

UNDERSTANDING CARBON SEQUESTRATION IN NORTH CENTRAL
MONTANA DRYLAND WHEAT SYSTEMS

by

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of the requirements for the degree

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in

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DEDICATION

I would like to take this opportunity to dedicate this work to my family and the loving memory of my brother. Without the love and support of my family, none of my educational conquests would have been possible. For their love and support I will be eternally grateful.

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ABSTRACT

Agricultural management practices that reduce tillage and/or increase crop intensity have been shown to promote soil carbon sequestration in many regions of the Great Plains. Comparatively little information is available on the impact of these practices on soil organic carbon (SOC) in Montana's semi-arid climate. The objective of this research was to measure rates of change in SOC in cropland for north central Montana's Golden Triangle related to conversion of crop-fallow to annual cropping, conversion to no-till management, and the implementation of both simultaneously. A second objective was to measure differences in soil microbial biomass carbon (SMBC) as an "early indicator" for soil carbon accrual after six years of management. Field experiments were established at six farm sites in fall 2002. Soil organic C was not affected by the treatments at three of the six sites after six years (2002–2008). Three of the six sites had soil carbon accrual associated with annual cropping ranging from 0.19 to 0.53 Mg ha⁻¹ yr⁻¹. Only one site showed soil carbon accrual associated with no-till management, accruing 0.26 Mg ha⁻¹ yr⁻¹. It proved unreliable to make quantitative comparisons for samples from different collection times using SMBC because stored soil samples had diminished SMBC correlated with months in storage, making it impossible to compare accurately freshly obtained SMBC with earlier baseline values from stored soil samples. It was concluded that annual cropping is likely to increase SOC in many instances; however a longer study period may be required to understand SOC response to soil management in this region.

1. REVIEW OF LITERATURE

The Global Carbon Cycle

The global carbon cycle consists of a short term biogeochemical cycle superimposed on a long term geochemical cycle (Berner, 1998, Govaerts, et al., 2009) operating on time scales which vary from minutes to millions of years (Berner, 1990). The long term carbon cycle occurs over a time-scale lasting millions of years and can be described by the pathways depicted in Figure 1.

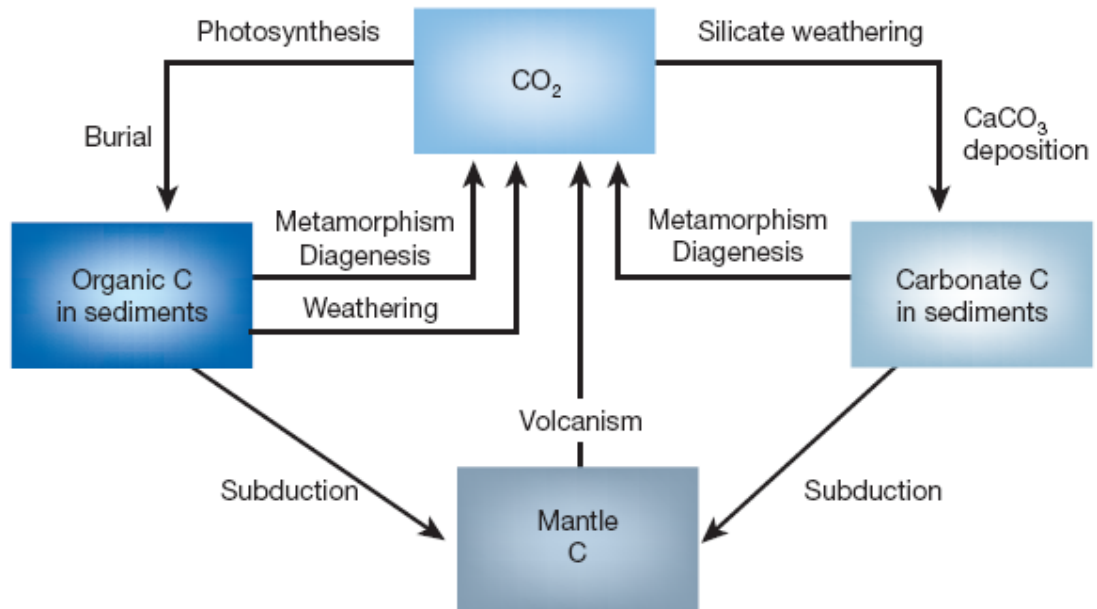


Figure 1. Long Term Carbon Cycle (Berner, 2003)

Carbon can be tracked through the right half of the pathway of the long term geochemical carbon cycle as mantle carbon is released as dioxide gas from deep within

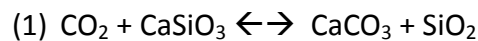
the earth through the natural process of volcanic eruptions. As the carbon dioxide gas collects in the atmosphere, silicates are exposed to the weathering forces of acidic rain. This process slowly dissolves inorganic carbon held tightly in mineral form, allowing it to eventually flow back towards the earth's oceans. Once dissolved in seawater, calcium carbonate (CaCO_3) is converted into carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) (Berner, 1987, Morse, and Mackenzie, 1998, Falkowski, et al., 2000). The cycle continues as certain marine organisms then fix the bicarbonate with calcium to once again create calcium carbonate (CaCO_3) in a biologic process to create their skeletal structures (Skulan, et al., 1997). These organisms eventually die and their bicarbonate skeletons or exoskeletons slowly make their way to the ocean floor (Falkowski, et al., 2000) where, over hundreds of thousands of years, they collect along with other sea deposits to eventually form calcareous sedimentary bedrocks (Morse, and Mackenzie, 1998). As the continental plates slowly shift over time, these sedimentary rocks are either pushed down toward the earth's core to later be returned to the surface as magma or forced upward and out of the seas to form land. Once the carbonate-rich rock is exposed to the forces of weathering, the mountain or magma slowly succumbs to the forces of nature and the rocks are weathered releasing soil sized particles which can be transported by wind and water. Once the weathering process has begun, the calcium carbonate may be again leached out of the soil and either be transported back to the oceans to continue the cycle or seep deep within the earth's crust to eventually be released from volcanoes as CO_2 or pushed back to the surface as solidified deposits as the continental plates

continue to move and new mountains are created. If not leached from the soil, the calcium carbonate can remain in the soil where it can react with acids. The product of the reaction between calcium carbonate and organic acids will result in CO_2 being released back to the atmosphere. This part of the cycle is largely controlled by time and operates on a timeline of millions or billions of years. Because of the extremely slow nature of this carbonate pathway, human interaction has had little direct effect to date on the overall cycling of carbon in this part of the slow geochemical cycle.

The left pathway in the long term geochemical cycle again starts out as CO_2 gas spewed from volcanoes and collected in the atmosphere. As the gas collects in the atmosphere a plethora of aquatic plants, algae, and bacteria fix the CO_2 gas with energy from the sun to create biomass. As these organisms die and sink below the water's surface they are buried by sediments further preventing oxygen from reaching the decaying organic matter. Over millions of years, the combination of anaerobic decay, heat from the earth's core and increasing pressure from the millions of years of continued sedimentary deposits forces a variety of liquid organic carbon compounds (hydrocarbons) from the organic matter where they seep out and collect in the surrounding bedrocks. As the continental plates continue to shift these organic carbon compounds can be pushed back to the surface in the form of oil seeps or coal outcroppings. Oil seeps and coal outcroppings are places where a combination of solid, liquid, and gaseous hydrocarbons spew from the earth and are exposed to oxygen. Once

in an aerobic environment the hydrocarbon-rich organic carbon sediments begin to break down as they are oxidized to eventually form CO₂ again.

The long term geochemical cycle can be summarized by the following generalized reactions (Berner, 1998, Berner, 2003):



Reaction (1) in the forward direction summarizes the process of atmospheric carbon dioxide as terrestrial calcium silicates are weathered, with the products of this reaction being the precipitation of calcium carbonates in marine sediments. The same equation in reverse describes the burial and decomposition of calcium carbonates, with the products of this reaction being carbon dioxide released back to the atmosphere. In the forward direction, reaction (2) represents uptake of CO₂ by photosynthesis, production of organic matter (CH₂O), and subsequent burial. The eventual product of this reaction is the hydrocarbon compounds formed. The reverse of reaction (2) follows the weathering and oxidation of organic matter, with the product of this reaction being the CO₂ produced (Berner, 2003). The short-term carbon cycle includes daily carbon cycling that occurs in the soil with the cumulative effects evidenced over a time-scale of hundreds to thousands of years as soils are created (Berner, 1998, Lal, 2003). Via photosynthesis, plants utilize energy from the sun to convert CO₂ in the atmosphere into carbohydrates that form the above- and below-ground (root) plant biomass. As

plant biomass undergoes decomposition by microbes and humification, it is converted into more recalcitrant carbon structures that create the soil organic carbon (SOC) pool, and which lead to the genesis of soil horizons or profiles. An overview of this cycle can be seen in Figure 2. The SOC pool once created can be lost to the atmosphere as CO₂, or transported in the environment by erosion (wind, water) or leaching of dissolved organic carbon (DOC).

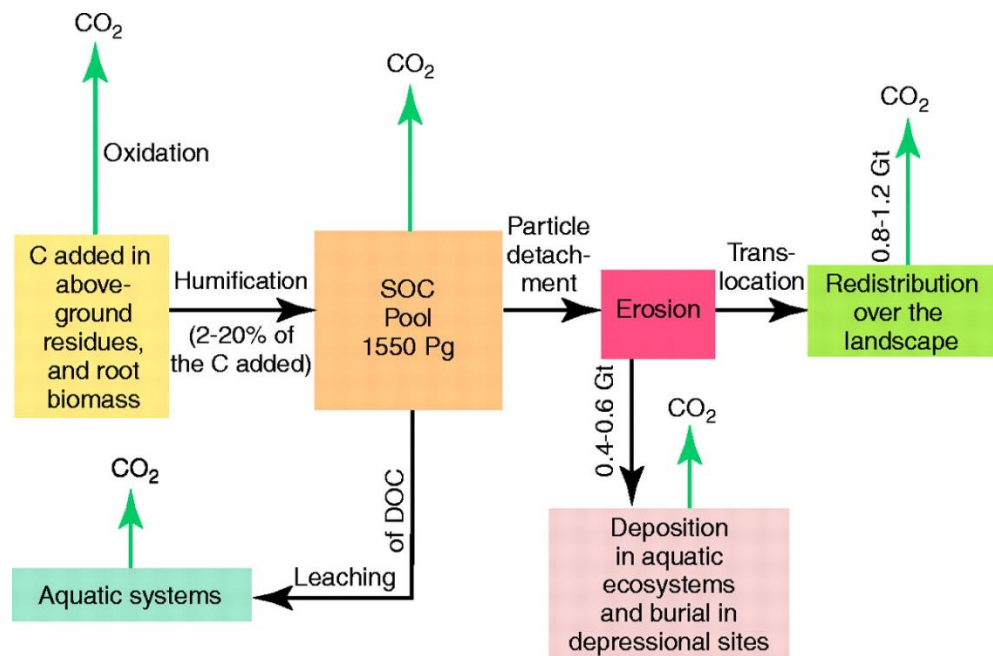


Figure 2. Short Term Carbon Cycle (Lal, 2004)

The short-term carbon cycle can be greatly impacted by human disturbances to the soil (Bricklemyer, et al., 2006). This cycle can respond quite rapidly to land management practices and often, disruptions in the cycle result in negative effects on soil quality. These deleterious effects can be detected by assessing parameters such as

soil structure (Lal, 2009a), aggregate stability (Tisdall, and Oades, 1982, Benjamin, et al., 2008), bulk density (Gupta, and Larson, 1979, Benjamin, et al., 2008), and hydraulic conductivity (Bowman, et al., 1990, Lado, et al., 2004, Benjamin, et al., 2008), as well as the retention of both water and nutrients (Vitousek, and Reiners, 1975, Gupta, and Larson, 1979, Pierzynski, et al., 2005, Lal, 2009a). Because of the sensitivity and importance of this cycle as illustrated by the number of soil quality parameters affected by it, attempting to understand this short term biogeochemical carbon cycle is of great importance.

Carbon Pools

Carbon pools are large reservoirs of carbon found throughout the global carbon cycle. For both the long-term and short-term carbon cycles, there are fluxes of carbon among pools. These pools can be further defined as either possessing the qualities of a sink, or a source, of carbon based on carbon gain or loss under any given timeframe. If a pool has a larger input of carbon than it loses, then the pool is defined as a carbon sink. If the pool has greater losses than inputs, the carbon pool is defined as a carbon source. The entire global carbon cycle was first generalized as consisting of fossil, atmospheric, oceanic, and terrestrial biosphere carbon pools (Schimel, 1995). Recently, in an effort to better track fluxes among pools, the global carbon cycle has been divided into five pools including oceanic, geologic, soil, biotic, and atmospheric (Lal, 2003). The amount of carbon varies from pool to pool and estimates of the various pools appear in Table 1.

