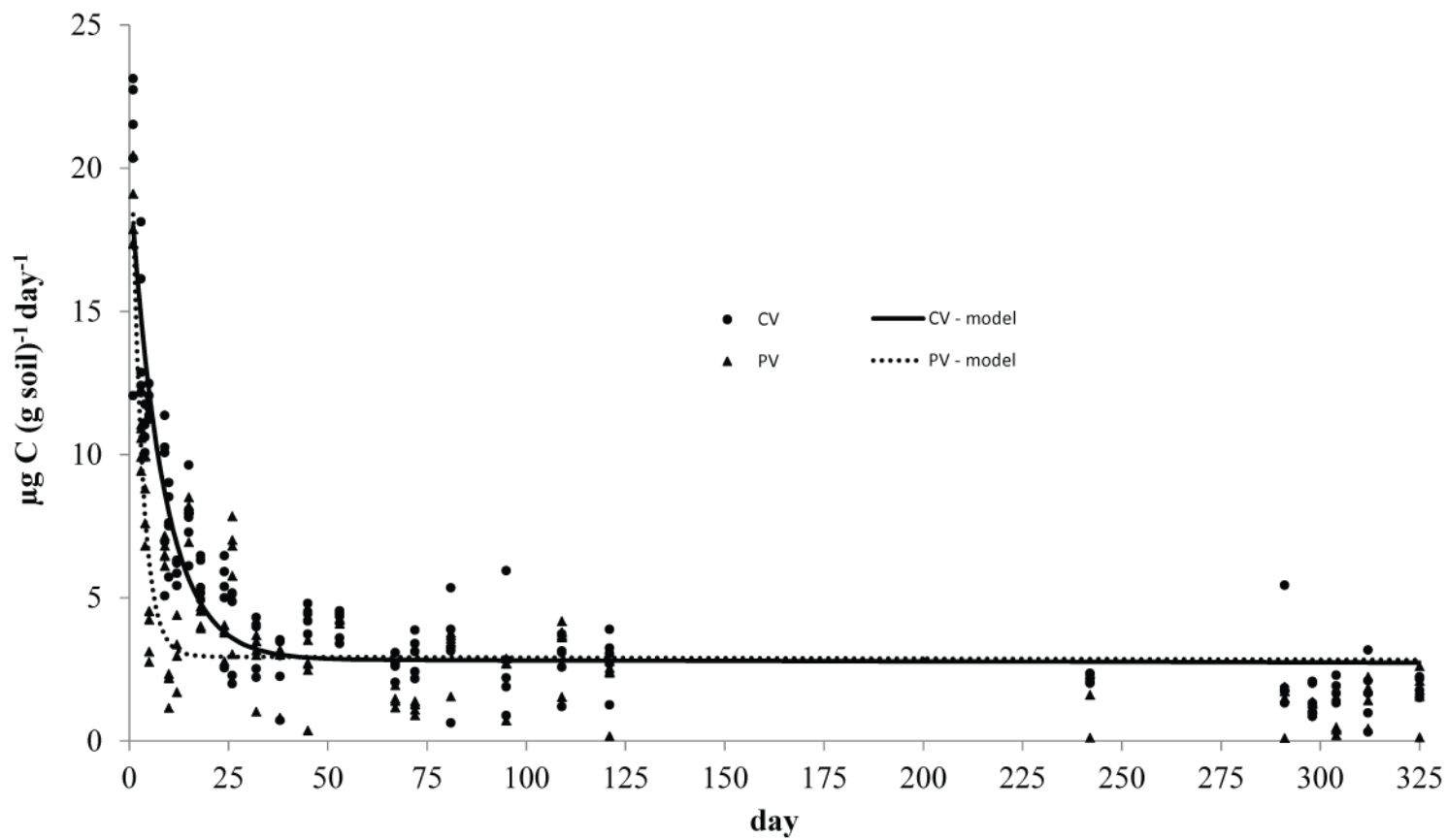Figure 3.3. Pre- and Post- venting CO_2 flux in the VENT experiment showing the low pre-vent flux rates (y axis on left) compared to the excessively high post-vent flux rates (y axis on right). Differing letters within