Table 1. Estimates of Carbon Pools (Lal, 2003, Lado, et al., 2004, Lal, 2004).

Oceanic	38,000 Pg
Geologic	5,000 Pg
Soil (1m deep)	2,500 Pg (SOC 1550 Pg, SIC 950 Pg)
Biotic	560 Pg
Atmospheric	760 Pg

Carbon Inputs

When looking at the long term geochemical carbon cycle, increases in actively circulating carbon for both pathways are typically the result of volcanoes periodically releasing large amounts of CO₂ into the atmosphere. A lesser natural input to the second pathway of the long-term carbon cycle, the formation of hydrocarbon substances, is the result of hydrocarbons periodically making their way back to the earth's surface as seeps. At these sites a combination of solid, liquid, and gaseous hydrocarbons are released to the environment from deep within the earth. Once the compounds are in the presence of oxygen, they can begin to be oxidized to CO₂ that returns to the atmosphere. However, as the atmospheric CO₂ concentrations increase, the ocean waters absorb more of the gas and the aquatic plants, algae, and bacteria fix the excess CO₂. Eventually a new CO₂ concentration equilibrium is reached which will typically be greater than pre-release values. Inputs to this cycle have historically been controlled by the movement of the continental plates. Since this cycle is a true global cycle there are very few true additions to the system; rather a dynamic imbalance between release and storage rates. In the short-term biogeochemical carbon cycle,

carbon inputs to a soil system can be either the result of natural occurrences or land-management strategies. In addition, the origination source of the carbon inputs to this cycle can be either *in situ* or *ex situ*. In a natural, undisturbed system, as plants grow and convert CO₂ gas into biomass, the soils become more carbon rich with the decomposing biomass with time. Over the course of hundreds and thousands of years, carbon in various forms of decay slowly accumulates annually in the soil faster than it is lost. This type of carbon input is an example of a natural *in situ* carbon input to a soil. Carbon inputs to a soil can also result from *ex situ* sources and be translocated by nature or man. A common example of a natural *ex situ* carbon input can be found by looking at the carbon rich soil often found along rivers and streams prone to periodic bank overruns. In this example carbon in the form of organic matter is carried to a downstream location by water, along with soil particles. The water begins to slow and the organic matter is deposited along with small soil particles as the water retreats back to the river banks.

From a management perspective, there are a number of management practices which will result in increased carbon inputs resulting in enhanced soil organic matter. Many of these management strategies are outlined in Figure 3.

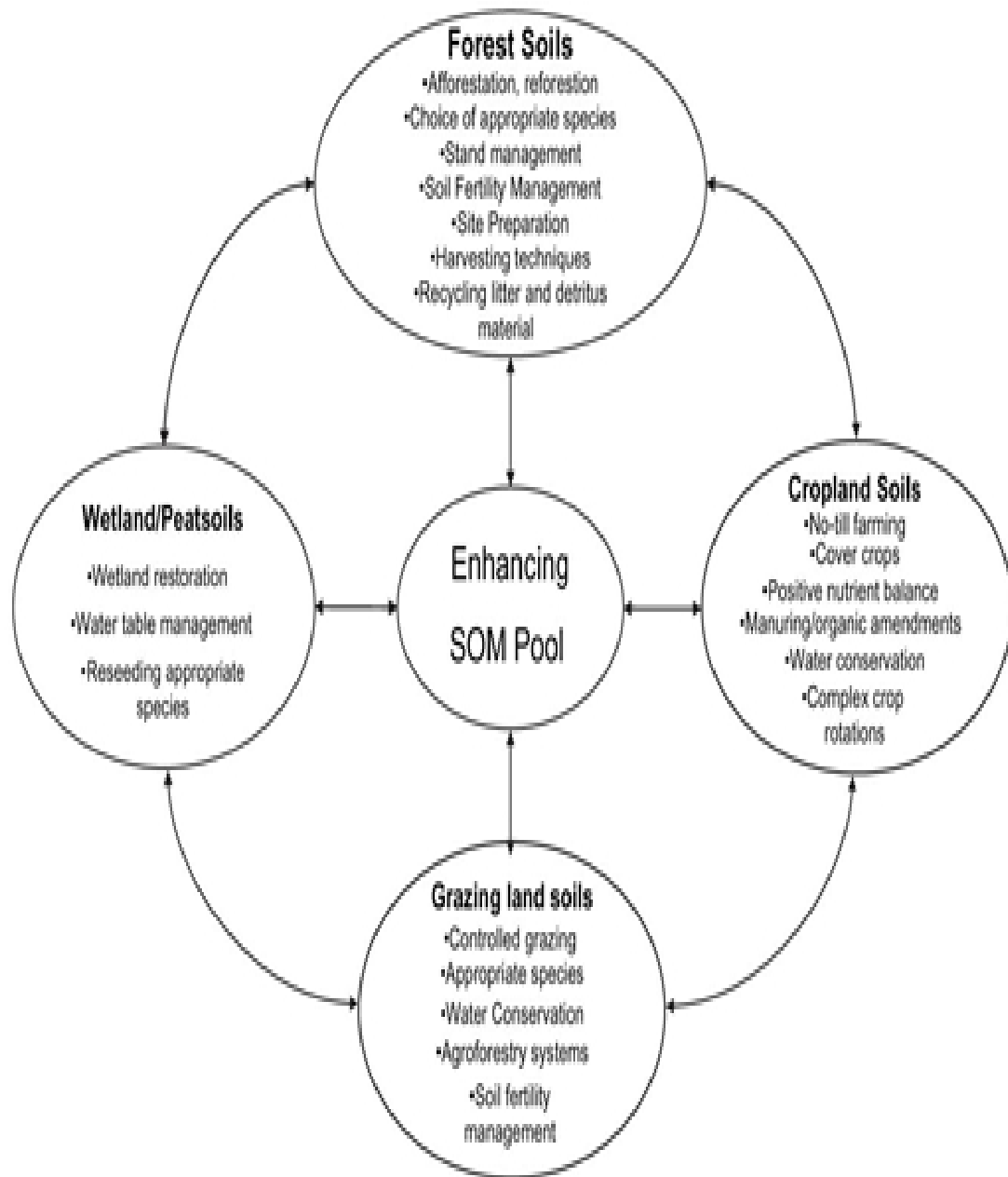


Figure 3. Management techniques for enhancing soil organic matter (Lal, 2009a)

Carbon Losses

The primary way in which carbon is removed from the long term geochemical cycle is through the slow accumulation of marine organisms deep within the ocean during the formation of either calcareous sedimentary rocks or hydrocarbons. If the sedimentation occurs faster than stored carbon is being released from volcanoes, the global atmospheric CO₂ concentrations can decrease in response to the reduction in carbon being actively cycled. Again, since the carbon cycle is global, this reduction in carbon cycling is not a true loss but rather a temporary reduction in available carbon being actively cycled in the system.

Since the short term biogeochemical carbon cycle operates on a small scale, carbon can be lost from a given location or system. In this cycle, carbon can be lost from the soil or it can be removed from a location before it has the opportunity to become incorporated into the soil. There are several ways in which carbon can be lost from the soil. For example, carbon can be translocated by erosive forces of wind (Lal, et al., 1999) or water, carbon can be illuviated down through the soil profile by percolating water, or it may be further degraded by a variety of biological and microbial processes and released back into the atmosphere as CO₂. While all these examples would be considered natural processes, the susceptibility of a soil to any of these forces and related carbon losses can be impacted by human activities which disturb the soil and ground cover (Raich, and Schlesinger, 1992, Schlesinger, and Andrews, 2000).

Another way carbon can be lost from a system is through active mining of the carbon from an area. A simple example of this is harvesting the carbon-rich plants which populate the area of interest. This activity removes fresh plant matter before it can be returned directly to the soil. Some of the activities which result in carbon loss include most agricultural operations, timber harvest, and peat moss mining. While all these activities can be done so that disruption to the carbon cycle is minimal, there are still many examples in which there is an unsustainable net loss of carbon from the system. There are also some activities such as peat moss mining that will always result in large amounts of carbon being lost from a locale because the rate of carbon removal far exceeds the rate of carbon accumulation (Cleary, et al., 2005).

When considering the total carbon cycle, comprised of both the long term and short term cycles, it is important to remember that the carbon cycle is truly one cycle and its discussion is broken down into sub-cycles in order to better describe the components of the total cycle. When conceptualizing the carbon cycle as one unified cycle a few things about the cycle become increasingly apparent. First, there are no true inputs or losses to the system as a whole but rather imbalances between the uptake and release of carbon associated with the individual phases of each of the carbon sub-cycles. Secondly, it becomes apparent that over the course of the earth's history, geology and plate tectonics have been the factors which naturally controlled the release of long-stored carbon into the atmosphere.

Carbon Dioxide as a Greenhouse Gas

As carbon cycles globally it takes various forms. Within terrestrial ecosystems, carbon can accumulate to form extremely rigid and nonreactive compounds such as diamonds (Mitchell, 1989), chemically reactive calcareous sedimentary rocks, or soft rocks, such as coal (Schopf, 1947). In addition, carbon may be considered the building block of life due to the importance of organic carbon compounds in biochemistry. However, it could be argued that one of the most important forms of carbon in the global carbon cycle might be as carbon dioxide gas (CO_2) because of the role of atmospheric CO_2 in historical global climate which ultimately controls the types of life forms which inhabit the earth.

Carbon dioxide is also a gas which belongs to a special group of seven gasses known as greenhouse gasses (GHG). There are six more anthropogenic GHGs within this group. These other gases are methane (CH_4), ozone (O_3), nitrous oxide (N_2O), hydrochlorofluorocarbons (HCFCs), perfluorocarbons (PFCs), and sulfur hexafluoride (SF_6) (Herzog, 2001). In addition to anthropogenic GHGs, water vapor is an important greenhouse gas; but it is often excluded from GHG lists because it is predominately the result of non-anthropogenic processes. Carbon dioxide is important in this group of anthropogenic gasses because it alone accounted for 82% of the total U.S. greenhouse gas emissions from 1991 to 2000 (Anderson & Newell, 2004).

Greenhouse gasses allow incoming shortwave solar radiation to pass through the atmosphere and reach the earth's surface. This shortwave radiation heats the earth

resulting in the release of long-wave radiation from the earth's surface back into the atmosphere. As the long-wave radiation radiates away from the earth it is absorbed by GHGs and re-radiated back to the earth producing an additional warming effect (Anderson, and Newell, 2004). The result of this process is that the current global mean temperature is substantially greater than the effective radiating temperature with the difference being referred to as the "greenhouse effect" (Mitchell, 1989). It is this basic process which has controlled and sustained a global climate capable of supporting life. Periodically throughout earth's history, increases or decreases in atmospheric carbon dioxide resulting from volcanic activity or lack thereof, have resulted in temperature shifts linked to mass extinction events such as ice ages (Susan, 2007). In the absence of the greenhouse effect, it has been estimated that the mean global temperature would be approximately -19°C which is substantially lower than the current mean of 15°C (Neftel, et al., 1985).

Starting in the early 1800s with the dawn of the industrial revolution, man has been utilizing reactions which release fossil energy stored in tightly bonded hydrocarbon molecules by oxidizing the carbon rich compounds to produce the energy required for mechanization (i.e. heat, power). The result of mechanization has had a twofold impact on the global carbon cycle and atmospheric CO_2 concentrations. First, as the energy stored within these bonds is transformed into kinetic energy, the reaction generates heat, water, and carbon dioxide as byproducts. This release of carbon in the form of carbon dioxide gas is typically from pools of fossilized carbon which have accumulated

extremely slowly. In recent human history this release of carbon dioxide from long term storage pools related to fossil energy production has been occurring at rates which far exceed the rates of natural carbon storage. Secondly, with the increase in mechanization from 1850 to present day, civilizations have been increasingly altering natural ecosystems by removing perennial plant communities to better suit their needs, effectively increasing the area of cultivated land by more than a factor of four (Wilhelm, et al., 1986). The disruption of the short-term biogeochemical cycle associated with cultivation and land use changes eventually results in the release of carbon from the soil carbon pool as CO_2 (Lal, 2005). Estimates of carbon release related to land use changes can be seen in Figure 4.