a given day represent the Bonferroni corrected significance at $\alpha = 0.05$. Bars represent one standard error of the mean.
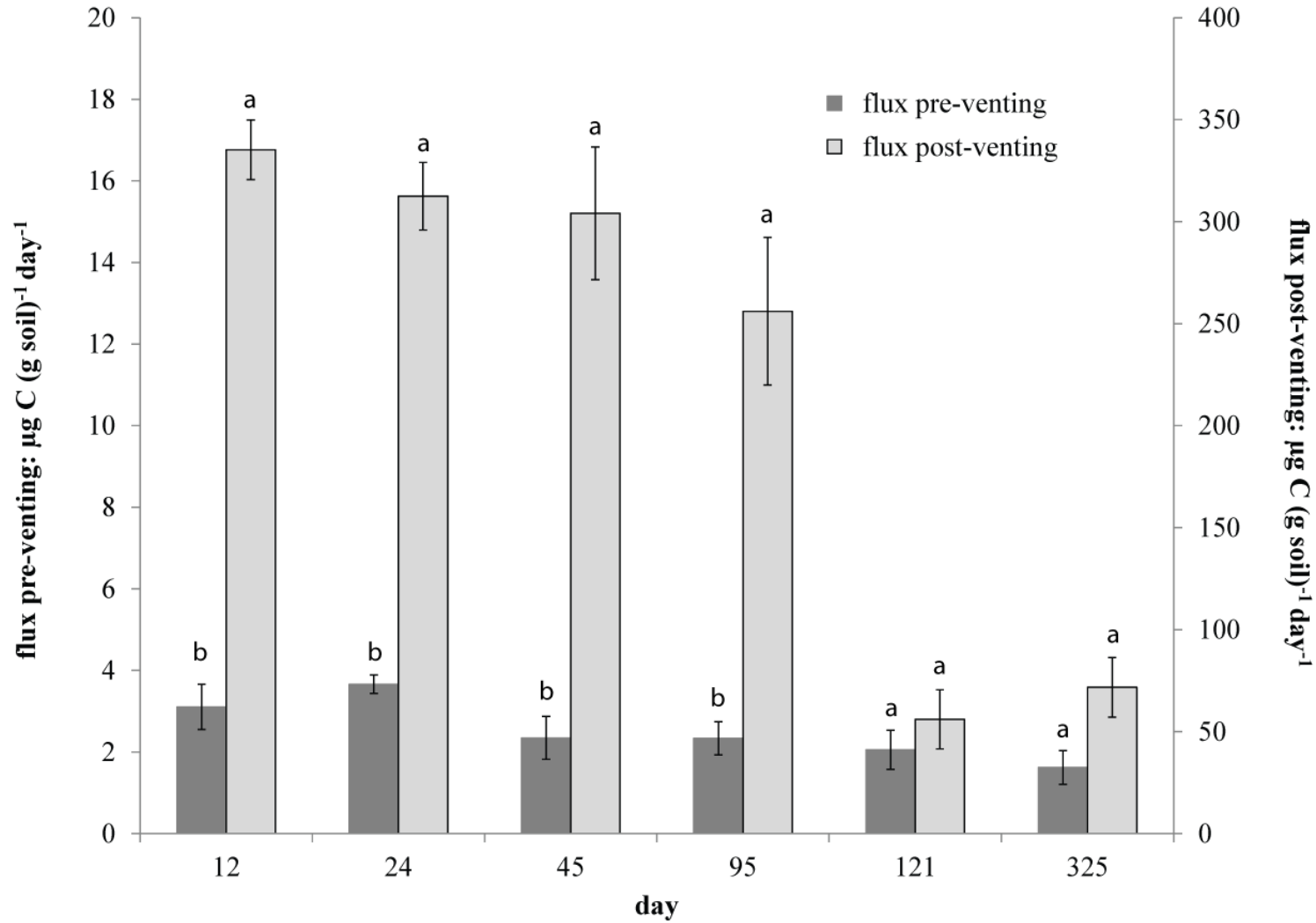
Figure 3.4. CO_2 respiration data (MIX) for the -fan/-vent (C), +fan/-vent (F), and –fan/+vent (V) treatments. Solid and dashed lines represent significantly different ($p < 0.0001$) non-linear regression models.
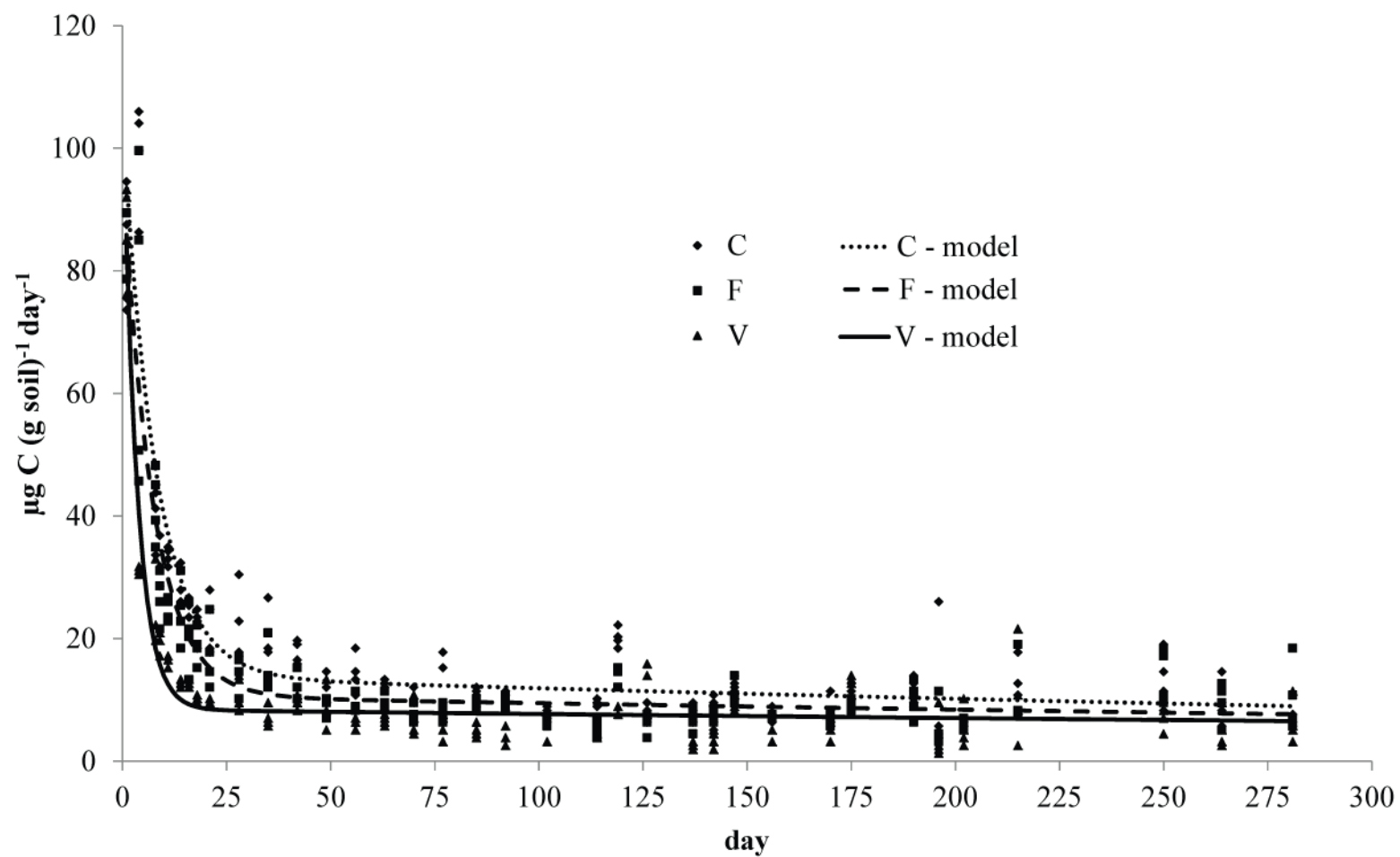
Figure 3.5. CO_2 concentration data (MIX) for the -fan/-vent (C), +fan/-vent (F), and –fan/+vent (V) treatments. Bars represent 1 standard error of the mean. Letters to the right of graph bars represent treatment level (3 heights combined), Bonferroni corrected significance at $\alpha = 0.05$.
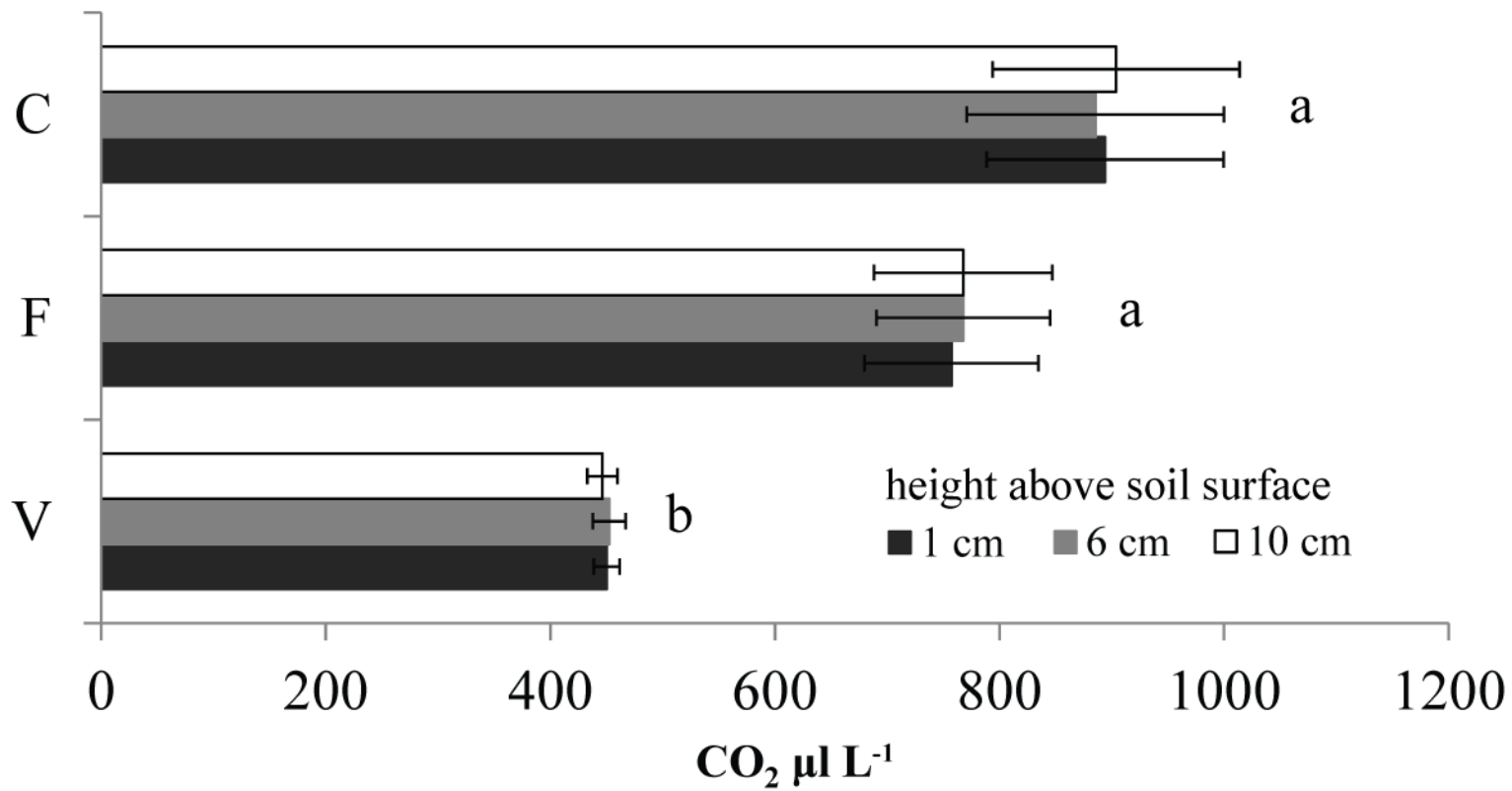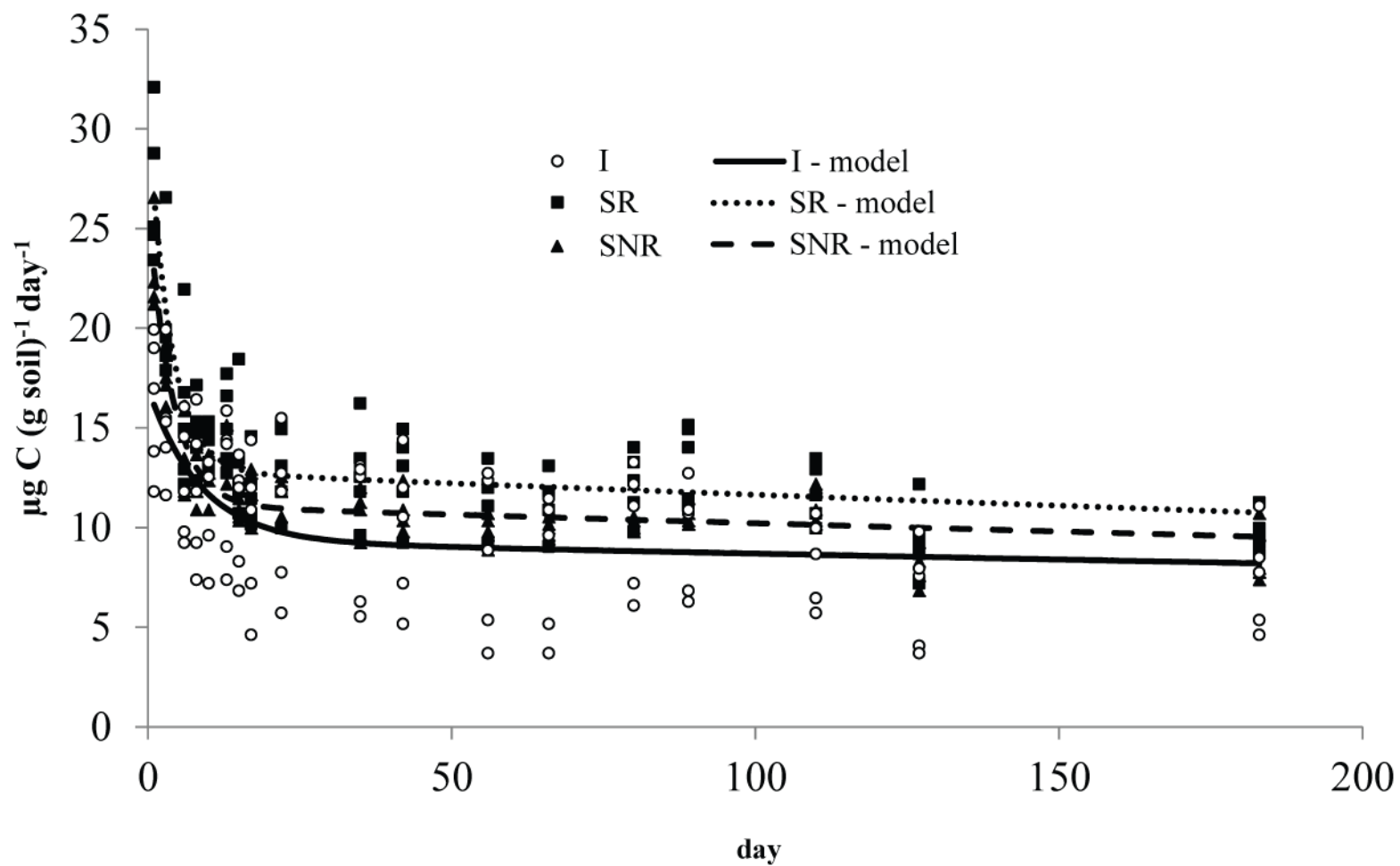
Figure 3.6. CO_2 respiration data (SIEVE) for the intact (I), sieved with roots (SR), and sieved with no roots (SNR) treatments. Solid and dashed lines represent significantly different ($p < 0.0001$) non-linear regression models.