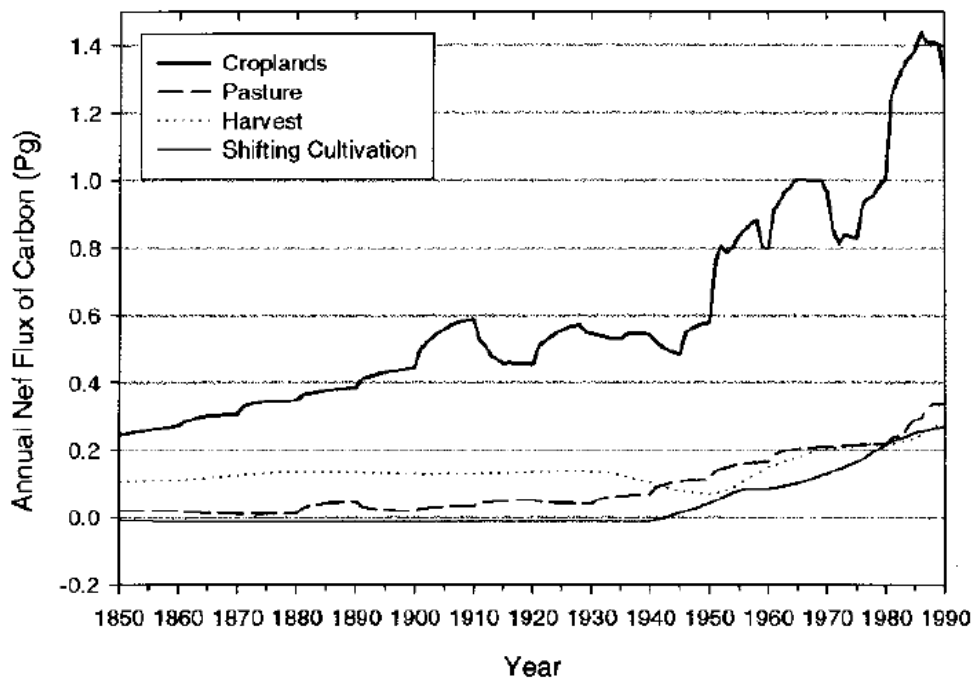


Figure 4. Annual carbon fluxes (Houghton, 1999).

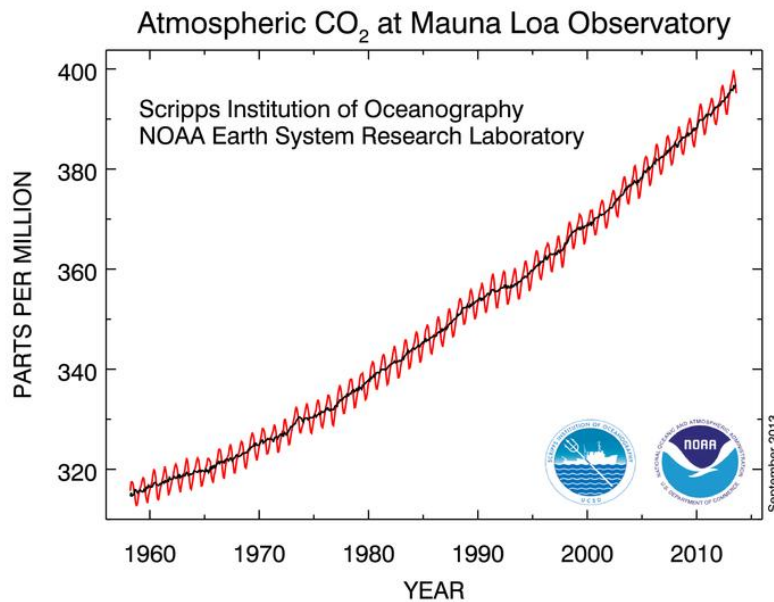


Figure 5. Changes in Atmospheric Carbon Dioxide at the Mauna Loa Observatory (Tans, et al., 2013).

The result of the imbalance between carbon storage and release related to anthropogenic activities has been an increase in atmospheric carbon dioxide concentrations which were originally detected in the 1950s and 1960s by the Mauna Loa Observatory on Hawaii (Brown, and Keeling, 1965). This increase in atmospheric CO₂ can be seen in Figure 5, which shows atmospheric CO₂ concentrations as measured by the Mauna Loa Observatory since monitoring began in 1958 at this location. Since the discovery of the Keeling Curve, researchers have been able to track the annual increases in CO₂ back to the beginning of the industrial revolution by means of various biological proxies (Wallace, et al., 1990, Andres, et al., 1999). The emissions related to anthropogenic activities since 1850 resulted in an estimated increase of atmospheric

CO₂ concentrations from a preindustrial level of 260 ppm to 360 ppm by 1990 (Wallace, et al., 1990), and 395 ppm in 2013 (Tans, et al., 2013). Armed with the understanding that the atmospheric CO₂ concentrations were increasing in response to anthropogenic activities, scientists began to research the consequences of the changing atmospheric CO₂ gas concentrations. By the early 1990s, it was determined that accumulating CO₂ was having an impact on global mean temperatures and local weather patterns (Smith, 1990, Hammerle, et al., 1991, Leygonie, 1991, Garrett, 1992, Oeschger, 1992, Smith, 1993) from emissions of more than 330 Pg C from fossil fuel combustion and 155 Pg C from land use conversion (Canadell, et al., 2007).

Role of Agriculture In GHG Mitigation

Given the concerns about increasing atmospheric CO₂ concentrations and the understanding of the effects that these increases have on global climate change, scientists have been investigating ways to mitigate this important and troublesome greenhouse gas (Riemer, 1996, Lenton, and Cannell, 2002, Gitz, and Ciais, 2003). Currently, a key research direction to mitigate atmospheric carbon dioxide concentration is in the area of carbon sequestration. Carbon sequestration is defined as the transfer of atmospheric CO₂ into long-lived pools to inhibit its re-entry to the atmosphere (Lal, 2008). Figure 6 outlines the various biotic, engineering, and chemical techniques available for carbon sequestration. The major sinks which have been examined as potential carbon sequestration options are oceanic, geologic (i.e., deep

saline formations, depleted oil and gas reservoirs, coal seams), terrestrial, and utilization (Herzog, 2001). The estimated capacities of each of the major carbon reservoirs listed are summarized in Table 2.

Table 2. Carbon Sequestration Capacity (Herzog, 2001).

Storage Option:	Worldwide Capacity:
Oceans	1000s GtC
Deep saline formations	100s–1000s GtC
Depleted oil reservoirs	100s GtC
Coal seams	10s–100s GtC
Terrestrial	10s GtC
Utilization	<1 GtC/Year

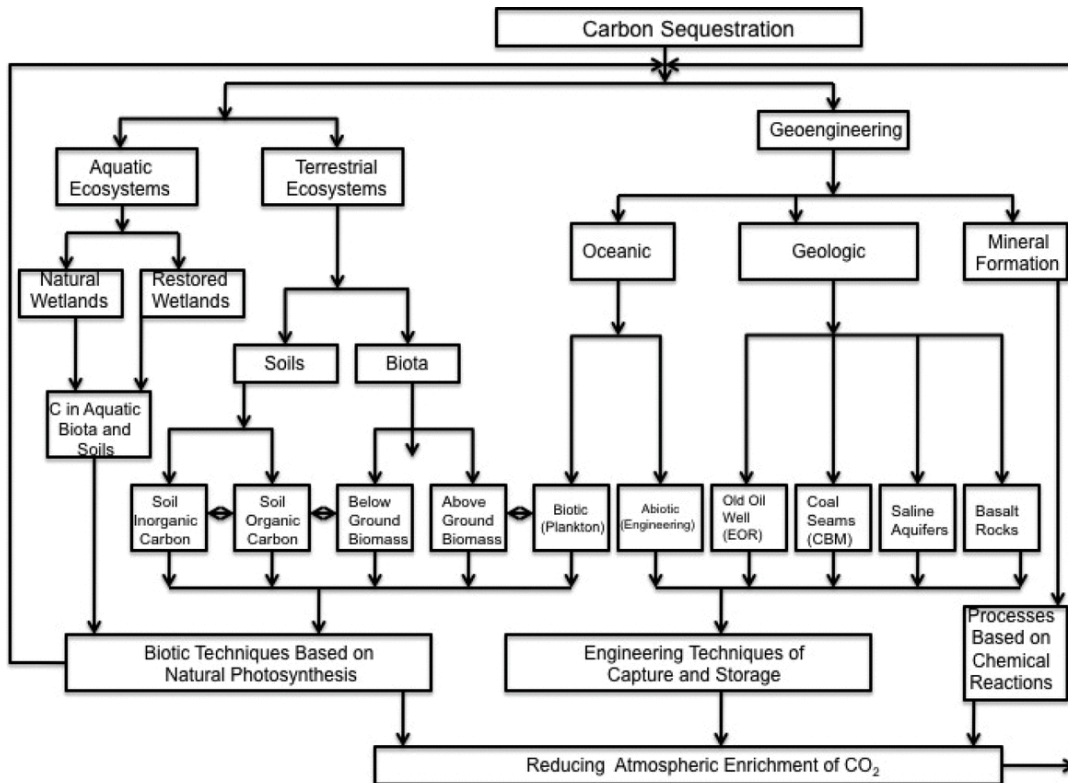


Figure 6. Carbon sequestration pathways (Lal, 2009b).

Although terrestrial carbon sequestration is not the largest of the available sinks, it is currently the safest, cheapest, and most immediate of all available options.

Terrestrial carbon sequestration is the basic process of using plants to convert atmospheric CO₂ into biomass and in concert with managing ecosystems to enable accretion of soil organic matter. Implementation of this process in dryland agricultural production involves two key concepts; reducing tillage, thereby slowing the biomass decay process (Campbell, et al., 1995, Angers, et al., 1997, Six, et al., 2000, Halvorson, et al., 2002, Mcconkey, et al., 2003) and increasing the cropping frequency, thereby accelerating the rate of C inputs (Halvorson, et al., 2002, Mcconkey, et al., 2003, Campbell, et al., 2005b). Because this simple process is driven by energy from the sun and uses modifications to current farming practices, it is one of the most affordable and feasible options currently available. By making the conversion to no-till management in agricultural soils, it is estimated that the U.S. has the ability to sequester 75–208 MMT of C per year (Lal, et al., 1999) and, over two decades the U.S. and Canada have the ability to sequester nearly 765 Tg C (Bruce, et al., 1999, Lal, et al., 1999). With an estimated historical global loss of 35–80 Gt C from the soils of cultivated lands (Cole, et al., 1997, Lal, 2004, Lal, 2005), the terrestrial carbon sequestration ability is immense. The Intergovernmental Panel on Climate Change (Lindstrom, 1986) identified soils as an important part of the global carbon cycle and climate change due to the fact that soils absorb and release the greenhouse gasses carbon dioxide and methane and store 80% of the earth's terrestrial carbon stock (Figueroa, et al., 2008).

While there are other methods outlined in Table 2 which may have much greater carbon sequestration capabilities, it will most likely be at least a decade or two before the geologic carbon sequestration options (deep saline injection, depleted oil reserve injections and coal seam injection) can be implemented on a large scale due to the infrastructure requirements, associated costs, scientific understanding of the processes, and risk analysis required to implement them safely. This timeline is based on the Department of Energy's goal to have technologies developed and ready to begin testing by 2012 (Figueroa, et al., 2008).

Physical Benefits From Carbon Sequestration

Terrestrial carbon sequestration in soils is the direct result of increased soil organic matter and consequently increased soil organic carbon (Lal, 2005). Increases in SOM achieved by implementing recommended soil management practices result in SOC sequestration through the individual processes of aggregation, humification, translocation into the subsoil, formation of secondary carbonates, burial of SOC laden sediments, and deep rooting plants (Wilhelm, et al., 1986, Wilhelm, et al., 2007, Benjamin, et al., 2008, Blanco-Canqui, and Lal, 2009). The benefits associated with SOM increases in the soil profile are a direct response to numerous soil quality parameters which can directly affect plant growth and harvestable yields. Some of these soil quality parameters include water storage, biological activity, enhanced soil aggregation and aggregate stability, increased nutrient cycling, and improved soil tilth (Wilhelm, et al.,

1986, Wilhelm, et al., 2007, Benjamin, et al., 2008, Blanco-Canqui, and Lal, 2009).

Increases in SOM can also lead to key environmental benefits such as improved water and air quality related to decreased erosion and increased filtration of runoff as well as increased buffering capacity against the impact of pollutants (Lindstrom, 1986, Mickelson, et al., 2001, Blanco-Canqui, and Lal, 2009).

2. MEASURING ON-FARM SOIL CARBON CHANGE DUE TO TILLAGE AND CROPPING INTENSITY IN NORTH CENTRAL MONTANA

Mounting societal concerns over increasing atmospheric CO₂ concentrations (Keeling, et al., 1995, Kleyvas, et al., 1999, Pearson, and Palmer, 2000, Palmer, and Ralsanen, 2002, Solomon, et al., 2009) and the continued disruptions to the global carbon cycle from land-use changes (West, and Wali, 2002, Cochran, et al., 2007, Lal, 2008, Wise, et al., 2009) and from fossil fuel consumption (Holtzeakin, and Selden, 1995, Fan, et al., 1999, Bengochea-Morancho, et al., 2001, Say, and Yucel, 2006, Wise, et al., 2009) have intensified interest in land management practices that sequester carbon in agricultural soils. Soil C is one of the five principal global C pools and, as such, is an important component of the global C cycle. The size of the soil C pool (one meter depth) is 2500 Pg of C or 3.3 times greater than the atmospheric C pool. Historically, cultivation of soils has resulted in the oxidation of soil C and its release as CO₂ to the atmosphere (Doran, 1980). An estimated 20–50% of the native soil organic carbon was lost during the first 20–50 years of cultivation (Tiessen, et al., 1982, Mann, 1986, Rasmussen, and Parton, 1994). Soils depleted in organic carbon, have the capacity to act as a C sink through a change in management practices. Adoption of reduced tillage and greater cropping intensity are two management practices that have been adopted to promote soil C sequestration in the semiarid northern Great Plains (NGP) (Campbell, and Zentner, 1993, Halvorson, et al., 1999b, Nyborg, et al., 1999, Campbell, et al., 2000, Halvorson, et al., 2002).

Terrestrial carbon sequestration research in the Great Plains has focused on available nitrogen (N) and carbon sequestration relationships (Campbell, and Zentner, 1993, Halvorson, et al., 1999b, Nyborg, et al., 1999, Campbell, et al., 2000, Halvorson, et al., 2002) and how best management practices (BMPs), such as annual cropping and no-till, can be adopted to enhance carbon sequestration (Lal, 1998, Curtin, et al., 2000a, Curtin, et al., 2000b, Campbell, et al., 2001, Antle, et al., 2002, Eve, et al., 2002, Mcconkey, et al., 2003, Liebig, et al., 2004). Although, management practices to sequester C in agricultural soils of the NGP have been identified, the sequestration rates remain unknown for some regions, including Montana's Golden Triangle. This region is defined on the north by the border with Canada, on the west by the Rocky Mountain front, and approximately on the east by a line-transect between Great Falls and Havre. This is Montana's largest grain-growing region with more than two million hectares of land annually in wheat production. Largely managed under alternate-year cropping rotations with varying levels of tillage intensity, the soils of this region have the potential to act as a carbon sink (Bricklemyer, et al., 2005, Watts, et al., 2011). The purpose of this research was to quantify soil carbon sequestration rates in response to agricultural BMPs, including no-till and annual cropping, on six representative well-managed farms in Montana's Golden Triangle.

Materials and Methods

Site Description and Experimental Design

Field studies were conducted at six farmer-managed sites (~32 ha) within Montana's Golden Triangle (Figure 7, Table 3). The six sites were under no-till management prior to the inception of this study in 2002. Soil particle size distribution,



Figure 7. Location of field sites in north central Montana's Golden Triangle marked by asterisks.

textural classification, and pH for the 0–10, 10–20, and 20–50-cm depths are summarized in Table 3 along with the site coordinates. Soil texture analysis revealed the soils at these sites to be fine textured with clay content in the surface 0–10-cm ranging

from 27% to 56%. Soil pH (0–10-cm depth) was greater than 7.0 at all sites, reflecting the occurrence of Ca and Mg carbonates common to the soils of this region.

Table 3. Soil physical properties by depth for six field sites in north central Montana.

Location	Latitude, Longitude	Depth	% Sand	% Silt	% Clay	Textural Class	pH
Dutton	47°58'37.08"N, 111°44'42.87"W	10 cm	30	23	46	Clay	7.8
		10–20 cm	27	21	52	Clay	8.1
		20–50 cm	25	21	54	Clay	8.6
Power	47°40'50.46"N, 111°34'38.47"W	0–10 cm	19	38	43	Silty clay	8.2
		10–20 cm	17	36	47	Silty clay	8.2
		20–50 cm	22	29	50	Clay	8.5
Chester	48°43'07.20"N, 110°51'45.95"W	0–10 cm	42	28	30	Clay loam	7.8
		10–20 cm	35	30	35	Clay loam	8.2
		20–50 cm	29	34	37	Clay loam	8.6
Conrad	48°18'44.37"N, 111°55'50.20"W	0–10 cm	34	32	35	Clay loam	7.4
		10–20 cm	30	29	41	Clay	7.7
		20–50 cm	27	32	41	Clay	8.2
Fife	47°29'13.59"N, 111°00'17.92"W	0–10 cm	20	24	56	Clay	7.6
		10–20 cm	21	22	56	Clay	8.2
		20–50 cm	17	20	63	Clay	8.6
Kremlin	48°31'41.36"N, 110°01'56.91"W	0–10 cm	38	34	27	Clay loam	7.8
		10–20 cm	35	31	34	Clay loam	8.1
		20–50 cm	34	30	36	Clay loam	8.7

Each field site was divided into two whole plots. The first remained under no tillage and the second was converted to minimum tillage. The whole plots were divided into two cropping intensity subplots, annual cropping (1.0) vs. alternate year cropping (0.5). The plot arrangements are illustrated in Figure 8. No-till main plots were generally

oriented on the west or upwind side of the field sites to avoid bias due to snow drift from tilled to no-till plots. Annually cropped treatments consisted of annual legumes in odd-numbered years and formed the center of the field (except for Chester) to facilitate farming operations. All field plots were sown to spring or winter wheat during even-

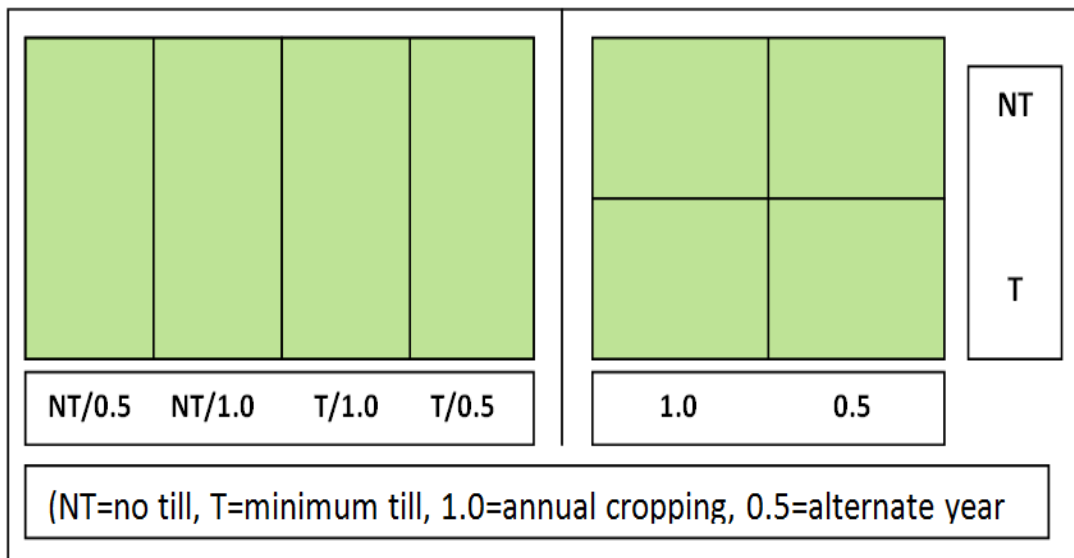


Figure 8. Field plot design at Conrad, Dutton, Fife, Kremlin, and Power (left) and Chester (right).

numbered years (i.e. 2002, 2004, 2006, and 2008). Aside from the field design constraints, all field management decisions, including tillage type and frequency, were left to the discretion of the producer. Annual legumes were seeded in the annual cropped treatments during odd-numbered years (2003, 2005, 2007).

Soil Sampling Protocol

The soil sampling protocol was designed to minimize the effects of spatial variability on soil C by analyzing the carbon content of five composite cores from permanent microplots (4 per subplot). Soil sampling microplots for each field site were identified in 2002 using methods adapted from previous work (Brickley, et al., 2005). Initially a digital grid was superimposed over the entire study area. The grid had a 30-meter buffer around the perimeter to minimize potential confounding edge effects. The cells of the grid were 30 x 30 m, the intersection points were numbered, and a random number generator was used to identify 12 sampling locations from each subplot. A single soil core (0–50 cm) was collected from each of the 12 sampling locations per subplot. Depth to soil carbonates (dilute HCl effervescence test) and soil texture in the control section (hand texture) were determined in the field and compared to the other 47 cores (total of 48 cores per field site). Four locations per treatment were selected as the center-axis for each microplot such that depth to carbonates and soil texture were most similar across microplots within each field. The location of the microplot center was marked with a buried (20 cm long x 2 cm diam.) steel bolt and geo-referenced using a Trimble GeoExplorer 3 (Sunnyvale, CA).

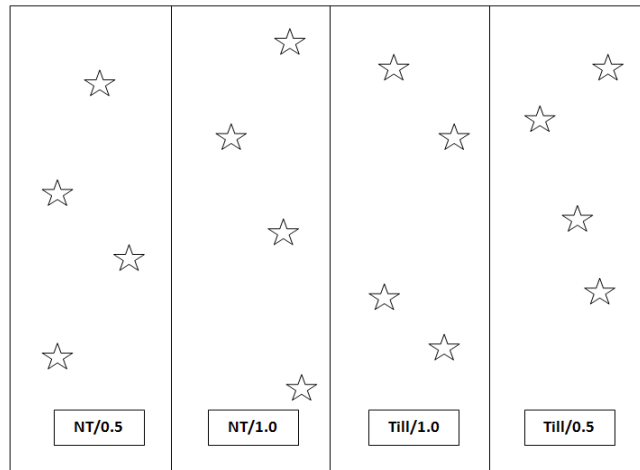


Figure 9. Example of field layout.

A five-pointed star (5 m radius) pattern was created around the center-axis of each microplot to mark soil sampling points (Figure 9). In 2002, the five points of the star were oriented 0° (true north), 72° , 144° , 216° , and 288° , and 5 m from the microplot center (Figure 10). The five points of the star were rotated clockwise 3.4° in 2008. If an obstruction (i.e., rock) was encountered while sampling, a second attempt to collect a sample was made 30 cm toward the center of the circle. If any of the sampling points fell in an obvious tractor wheel track, the sampling point was moved sufficiently toward the center of the star to avoid the compressed soil. Surface litter was gently cleared by hand from the area to be sampled in an effort to minimize disturbance and avoid litter contamination to the lower sample depths.

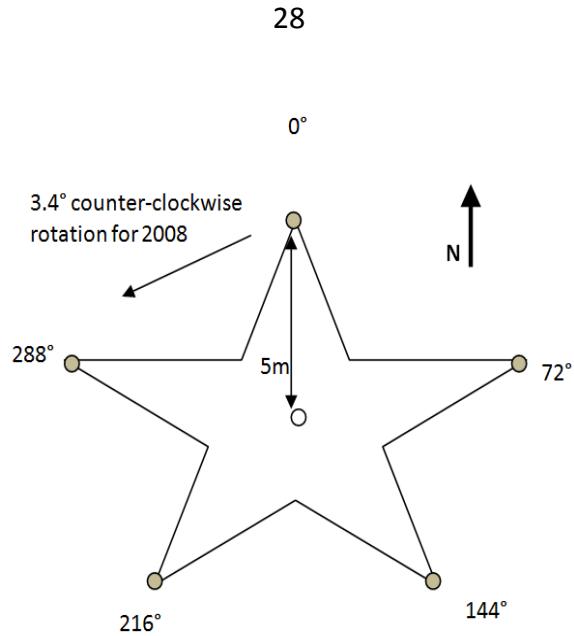


Figure 10. Sample layout and rotation of soil cores around microplots.

A Concord Environmental Equipment hydraulic sampler (Hawley, MN) equipped and pneumatic hammer was used to penetrate the soil profile to a 50-cm depth. In 2002, a solid steel probe fitted with a clear plastic sleeve was used to collect soil cores (7.3 cm dia.). The soil cores were transported to the lab intact and later cut into 0–10, 10–20, and 20–50-cm depth layers. In 2008, soil cores (5.1 cm dia.) were pressed into a slotted steel soil probe that enabled the operator to visually inspect the core for compaction while sampling. This safeguard was not possible in 2002 because of the use of the solid steel core containing the plastic sleeve inserts. Extracted soil cores were separated into 0–10, 10–20, and 20–50-cm depths in the field in 2008.