CHAPTER 4

GENERAL CONCLUSIONS

4.1 Introduction

Soil organic carbon is a valuable resource, not only for the men and women that rely on the land for their livelihood but also for society at large who, whether knowingly or unknowingly, reap the benefits associated with healthy, robust, and sustainable agricultural landscapes. The consequences of unsustainable soil management have been devastating to both ancient and contemporary culture; in some instances leading to the complete collapse of civilizations (Lowdermilk, 1953). As recently as the 1930s, severe drought, coupled with intensive, and unsustainable farming practices led to the exodus of hundreds of thousands of people from their homes in Texas and Oklahoma as 40.5 million hectares of land in the central United States was devastated by large scale wind erosion (Worster, 2012). In the 21st Century, we find ourselves once more at a point where climate and soils intersect, in this instance with climate change working to destabilize societies, and soils management at the forefront of the multi-tactic solution to this daunting global problem (Lal, 2004).

Soils interact with the global climate by both emitting CO_2 as SOC is mineralized and by sequestering atmospheric C as plants allocate biologically fixed carbon to root structures which ultimately senesce and enter the SOM continuum (Lal, 2008). Many of the world's agricultural soils contain far less SOC than they once did prior to conversion from native systems (Collins et al., 1999). Therefore, it may be hypothetically possible to rebuild SOC stocks to past levels through appropriate agricultural best management practices (Conant et al., 2007).

Many practices are touted as a means to sequester SOC. These practices include no-till farming and other conservation tillage practices (e.g. strip till, ridge till), application of manure and municipal bio-solids, cultivation of perennial crops, inclusion of cover crops, lengthening

and diversifying crop rotations, and even applying synthetic N fertilizers (Huggins et al., 1998; Nafziger and Dunker, 2011; Ogle et al., 2005). Many, though not all, such practices are supported by studies that have compared land use and land cover treatments side by side at a certain point after the initiation of a field trial (Ogle et al., 2005; Sanderman et al., 2010). While results from such studies shed light on the relative impacts of agricultural management, they cannot, without taking into account historic SOC levels, provide solid evidence about whether or not such practices have indeed led to SOC sequestration. It is troubling, therefore, to consider that less than 50% of the studies in major reviews of SOC stock changes have actually followed a change in management through time (Sanderman and Baldock, 2010).

Data generated by the scientific community on agricultural practices and SOC sequestration is used by both the public and private sector, as well as by other scientists. These data influence decisions about the design of cap and trade systems for CO_2 and how and for what practices farmers might be compensated. It also serves to provide input parameters for biogeochemical modeling efforts directed at studying land use and global climate change. The lack of scientific studies that have accounted for changes in SOC over time leads one to question the validity of policies intended to balance CO_2 emissions by paying farmers for conservation practices when it is not clear whether or not such practices lead to a sequestration of atmospheric C. Biogeochemical models may also suffer if assumptions about conservation agricultural practices do not hold.

Without an understanding of how different pools of SOC react to soil mineralogy, temperature, moisture and land management, it will not be possible to develop a robust model of SOC stabilization and longevity.   In fact, many of the apparent discrepancies regarding estimates of SOC sequestration and loss found in the literature may be atributed to a "one size

fits all" approach to managing our SOC resources. While NT grain production might foster SOC sequestration on one soil in one region, such management may result in significant loses of SOC in another. Methods that enable us to go beyond studying total SOC are of critical importance to our understanding of SOC dynamics.

4.2 Total SOC

The total SOC work conducted at the Wisconsin Integrated Cropping Systems Trial highlighted the importance of accurate C accounting when drawing conclusions about the sequestration potential of best management practices. Results from this study showed a clear loss of SOC over the 20-year period between 1989 and 2009, irrespective of management. While NT, perennial, and grass-based systems all slowed the loss of SOC, none of the systems stabilized C throughout the soil profile (0 to 90 cm). The losses at WICST likely stem from a combination of factors including a very high background level of SOC, conversion from perennial forage systems in 1989, a lack of belowground carbon inputs, and possibly a link to the warming Wisconsin climate. Because we used only two points in time (1989 and 2009), it is not possible to determine whether the soils are on a continued negative trajectory relative to SOC or whether there was a large drop in SOC following conversion from perennial dairy systems followed by a stabilization of SOC levels. A continued monitoring of the WICST systems on a 5 to 10 – year cycle should improve our understanding of these systems and their impact on SOC dynamics.