Soil Sample Processing, Carbon Analyses, Bulk Density Protocol

Soil samples for all years were oven-dried at 50°C for 4 days and weighed for bulk density determination. Bulk density was calculated by dividing ground soil (< 2 mm) dry weights by core volumes minus rock weight and volume.

Soil cores for 2002 and 2008 were processed for soil C analyses according to the schemes outlined in Figures 11 and 12. In 2002, soil samples from each star point were partially ground using a flail mill. A subsample from each of the five star points (~60 g) was then combined into a single composite sample. The rock fraction was then estimated after passing the composite sample through a 2-mm sieve. In 2008, the soil samples from each star point were flail milled, and 100% of the flail milled material was hand-ground with a mortar and pestle. Rock fragments, surface plant litter, and coarse root material were separated from soil material by passing the hand-ground material through a 2-mm sieve. At the time a subsample was prepared for ball milling, the visible litter and root matter that passed through the 2-mm sieve was removed with a tweezers until none could be identified with the naked eye. The rock fraction was calculated by weighing the fragments which would not pass through the screen. Ground soil samples, 30.0 g (+/- 0.1 g) from each star point, were combined into a single composite sample (~150 g).

A representative subsample (~30 g) from each of the composite samples was milled to fine powder (<200µm) in a ball mill (Pica Blender Mill model 2601, Cianflone Scientific Instruments Corp., Pittsburg, PA) prior to C analysis. Total C was measured

using ~0.2 g of the milled subsample on a LECO TruSpec CN combustion furnace analyzer (LECO Corp., St. Joseph, MI). Inorganic C was measured using ~1.0 g +/- 0.05 g of the milled subsample for the modified pressure calcimeter method (Sherrod, et al., 2002). Organic C was calculated by subtracting inorganic C from total C.

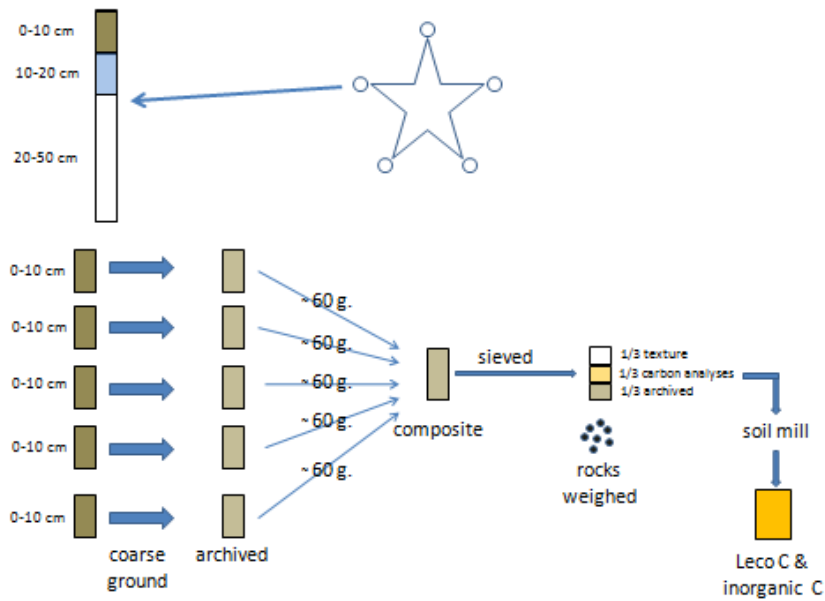


Figure 11. Sample processing scheme used for cores collected in 2002.

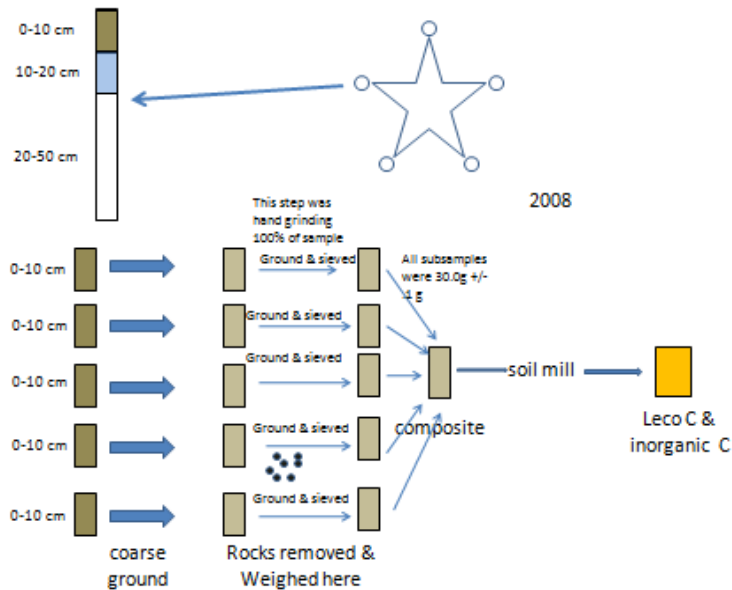


Figure 12. Soil sample processing scheme used for cores collected in 2008.

Biomass Sampling and Plant Carbon Analysis Protocol

Crop biomass for each treatment was estimated by hand-harvesting three areas totaling 2 m² around the center of each microplot. The sampling areas were located at positions 0° (due north), 120°, and 240°, and 3 m from the microplot center. Samples were composited and oven-dried at 50°C for 4 days to estimate biomass production. Wheat and legume grain yields were estimated after threshing biomass samples with a small plot combine (Wintersteiger, Ried, Austria) and hand-threshing, respectively. Shoot biomass was coarse-ground by first processing the dried sample with a Wiley Mill, (Thomas Scientific, Swedesboro, NJ) fitted with a 5-mm screen. Representative coarse-ground shoot subsamples were fine-ground with a UDY Cyclone sample mill (Fort Collins, CO) fitted with a 1-mm mesh screen. Subsamples (0.2 g) of the plant material

were analyzed for C and N on a LECO Truspec dry combustion analyzer (LECO Corp., St. Joseph, MI). Weeds were weighed separately but then reincorporated into the appropriate biomass sample prior to sampling grinding. If the weeds represented >10% of the total biomass (very rarely the case) they were ground and analyzed separately. If a producer chose to hay their annual legume crop, biomass estimates were made for plant material above and below hay cutting height (10 cm). In order to estimate total plant carbon inputs, it was necessary to estimate C inputs associated with roots of legume crops and wheat. Legumes grown for seed were estimated to have a root:shoot ratio of 0.2 and legumes grown for green manure or forage were estimated to have a root:shoot ratio of 0.4 (Paez, et al., 1983, Herdina and Silsbury, 1990, Wichern, et al., 2008). The carbon concentration was set equal to that measured in the shoot (44%). Wheat was considered to have a root:shoot ratio of 0.15 (Chaudhuri, et al., 1990, Bolinder, et al., 2007) and carbon concentration was set equal to that measured in the shoot (45%).

Soil Organic Carbon Calculations and Mass Equivalency Adjustment

Soil organic carbon mass per unit area expressed in units of Mg ha^{-1} were calculated by multiplying SOC concentration by the product of bulk density and soil depth. SOC calculations for 2002 and 2008 utilized bulk density values estimated from 2008 only. Inspection of 2002 bulk density values revealed that many estimates were nonsensical (too high or too low). This may have been a result of the requisite “blind”

sampling associated with the plastic sleeve method (i.e., inability to view compaction of the soil core during sampling) and dispersion of cores within the tubes during transport to, and storage in, the lab. Hence, bulk density values from 2008 were applied to the 2002 SOC concentrations to calculate Mg of SOC ha⁻¹.

The calculations for mass of SOC per hectare for each treatment and at each field site were next normalized for mass equivalency (Ellert, and Bettany, 1995). Mass equivalency adjustments are necessary to compensate for spatial variability across a field site as well as the application of treatments (e.g., tillage may affect soil bulk density). Estimates of mass of soil organic carbon per unit area are bulk density dependent. Hence, haphazard variations in bulk density may obscure differences in SOC storage. Mass-equivalency adjustments were made by using the lightest core concept which is a modification of work previously described in the literature (Ellert and Bettany, 1995). Modifications were done by adjusting downward the mass of C per depth increment (i.e., 0–10, 0–20, and 0–50 cm) based on the bulk density of the lightest microplot core. Bulk densities used for this adjustment were those which were calculated by removing both the mass and volume of the rock fragments from the mass and volume of the sample collected.

Statistical Analysis

Soil C analyses were conducted on the change in SOC from 2002 to 2008 for each of the six farm sites. Each site was analyzed separately due to management differences

among farm sites in tillage intensity practiced under the minimum tillage treatment and legume crops. Statistical analysis was conducted using the SAS® software (SAS Systems for Windows, Release 9.2, SAS Institute, Cary, NC) and the GLM procedure (PROC GLM). The ANOVA model for each site analyzed tillage, cropping intensity, and tillage by cropping intensity interaction using stars as independent experimental unit. Shoot biomass, wheat grain yield, crop residue biomass, and estimated carbon in biomass were summed for six years for all treatments and sites and analyzed with Proc GLM same ANOVA model above. A *P*-value of 0.10 was the cutoff for significance for all analyses.

Site Descriptions

Crop species and cultivar selections seeded at each site are summarized in Table 4. This table begins to show some of the variability that can exist from site to site when production decisions are left to the individual producers enrolled in the study. While letting the producers decide the specifics related to the crop production on their land can result in site to site variation, it ensured that producers were growing cultivars they felt were best suited for their site-specific growing conditions, which may vary across north central Montana. Soil textures were determined experimentally utilizing the hydrometer method(Gee, and Bauder, 1986) for all sites (Table 3).

The Dutton site is described in a web-based soil survey as receiving 28 to 36 cm of annual precipitation with a frost-free period of 105 to 125 days and an annual mean

temperature of 1–8°C (Soil Survey Staff, 2009). This soil survey classifies the map unit name for the area of the study as belonging to the Scobey-Kevin clay loam series. This map unit is described as a moderately deep soil having more than 2 m of soil before reaching restrictive features or ground water. Prior to 2001, the Dutton field site was managed as conventional tillage with no information available regarding historic crop rotation due to a change in ownership in 2001. Beginning in 2002 this site was managed by experienced no-till farmers.

Table 4. Crops and *cultivar* seeded at six farm sites by year.

Site	Year					
	2003 †	2004	2005 †	2006	2007 †	2008
Dutton	<i>Grande</i> pea	<i>Fortuna</i> spring wheat	<i>Salute</i> pea	<i>Fortuna</i> spring wheat ‡	<i>Cruiser</i> pea	<i>Falcon</i> winter wheat
Power	<i>Arvika</i> pea ¥	<i>Fortuna</i> spring / <i>Vanguard</i> winter wheat§	<i>Richlea</i> lentil	<i>Pryor</i> winter wheat	<i>Richlea</i> lentil	<i>Fryer</i> spring wheat
Chester	winter pea (vns)	<i>Fortuna</i> spring wheat	winter pea (vns)	<i>Fortuna</i> spring wheat	<i>Richlea</i> lentil	<i>Fortuna</i> spring wheat
Conrad	lentil (vns) Ω	<i>Ernest</i> spring wheat	pea (vns)	<i>Timer</i> winter wheat	<i>Salute</i> pea	<i>Conan</i> spring wheat
Fife	<i>Arvika</i> pea & <i>Harrington</i> barley ¥	<i>Ernest</i> spring wheat	<i>Melrose</i> winter pea	<i>Promontory</i> winter wheat	Pea (vns)	<i>Carlise</i> winter wheat
Kremlin	<i>Arvika</i> pea	<i>Conan</i> spring wheat	<i>Escape</i> pea	<i>Choteau</i> spring wheat	<i>Cruiser</i> pea	<i>Genou</i> winter wheat

† Crops listed in odd years were only grown in annually cropped (=1.0 crop intensity)

‡ Crop planted after winter wheat failed

§ Spring wheat planted on continuous crop plots, winter wheat on fallow plots

¥ Crop harvested as forage

Ω Crop terminated as green manure

The Power site is located south of Power, MT, and is described in a web-based soil survey as receiving 28 to 36 cm of annual precipitation with a frost-free period of 110 to 135 days and an annual mean temperature of 4–8°C (Soil Survey Staff, 2009). This soil survey describes the map unit which covers the area of the study as the Cargill silty clay loam series; a well-drained soil with 2 m to the water table and approximately 50 to 100 cm of soil before reaching paralithic bedrock. The depth to bedrock makes this the shallowest of the six soils which was consistent with our soil-sampling experience. This site was historically in a wheat-fallow rotation prior to entering this study with conventional tillage used through 1999. No-till management was initiated in 2000.

The Chester site is located 25 km north of Chester. This site is described in a web-based soil survey as receiving 28 to 36 cm of annual precipitation with a frost-free period of 105 to 125 days and an annual mean temperature of 4–8°C (Soil Survey Staff, 2009). This survey classifies the map unit of the study area as belonging to the Joplin-Hillon loams series; a well-drained soil with 2 m to the water table or before reaching restrictive features. This site was historically in a wheat-fallow rotation prior to enrolling in this study, with conventional tillage used through 1993, reduced-till management 1994–1997, and no-till management begun in 1998.

The Conrad site is located 12 km north of Conrad, MT. This site is described in a web-based soil survey as receiving 28 to 36 cm of annual precipitation with a frost-free period of 105 to 125 days and an annual mean temperature of 1–8°C (Soil Survey Staff, 2009). This series describes the map unit of the area of study as belonging to the

Scobey-Kevin clay loam series; a well-drained soil with more than 2 m to the water table or before reaching restrictive features. This site was historically in a wheat-fallow rotation prior to entering this study, with conventional tillage used through 1993. In 1994, land management changed to no-till and remained in no-till until this site was enrolled in this study.

The Fife site is the wettest of the six sites enrolled in this study and is located 20 km east of Great Falls. The area of this site is described in a web-based soil survey as receiving 35 to 46 cm of annual precipitation with a frost-free period of 105 to 130 days and an annual mean temperature of 3–8°C (Soil Survey Staff, 2009). This survey describes the map unit of the area of the study as belonging to the Lawthery silty clay series; a well-drained soil with more than 2 m to the water table or before reaching restrictive features. This site also has the greatest clay content of the six sites studied, with 56% clay dominated by 2:1 expanding type montmorillonite clays.

The Kremlin site is located 5 km east of Kremlin, MT, and is described in a web-based soil survey as receiving 25 to 33 cm of annual precipitation (making this the driest of the six sites) with a frost-free period of 105 to 120 days and an annual mean temperature of 4–8°C (Soil Survey Staff, 2009). This survey lists several soil series names for the sampling area of the study with the Phillips-Elloam complex being the dominant soil series at this site accounting for 63% of the area. The other two series listed in this survey are the Kevin-Hillon clay loam accounting for 20% of this site and the Scobey-Kevin clay loam accounting for 13% of the site. This site is also described as a well-

drained soil with more than 2 m to the water table or before reaching restrictive features. This site was historically in a wheat-fallow rotation with no-till management dating back to 1993.

Results and Discussion

Biomass and Grain Yields

Cumulative shoot biomass, grain yield, and estimated carbon inputs by site and as affected by tillage and cropping intensity are summarized in Tables 5 and 6. Table 5 illustrates the site-specific production variability that exists across an area the size of Montana's Golden Triangle (2 million ha). Table 5 shows that after six years of continuous observation, production variation exists from site to site in the total shoot, shoot residue, and shoot C input categories. The shoot C input column (last column) in Table 5 shows that each site has significantly different amounts of carbon inputs being returned to the soil after harvest. This difference is important; it has been shown that for soils which are not carbon saturated (an equilibrium where greater C inputs only result in C losses), the quality and quantity of crop residue returned to the soil is directly related to changes in SOC (Lal, 2004). Based on this relationship, the long history of carbon-depleting production agriculture (Mcconkey, et al., 2003) at these sites, the direct relationship between soil texture and sequestration rates (Bonfil, et al., 1999, Halvorson, et al., 1999a), and the shoot C inputs by site (Table 5), the Fife site might be expected to sequester the most carbon and the Power and Chester sites sequestering

the least. Given the soil texture differences seen in Table 3 and the site-specific weather differences to be discussed later, these site specific production differences were generally not a surprise.

Table 5. Cumulative shoot biomass, grain yield, and carbon inputs over six years (2003–2008) and as affected by site and tillage.

Site	Total Shoot	Shoot residue	Shoot C input
Site (Mg ha⁻¹)			
Dutton	24.1 BC	15.8 C	7.11 C
Power	20.5 C	11.8 E	5.35 E
Chester	19.9 C	12.9 D	5.91 D
Conrad	26.0 B	15.7 C	7.12 C
Fife	40.8 A	24.4 A	10.94 A
Kremlin	26.9 B	17.1 B	7.75 B
No-till vs Tilled (Mg ha⁻¹)			
No-till	26.9 A	16.5 A	7.48 A
Tilled	25.9 B	16.0 A	7.25 A

Means within columns followed by the same letter do not differ according to Protected LSD ($P < 0.10$).

The tillage comparison (lower part of Table 5) shows greater total shoot biomass production for the no-till treatments averaged across all six sites. The increased yields seen here are consistent with work previously done in this type of arid environment (Bonfil, et al., 1999, Cantero-Martinez, et al., 1999, Halvorson, et al., 1999a), where no-

till management has been shown to increase spring and winter wheat yields over conventional tillage yields, especially under low precipitation levels. The advantage to the no-till system in an arid environment is an increase in water collection and conservation, resulting from the accumulation of crop stubble and residues at the soil surface (Miller, et al., 2002, Huang, et al., 2008), resulting in increased water and N use efficiencies (Sainju, et al., 2009). However, shoot residue biomass and shoot C input did not differ between the tillage systems.

Table 6 illustrates that the cumulative total carbon input (last column to the right) was greater for the continuous cropped treatments than for the treatments which included fallow for three of six sites. Total carbon inputs did not differ by cropping intensity for two of the sites, and one site (Conrad) had greater carbon input under the fallow system. This would be the anticipated outcome with a crop being grown every year, if sufficient precipitation occurred to grow viable crops annually.

Previous research has shown that the differences in the amount of annualized biomass residue returned to the soil is an important factor in directly explaining much of the variability seen in SOC and total nitrogen (Campbell, et al., 2000, Halvorson, et al., 2002, West, and Post, 2002, Mcconkey, et al., 2003, Campbell, et al., 2005a, Shrestha, et al., 2013).

Table 6. Total shoot biomass , total shoot residues, total shoot C input, and estimated total C input (shoot + root), for different tillage and cropping intensity regimes at six field sites in north central Montana, summed from 2003 through 2008.

Site	Total Shoot	Shoot residue	Shoot C input	Estimated Total C
Cropping Intensity by Site (Mg ha⁻¹)				
Dutton - 0.5	23.6 a	15.6 a	7.1 a	8.7 a
Dutton - 1.0	24.7 a	16.0 a	7.1 a	9.0 a
Power - 0.5	16.4 b	9.6 b	4.4 b	5.5 b
Power - 1.0	24.6 a	13.9 a	6.3 a	8.3 a
Chester - 0.5	19.6 a	12.3 b	5.7 a	7.0 b
Chester - 1.0	20.3 a	13.5 a	6.1 a	7.6 a
Conrad - 0.5	27.6 a	16.5 a	7.5 a	9.4 a
Conrad - 1.0	24.5 b	14.8 b	6.7 b	8.6 b
Fife - 0.5	40.8 a	25.0 a	11.2 a	14.0 a
Fife - 1.0	40.8 a	23.9 b	10.6 b	14.2 a
Kremlin - 0.5	26.1 a	15.8 b	7.2 b	9.0 b
Kremlin - 1.0	27.7 a	18.4 a	8.3 a	10.4 a

Means within columns and site followed by the same letter do not differ ($P < 0.10$).

SOC Changes, 2002–2008

Post-harvest soil samples collected in 2008 reflect six years under each of the prescribed management regimes. It was anticipated that refinement of the sampling and analytical procedures would result in an increased signal-to-noise ratio which would

ultimately benefit comparisons between the 2002 baseline SOC and the 2008 SOC values. Summary data tables for SOC by site and year, along with the results from ANOVAs conducted using the SAS® software (Proc GLM) can be seen in Tables 7 through 24 below. These tables are grouped by site and show estimated changes in organic carbon by site and depth over 6 years with the corresponding ANOVAs.

Dutton

No change in SOC was evident at the Dutton site (Tables 7 and 8). The Dutton site showed no main effects, or interaction, in δC for any depth (Table 7). Table 8 displays the SOC values for 2002, 2008, and δC by individual depths. No obvious sequestration trends are observed at this site for any depth.

Table 7. Summary ANOVA table for delta SOC, by depth (2002, 2008) at Dutton.

Depth	Source	DF	Sum of Squares	Mean Square	F value	P value
0–10cm	Tillage	1	0.74	0.74	0.83	0.38
	Cropping intensity (CI)	1	<0.00	<0.00	0.00	0.97
	Tillage X CI	1	1.68	1.68	1.90	0.20
	Residual	11	9.75	9.75		
10–20cm	Tillage	1	0.06	0.06	0.12	0.73
	Cropping intensity (CI)	1	0.33	0.33	0.71	0.41
	Tillage X CI	1	0.16	0.16	0.35	0.56
	Residual	12	5.50	5.50		
20–50cm	Tillage	1	6.81	6.81	0.37	0.55
	Cropping intensity (CI)	1	0.73	0.73	0.04	0.85
	Tillage X CI	1	0.17	0.17	0.01	0.93
	Residual	12	220.58	18.38		

Table 8. Soil organic carbon at Dutton in 2002, 2008, and delta SOC (2002, 2008) as affected by tillage and cropping intensity.

Year	Treatment	Soil depth layer (cm)		
		0–10	10–20	20–50
		----- MT C ha ⁻¹ -----		
2002	NT 0.5	13.31	10.56	24.66
	NT 1.0	11.68	9.61	23.05
	TILL 1.0	14.13	10.05	22.68
	TILL 0.5	12.34	10.51	22.78
2008	NT 0.5	12.20	10.22	25.28
	NT 1.0	11.27	9.76	23.44
	TILL 1.0	11.60	9.89	24.18
	TILL 0.5	12.35	10.26	24.91
δC	NT 0.5	-1.11	-0.33	0.61
	NT 1.0	-0.41	0.16	0.39
	TILL 1.0	-2.53	-0.16	1.49
	TILL 0.5	0.01	-0.25	2.12

Power

No-till increased ($P = 0.02$) delta SOC in the 0–10-cm depth (Table 9) with a net gain of 1.6 MT C ha⁻¹ (Table 10). Annual cropping showed a marginally significant net gain of 1.1 MT C ha⁻¹ ($P=0.09$) in the top depth only (Table 10). No interactions between tillage and intensity ($P < 0.05$) were detected at any depth (Table 9).

Table 9. Summary ANOVA table for delta SOC, by depth (2002, 2008) at Power.

Depth	Source	DF	Sum of Squares	Mean Square	F value	P value
0–10cm	Tillage	1	10.79	10.79	7.38	0.02
	Cropping intensity (CI)	1	4.93	4.93	3.37	0.09
	Tillage X CI	1	2.59	2.59	1.77	0.21
	Residual	12	17.54	17.54		
10–20cm	Tillage	1	1.39	1.39	0.47	0.51
	Cropping intensity (CI)	1	3.90	3.90	1.33	0.27
	Tillage X CI	1	0.46	0.46	0.16	0.70
	Residual	12	35.31	35.31		
20–50cm	Tillage	1	17.85	17.85	0.42	0.53
	Cropping intensity (CI)	1	3.98	3.98	0.76	0.40
	Tillage X CI	1	0.05	0.05	0.00	0.97
	Residual	12	504.60	42.050		

Table 10. Soil organic carbon at Power in 2002, 2008, and delta SOC (2002, 2008) as affected by tillage and cropping intensity.

Year	Treatment	Soil depth layer (cm)		
		0–10	10–20	20–50
----- MT C ha ⁻¹ -----				
2002	NT 0.5	16.40	13.99	29.00
	NT 1.0	14.38	13.36	32.35
	TILL 1.0	17.47	14.54	32.29
	TILL 0.5	18.28	15.30	27.73
2008	NT 0.5	16.31	12.63	27.09
	NT 1.0	16.21	12.65	27.49
	TILL 1.0	16.84	14.76	29.66
	TILL 0.5	17.36	14.18	27.81
ΔC	NT 0.5	-0.09	-1.36	-1.91
	NT 1.0	1.83	-0.72	-4.86
	TILL 1.0	-0.62	0.21	-2.63
	TILL 0.5	-0.92	-1.11	0.08

Chester

The analysis of the delta SOC data from the Chester site displays a strongly significant cropping intensity effect ($P < 0.05$) on delta SOC in the 0–10 and 10–20-cm depths. Higher cropping intensities resulted in accretion of SOC. Under continuous cropping, SOC increased 0.91 and 0.70 MT ha⁻¹ for the 0–10 and 10–20-cm depths (Table 12), respectively. Conversely, a net SOC loss of 0.77 and 0.77 MT ha⁻¹ was observed in the 0–10 and 10–20-cm depths, respectively. Cropping intensity effect on SOC in the 20–50-cm depth was moderately affected ($P < 0.10$) by cropping intensity. There was also a moderately significant ($P < 0.10$) interaction observed between tillage and cropping intensity in the 10–20cm depth. No other significant effects were observed.