4.3 SOC Pools

The work on SOC pools and their dynamics was interesting and somewhat surprising. In this study SOC dynamics in seemingly different production agricultural systems were not significantly different from one another, while SOC dynamics in the perennial grass systems were. The method was highly sensitive to small differences in land management, generally supporting the idea that grass systems build labile pools of carbon relatively rapidly while production agricultural systems tend to deplete such pools in favor of those that are less accessible to microbial degradation and loss. It is increasingly clear that stabilization of SOC is an issue of accessibility and less one of chemical recalcitrance (Schmidt et al., 2011). Grass systems are more likely to lead to the long term accumulation of stable carbon in these soils for two main reasons. First, grass based systems produce belowground biomass (roots and root exudates) sufficient to outpace the microbial community's ability to degrade these residues, which would result in substantial loss of SOC as CO_2. This balance favors the eventual interaction of plant derived C with soil mineral surfaces where it can be trapped in macro- and micro- aggregates or adsorbed – all of which would favor long term stability. Second, perennial grass roots are morphologically quite different from those of many crop plants in that there is a preponderance of fine roots with innumerable root hairs. The large surface area associated with fine root biomass increases the interaction of both fine roots and root hairs with soil micro-pores and micro-aggregates. Penetration of root hairs into such micro sites, where anoxic conditions prevail and that are otherwise inaccessible to microbial decomposers, preferentially stabilizes these stocks of root derived C (Rasse et al., 2005).

This study also highlighted the potential sensitivity of grassland systems to future disturbance. Of particular concern is the large scale conversion of Conservation Reserve

Program (CRP) lands back to row-crop agriculture. It is very likely that such land has, in the past 30 years, accumulated labile stores of SOC (at least in the top 30 cm), which may be lost quite rapidly, negating the C gain realized under the program.

4.4 Methodological consideration

Because the SOC parameters estimated via the AHI method are incorporated into many biogeochemical models it is important that the technique is executed in a way that minimizes laboratory artifact. The results from this study demonstrated the strong effect that CO_2 concentration gradient has on CO_2 efflux measured in vitro. Depending on how flux is measured, periodically vented chambers resulted in a substantially suppressed flux (flux measured before venting) or a substantially exaggerated flux (flux measured post venting). The continuously vented chamber provided a more consistent measure of CO_2 flux and did so without sacrificing ideal soil moisture levels. It is important to recognize that these differences may not be important if one is interested in simply comparing treatment effects. If results are to be compared between studies, or if AHI parameter estimates are to be used for modeling purposes, these findings are critically important. A standardized protocol should be established.

4.5 Follow-up studies and future research

Given the results from the current study it is clear that there are many important questions about SOC dynamics and stabilization which require further investigation. In addition to continuing the 5 and 10-year monitoring of the WICST systems it would be interesting to couple results from the AHI method with results observed between 1989 and 2009 at WICST. Of particular interest is the observation at WICST that while SOC accumulated in the 0 to 30 – cm

horizon of the pasture system, there was a negative, although non-significant, trend below 30 cm. The AHI method might be employed using the pasture system and prairie systems at WICST to look at whether SOC dynamics differ at depth between C3 grass pasture and C4 grasslands. Results from such a study would shed further light on whether SOC sequestration as a result of improved land management is a reasonable expectation in the C rich Mollisols of southern Wisconsin.

The results obtained from chapters 2 and 3 demonstrated how sensitive the coupling of acid hydrolysis and long term incubation was to both land management and methodological execution. As one of the important utilities of the AHI method is the parameterization of biogeochemical models it would be a valuable exercise to evaluate whether parameter estimates from periodically- or continuously-vented chambers resulted in more accurate predictions of CO_2 flux when compared against field measurements. Results from such a study would be essential in the development of a standardized protocol for the AHI method.