Table 11. Summary ANOVA table for delta SOC by depth (2002, 2008) at Chester.

Depth	Source	DF	Sum of Squares	Mean Square	F value	P value
0–10cm	Tillage	1	0.02	0.02	0.12	0.74
	Cropping intensity (CI)	1	2.74	2.74	18.67	<0.01
	Tillage X CI	1	0.27	0.27	1.85	0.20
	Residual	11	1.61	1.61		
10–20cm	Tillage	1	0.90	0.90	1.47	0.25
	Cropping intensity (CI)	1	8.60	8.60	14.09	<0.01
	Tillage X CI	1	2.33	2.33	3.82	0.07
	Residual	12	7.32	7.32		
20–50cm	Tillage	1	7.63	7.63	0.59	0.46
	Cropping intensity (CI)	1	2.20	2.20	0.17	0.07
	Tillage X CI	1	25.83	25.83	2.01	0.18
	Residual	12	153.98	12.83		

Table 12. Soil organic carbon at Chester in 2002, 2008, and delta SOC (2002, 2008) as affected by tillage and cropping intensity.

Year	Treatment	Soil depth layer (cm)		
		0–10	10–20	20–50
		----- MT C ha ⁻¹ -----		
2002	NT 0.5	12.13	10.93	24.77
	NT 1.0	10.28	11.11	25.99
	TILL 1.0	11.62	11.40	24.30
	TILL 0.5	11.94	12.30	26.91
2008	NT 0.5	11.53	10.79	23.33
	NT 1.0	11.90	11.67	22.75
	TILL 1.0	11.81	12.25	24.98
	TILL 0.5	11.00	10.92	24.31
ΔC	NT 0.5	-0.60	-0.15	-1.44
	NT 1.0	1.62	0.55	-3.24
	TILL 1.0	0.19	0.85	0.66
	TILL 0.5	-0.94	-1.38	-2.60

Conrad

The analysis of the delta SOC data from the Conrad site showed no significant effects in the 0–10- and 10–20-cm soil depths for cropping intensity, tillage, or cropping x tillage (Table 13). Delta SOC in the 20–50-cm depth was significantly affected by cropping intensity and cropping intensity x tillage. The loss in SOC in the tilled annual cropping system was equivalent to 7.0 MT ha⁻¹, and gain in SOC in the till alternate cropping system was equivalent to 4.4 MT ha⁻¹. Both these differentials are impossibly

large, assuming no significant soil erosion or deposition. It is not known why this occurred, but it may be related to soil sampling and processing errors, spatial/topographical variability across the site, and bias created by the star locations (i.e., tilled star locations were biased by slope position, Figure 13).

Table 13. Summary ANOVA table for delta SOC, by depth (2002, 2008) at Conrad.

Depth	Source	DF	Sum of Squares	Mean Square	F value	P value
0–10cm	Tillage	1	2.52	2.52	0.38	0.55
	Cropping intensity (CI)	1	18.86	18.86	2.85	0.12
	Tillage X CI	1	0.02	0.02	0.00	0.95
	Residual	12	79.37	79.37		
10–20cm	Tillage	1	0.43	0.43	0.13	0.73
	Cropping intensity (CI)	1	0.40	0.40	0.12	0.74
	Tillage X CI	1	2.41	2.41	0.72	0.41
	Residual	12	40.31	40.31		
20–50cm	Tillage	1	22.21	22.21	1.86	0.20
	Cropping intensity (CI)	1	113.05	113.05	9.48	0.01
	Tillage X CI	1	148.78	148.78	12.47	<0.01
	Residual	12	143.12	11.93		

Table 14. Soil organic carbon at Conrad in 2002, 2008, and delta SOC (2002, 2008) as affected by tillage and cropping intensity.

Year	Treatment	Soil depth layer (cm)		
		0–10	10–20	20–50
		----- MT C ha ⁻¹ -----		
2002	NT 0.5	16.09	13.76	33.15
	NT 1.0	15.24	12.86	37.19
	TILL 1.0	13.88	12.99	42.34
	TILL 0.5	18.39	14.52	33.45
2008	NT 0.5	15.98	13.64	33.83
	NT 1.0	17.37	13.20	38.66
	TILL 1.0	15.14	12.24	35.35
	TILL 0.5	17.55	14.86	37.88
ΔC	NT 0.5	-0.12	-0.11	0.68
	NT 1.0	2.13	0.35	1.46
	TILL 1.0	1.26	-0.75	-6.99
	TILL 0.5	-0.84	0.34	4.42

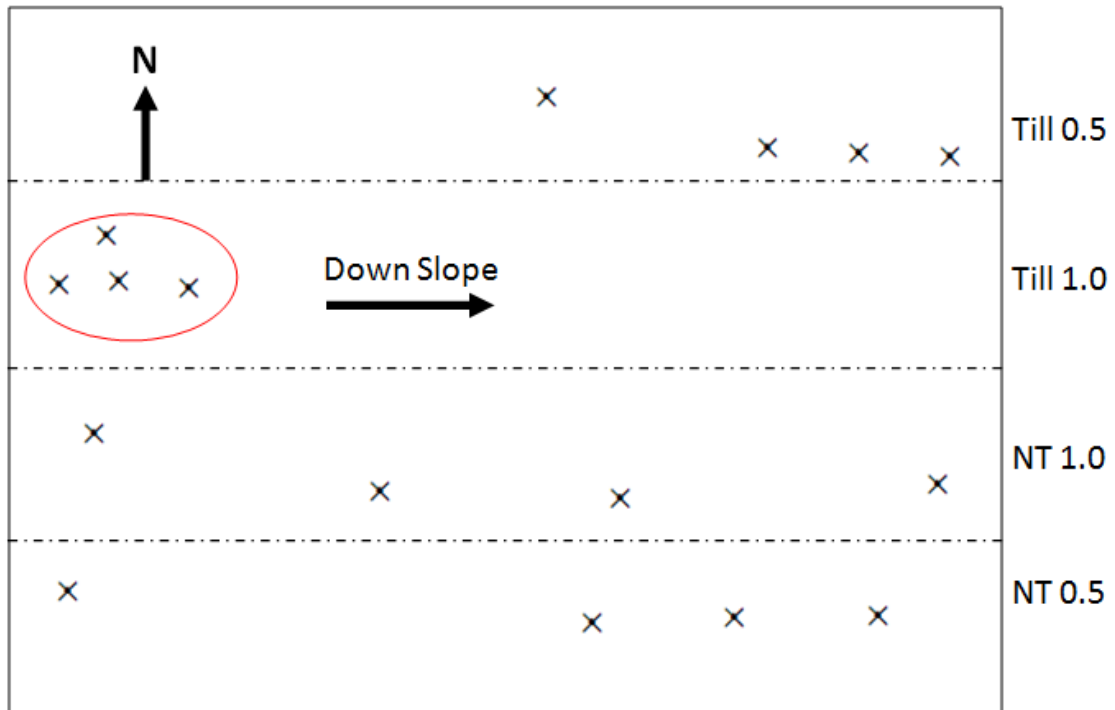


Figure 13. Conrad microsite locations. Treatment annotated with dashed lines.

Fife

Cropping intensity strongly affected delta SOC in the 0–10-cm depth (Table 15). Under continuous cropping there was a net SOC gain of 2.30 MT ha⁻¹ for the 0–10-cm depth. Under alternate-year cropping, the SOC gain was equivalent to 0.41 MT C ha⁻¹ for the 0–10-cm depth. No other significant SOC changes were observed for treatments. The field site at Fife was characterized by high clay content (55%) which created sampling difficulties, especially in the 20–50-cm sampling depth. These sampling difficulties led to large apparent differences in SOC at 20–50-cm between 2002 and 2008.

Table 15. Summary ANOVA table for delta SOC, by depth (2002, 2008) at Fife.

Depth	Source	DF	Sum of Squares	Mean Square	F value	P value
0–10cm	Tillage	1	1.49	1.49	1.510	0.24
	Cropping intensity (CI)	1	14.40	14.40	14.640	<0.01
	Tillage X CI	1	1.18	1.18	1.200	0.30
	Residual	12	11.81	11.81		
10–20cm	Tillage	1	1.21	1.21	0.930	0.36
	Cropping intensity (CI)	1	1.84	1.84	1.400	0.30
	Tillage X CI	1	1.59	1.59	1.210	0.29
	Residual	12	15.69	15.69		
20–50cm	Tillage	1	1.74	1.74	0.190	0.67
	Cropping intensity (CI)	1	27.01	27.01	2.890	0.12
	Tillage X CI	1	0.17	0.17	0.020	0.89
	Residual	12	112.12	9.34		

Table 16. Soil organic carbon at Fife in 2002, 2008, and delta SOC (2002, 2008) as affected by tillage and cropping intensity.

Year	Treatment	Soil depth layer (cm)		
		0–10	10–20	20–50
		----- MT C ha ⁻¹ -----		
2002	NT 0.5	19.18	13.90	27.30
	NT 1.0	19.45	13.37	27.00
	TILL 1.0	19.25	14.84	25.78
	TILL 0.5	19.48	13.66	26.50
2008	NT 0.5	20.16	14.89	33.19
	NT 1.0	21.79	15.67	35.28
	TILL 1.0	21.51	15.96	34.93
	TILL 0.5	19.31	14.73	32.84
ΔC	NT 0.5	0.98	0.99	5.89
	NT 1.0	2.33	2.30	8.28
	Till 1.0	2.26	1.12	9.15
	Till 0.5	-0.17	1.07	6.34

Kremlin

No changes in SOC were evident at the Kremlin site for any depth or any treatment or interactions between treatments (Tables 17 and 18).

Table 17. Summary ANOVA table for delta SOC, by depth (2002–2008) at Kremlin.

Depth	Source	DF	Sum of Squares	Mean Square	F value	P value
0–10cm	Tillage	1	<0.01	<0.01	0.000	0.99
	Cropping intensity (CI)	1	7.84	7.84	2.380	0.15
	Tillage X CI	1	3.12	3.12	0.950	0.35
	Residual	12	39.54	39.54		
10–20cm	Tillage	1	0.38	0.38	0.240	0.63
	Cropping intensity (CI)	1	4.63	4.63	2.970	0.11
	Tillage X CI	1	4.76	4.76	3.060	0.11
	Residual	12	18.69	18.69		
20–50cm	Tillage	1	2.47	2.47	0.050	0.83
	Cropping intensity (CI)	1	1.70	1.70	0.030	0.86
	Tillage X CI	1	62.06	62.06	1.190	0.30
	Residual	12	625.70	52.14		

Table 18. Soil organic carbon at Kremlin in 2002, 2008, and delta SOC (2002–2008) as affected by tillage and cropping intensity.

Year	Treatment	Soil depth layer (cm)		
		0–10	10–20	20–50
		----- MTC ha ⁻¹ -----		
2002	NT 0.5	9.74	9.21	22.48
	NT 1.0	10.75	9.43	22.22
	TILL 1.0	9.75	9.26	24.45
	TILL 0.5	10.31	9.97	24.47
2008	NT 0.5	9.21	9.74	28.24
	NT 1.0	7.93	9.95	31.26
	TILL 1.0	7.80	11.17	28.77
	TILL 0.5	8.89	9.71	33.38
ΔC	NT 0.5	-0.53	0.53	5.76
	NT 1.0	-2.81	0.51	9.04
	TILL 1.0	-1.94	1.92	4.32
	TILL 0.5	-1.42	-0.25	8.91

Sequestration Rates

A wider review looked at sequestration rates for a variety of crops from a global dataset (276 paired treatments) consisting of data from published studies (West & Post, 2002). Results indicate that converting wheat-fallow to a continuous cropped system, with one or more different crops (i.e., replacing fallow with an alternative crop), resulted in SOC sequestration rates of 0.51 +/- 0.47 Mg C ha⁻¹ yr⁻¹. This survey also found no gains in SOC under wheat-fallow when conventional till was converted to no-till. However, if wheat-fallow rotations were excluded from their data set, conversion from

conventional till to no-till resulted in C sequestration rates of $0.57 \pm 0.14 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$.