One additional and equally important modeling study would be to parameterize AgroIBIS or DAYCENT with parameter estimates for the five distinct agroecosystem groupings identified in chapter 2. It would be important to determine whether model CO_2 flux rates in these systems matched those measured in the field and if such fine resolution is required or if a simpler approach (grasslands and ag-land) would suffice. The utility of AHI for predictive modeling requires additional scrutiny before its widespread use is promoted.

One of the most important areas for future SOC research involves developing robust mechanistic and quantitative models to explain SOC dynamics. Current models are based primarily on empirical data, and as a result there is no guarantee that their predictive capacities will hold in light of a changing climate. Techniques that should facilitate building a more

mechanistic model include solid state NMR, ^{13}C and ^{14}C radioisotope dating, physical SOC fractionation, long term soil-incubations, and methods to determine the type, quantity, and functionality of the soil microbial community. In general chemical fractionation techniques that rely on strong acids or bases should be avoided as the data obtained from such methods will likely reflect the laboratory method as much or more than in situ SOC reality. Coupling long-term soil incubations with periodic destructive sampling for NMR and microbial analysis would greatly improve our understanding of the forms of SOC that are preferentially oxidized and what microbial communities shifts occur as substrate quantity and quality changes. Irrespective of the specifics, future SOC research needs to move away from two pernicious assumptions: 1) that we can treat SOC as a black box and assume that there is enough substrate and microbial redundancy that all soils will respond similarly to environmental and management induced stresses, and 2) that SOC stabilization is a result of recalcitrance imparted by the chemical complexity of a substrate.

4.6 References

Collins H.P., Blevins R.L., Bundy L.G., Christenson D.R., Dick W.A., Huggins D.R., and E.A. Paul. 1999. Soil carbon dynamics in corn-based agroecosystems: Results from carbon-13 natural abundance. Soil Science Society of America Journal 63: 584-591.

Conant R.T., Easter M., Paustian K., Swan A., and S. Williams. 2007. Impacts of periodic tillage on soil C stocks: A synthesis. Soil and Tillage Research 95: 1-10.

Huggins D.R., Buyanovsky G.A., Wagner G.H., Brown J.R., Darmody R.G., Peck T.R., Lesoing G.W., Vanotti M.B., and L.G. Bundy. 1998. Soil organic C in the tallgrass prairie-derived region of the corn belt: effects of long-term crop management. Soil & Tillage Research 47: 219-234.

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

Lal R. 2008. Carbon Sequestration. Philosophical Transactions of the Royal Society 363: 815-830.

Lowdermilk W.C. 1953. Conquest of the land through 7000 years. Agriculture Information Bulletin 99, in: NRCS (Ed.), Washington, D.C. http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb1043789.pdf. (accessed, 9 August, 2012).

Nafziger E.D., and R.E. Dunker. 2011. Soil Organic Carbon Trends Over 100 Years in the Morrow Plots. Agronomy Journal 103: 261-267.

Ogle S.M., Breidt F.J., and K. Paustian. 2005. Agricultural management impacts on soil organic carbon storage under moist and dry climatic conditions of temperate and tropical regions. Biogeochemistry 72: 87-121.

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

Sanderman J., and J. Baldock. 2010. Accounting for soil carbon sequestration in national inventories: a soil scientist's perspective. Environmental Research Letters 5: 1-6.

Sanderman J., Farquharson R., and J.A. Baldock. 2010. Soil carbon sequestration potential: a review of Australian agriculture, in: C. L. W. Report (Ed.), www.csiro.au/resources/Soil-Carbon-Sequestration-Potential-Report.html. (accessed, 9 August, 2012).

Schmidt M.W., Torn M.S., Abiven S., Dittmar T., Guggenberger G., Janssens I.A., Kleber M., Kogel-Knabner I., Lehmann J., Manning D.A.C., Nannipieri P., Rasse D.P., Weiner S., and S. Trumbore. 2011. Persistence of soil organic matter as an ecosystem property. Nature 478: 41-56.

Worster D. 2012. Dust bowl. Handbook of Texas Online. Texas State Historical Society. http://www.tshaonline.org/handbook/online/articles/ydd01. (accessed, 9 August, 2012).