Recently, studies in the northern Great Plains have identified a range of SOC sequestration rates based on site-specific factors. One study conducted in the semiarid to sub-humid environment of Saskatchewan found annual rates of SOC sequestration in the surface 15 cm resulting from increased cropping intensities (alternate-year wheat contrasted with annually cropped wheat) ranged from 0.03 to $0.43 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ (Mcconkey, et al., 2003). The same study found rates of SOC carbon sequestration associated with no-till management ranged from $0.07 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ to $0.51 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ (Campbell, et al., 2005a). They attributed the variability in SOC sequestration rates from this study largely to the durations of the studies (8–25 years) and the soil texture differences at each site studied (28–63% clay). A second study conducted near Mandan, ND, over 12 years of continuous cropping found that changes in SOC storage (0–15.2-cm depth) associated with no-till, minimal till, and conventional till were occurring at 0.23 , 0.03 , and $-0.14 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ respectively (Halvorson et al., 2002). The effects of no-till management on SOC were consistent with long-term study results published by Campbell et al. (2000).

Our results were generally consistent with the findings from the above discussed research, though were likely hampered by the comparatively short term (i.e., 6 years) of this study. There was no response to tillage or annual cropping at three of six sites. Tillage affected SOC at only one site (Power) and for only one depth (0–10 cm) with no-

till management increasing SOC by $0.27 \text{ Mg ha}^{-1} \text{ yr}^{-1}$, a value that is mid-range relative to previous reports (Campbell et al. 2005). Annual cropping increased SOC in the surface 0–10 cm at three of six sites (Chester, Fife, Power), and additionally in the 10–20-cm soil depth at one of those sites (Chester). Accrual rates ranged from 0.19 to $0.53 \text{ Mg ha}^{-1} \text{ yr}^{-1}$, which is a slightly higher range than reported by McConkey et al. (2003). Interestingly the high value in this range occurred at Chester, the only site with a previous history (>3 years) of annual cropping, i.e., wheat-pulse. It is possible that SOC accrued over this period; then, with the conversion to a wheat-fallow system, SOC levels may have fallen, thus widening the differential between crop-fallow and annual cropping. Similar results have been observed in a cropping system study near Bozeman (Miller, et al., 2009). A review study examined carbon sequestration rates related to cropping frequencies and tillage practices for soils in the semiarid North American Great Plains (McConkey, et al., 2003). The authors of this review were able to identify gains in SOC under no-till management that were $0.20 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ to $0.25 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ greater than rates associated with tilled systems, regardless of cropping frequencies.

3. ACTIVE MICROBIAL BIOMASS CARBON TESTING

To detect change in SOC carbon using traditional analytical techniques, researchers have typically required a minimum of six years after treatments begin (Alvarez, and Alvarez, 2000, Mcconkey, et al., 2003, Bricklemyer, et al., 2005, Bremer, et al., 2008) and still the amount of natural variation among soil samples can confound detection of SOC changes (Robertson, et al., 1997, Al-Kaisi, et al., 2005, Bricklemyer, et al., 2005, Munoz, et al., 2007). Because of these difficulties, researchers have experimented with alternative methods for detecting or predicting changes in soil organic carbon pools (Powlson, et al., 1987, Sparling, 1992, Sparling, et al., 1992, Franzluebbers, et al., 1996, Sherrod, et al., 2005). Theoretically, by measuring a smaller, more dynamic subpool of carbon, it should be easier to detect differences over short time periods due to a reduced variance component associated with the subpool being measured.

To better describe the subpools of SOC, researchers have conceptually divided soil organic carbon into “passive,” “slow,” and “active” pools based on their turnover times in the soil. The passive pool, or resistant fraction, comprises 60–70% of SOC and has a turnover time of 1000–1500 years. This pool consists of lignin and chemically stabilized carbon compounds (Cochran, et al., 2007). The slow pool accounts for about 20–40% of SOC and has a mean turnover time of 25–50 years. This slow pool is comprised of structural plant compounds and physically stabilized carbon (Burke, et al., 1997). The active organic C (AOC) pools make up <5% of SOC and have a mean turnover

time ranging from hours to months (Cochran, et al., 2007). The active organic carbon fraction of soil is comprised of simple sugars, organic acids, and metabolic compounds from incorporated plant residues and soil microbial biomass (Franzluebbers, et al., 2000b). The AOC fractions are also considered to be important for defining plant available nutrient supply, soil structure, and decomposition of natural and synthetic organic amendments (Franzluebbers, et al., 2000b). By looking at the pools with the shortest half-lives, changes in organic carbon relating to treatments can often be detected on an annual or semiannual basis.

Many of the tests employed to measure the active fraction of the total SOC involve rewetting the dried soils in a reaction vessel capable of collecting the gas that evolves following rewetting. This type of test relies on natural microbial processes to convert organic carbon into a gas, which can be collected and measured. As soils are dried, it results in a rapid and total cessation of microbial activity in the sample, which is readily reversible under natural conditions (De Nobili, et al., 2006). Most of the organisms which are capable of surviving for extended periods in dry soils form resistant structures such as endospores, cysts, and other specialized structures, while some organisms such as *Arthrobacter* and some rod-shaped bacteria are capable of withstanding the conditions of desiccation as unmodified cells (Chen and Alexander, 1973, Jackson, et al., 1997). Consequently, the rewetting of the air-dried soil results in a flush of CO₂ attributed to the turnover of soil microbial biomass (Magid, et al., 1999) and the mineralization of soil organic matter made decomposable by the air-drying

process (Jenkinson and Powlson, 1976, Appel, 1998, Franzluebbbers, et al., 2000a, Wu and Brookes, 2005).

One specific test which employs this concept, the Active Microbial Biomass Carbon (AMBC) Test, (Franzluebbbers, et al., 1996, Franzluebbbers, et al., 2000a, Sherrod, et al., 2005) is used to measure a highly reactive fraction of the carbon pool. The AMBC test uses existing soil microorganisms to convert the sugars, starches, and proteins into biomass and respired CO₂. The differences in CO₂ produced from one treatment to the next can be used to estimate differences in the microbial biomass (which existed in the soil at the time the sample was taken). Any differences can then be used as an indicator of changes in the more recalcitrant forms of carbon in the soil such as recently incorporated biomass.

If comparisons could be made between freshly collected samples and samples from the same location stored from previous years, the test could prove more useful in early detection of directional changes which may be occurring in the SOC pool. It was our desire to make these comparisons between AMBC for samples from the Fife and Power sites with samples collected in 2002, and again with samples from the same microsites in 2008. The Fife site was chosen for this test because of the high productivity witnessed there (increased carbon inputs). The Power site was chosen for its long history of no-till management. Due to the dependence on viable organisms for this test to work, there was initial concern about the ability of this test to make comparisons between samples with different storage times due to the effects of soil sample storage.

Soil samples are typically air dried prior to storage. Storage of an air-dried soil sample results in a reduction of viable microbes proportional to the length of the storage period (Sparling and Cheshire, 1979). Any reduction in organisms could potentially result in a smaller fraction of the AMBC being turned over during the incubation period, resulting in lower CO₂ concentrations being evolved from the soil sample simply as an artifact of sample storage time. If there is a storage influence, it could be misconstrued as a treatment affect if the test response was not adequately understood.

Because of the potential sensitivity to detecting early changes with this test, our goal was to employ this test as a means of early detection of directional changes that may not be apparent with the traditional analytical techniques used in SOC analysis. Additionally, we hoped to better define the capabilities of this test for making comparisons between soil samples stored for various lengths of time. The specific objectives of this research were to:

1. Determine the feasibility of applying the methods of the AMBC test to soils stored for varying lengths of time.
2. Detect changes in AOC pools by using the AMBC test.

Materials and Methods

Soil microbial biomass carbon was analyzed using the AMBC test and methods described by L. A. Sherrod (Sherrod, et al., 2005). This involved incubating 20-g soil samples in sealed 1-L canning jars at 30°C and 50% water-filled pore space for 3 days.

Pore space percentage was calculated by weighing samples into 45-mm Wheaton screw cap jars with a line indicating the fill level that would result in a bulk density of 1.0 and using a particle density of 2.65 g cm^{-3} . Respired CO_2 was measured after 3 days using a Varian CP-3800 Gas Chromatograph (Walnut Creek, CA). Concentrations of carbon dioxide were converted to soil microbial biomass using the following equation where “x” is the amount of CO_2 respired expressed in mg C kg^{-1} soil (Sherrod, et al., 2005).

$$Y = 337 + 2.4 x$$

This analysis was done on soil samples collected at the Power and Fife sites, during the fall of 2008. If deemed appropriate by testing to be addressed, comparisons would also be made with soil samples from the same sites collected in, and stored since, 2002.

The results of the AMBC testing were analyzed using the SAS[®] software (SAS Systems for Windows, Release 9.2, SAS Institute, Cary, NC) to model a two-factor ANOVA looking at tillage, cropping intensity, and tillage x intensity interaction.

To determine the feasibility of using the AMBC test across time, the general methods for the AMBC test were conducted as described by Sherrod et al. (Van Elsas, 1995), with modifications to address the effect of storage time on the outcome of the test. Since our focus for this was on the actual testing procedures rather than results related to our specific field site, and in order to ensure adequate soil for repeated testing, the decision was made to use soil local field site. The soil used to assess the effect of storage time on AMBC testing was 20 kg of fresh soil which was collected from the 0–10-cm depth at a field site 6 km west of Amsterdam, MT, during June, 2008. This

soil was returned to the lab and air dried at 40°C for 4 days. After complete drying, the samples were cooled and processed for analysis by removing all plant litter and grinding the sample to ensure it was a homogeneous mixture.

The AMBC test was repeated every 3 months for 15 months using the soil collected from Amsterdam during June of 2008. Additional controls were added to the experiment to better understand the response of time on these tests. These controls consisted of three incubation chambers which contained autoclaved soil from the Amsterdam site rather than fresh soil. The soil for these controls was autoclaved for 1 hour and then allowed to cool overnight. This was repeated for three consecutive days on the same soil. After the third cycle, the 20-g sample was weighed out and placed into the incubation chamber and the incubation container and soil was autoclaved once more for 1 hour to ensure minimal chances of contamination of the soil and incubation chamber. Additionally, the deionized water used to wet the sample was autoclaved to prevent contamination from the water supply system.

Dilution series and spread plates for direct plate counts were also prepared every three months. Agar plates were spread at the time each of the repeated incubations began. Direct plate-count procedures were conducted using site-specific soil extract agar and the procedures outlined below. Spread plates were prepared using both fresh and autoclaved soil from the 2008 Amsterdam collection.

The methods required for quantification and isolation of the various microorganisms present in the soil were as described in the Soil Science Society of

America (SSSA) handbook (Van Elsas, 1995), with the following modifications. Soil extract was prepared by autoclaving 1 kg of soil in 1 L of deionized water for 30 minutes and then allowed to cool at 4°C. Soil used to make the extract was collected from the field site being tested, but not from the area of a sampling star. Once cool, 0.5 g of CaCO₃ was mixed into the extract to induce flocculation of the clay particles and then the mixture was allowed to settle for 12 hours. The liquid portion was decanted and then centrifuged in large Oakridge bottles for 30 minutes at 8000 g and 4°C to separate suspended soil. The supernatant was poured off and filtered through a #42 ashless filter paper. The filtered supernatant was autoclaved for 45 minutes and refrigerated at 4°C until used.

The soil extract agar was prepared using 17 g of agar in 1 L of site-specific liquid soil extract. Cooled plates were refrigerated at 4°C until used. Diluent for dilution series was prepared by mixing 1 L of liquid site-specific soil extract with 1 g of sodium pyrophosphate to make soil-extract-phosphate solution. The mixture was then autoclaved for 45 minutes and stored at 4 °C until needed.

Milk dilution bottles containing 10 g of soil and 90 ml of sterile diluent (10⁻¹ dilutions) were shaken vigorously by hand for 30 seconds and then on a horizontal shaker for 30 minutes. Bottles were allowed to settle for 30 seconds and then 1 ml was removed from just above the settled solid material and used in a 10-fold dilution series using soil extract-phosphate solution. For estimating viable cell counts, 0.1 ml aliquots from the serial dilutions were spread onto soil extract agar plates and then incubated at

28°C for 4 days, after which colony counts were recorded. This was done for both fresh soil and soil which had been autoclaved for three consecutive days. The autoclaved soil was weighed into the milk dilution bottles and autoclaved once more for 1 hour prior to preparing dilution series and spread plates.

These two procedures, the AMBC testing and the direct plate counts, were repeated in tandem every three months for a period of 15 months after soil air-drying.

Results and Discussion

The repeated testing of air-dried soil collected from the Amsterdam site in June of 2008 was done to illustrate the influence of storage time on the outcome of the AMBC test (Figure 14). This figure shows a decreasing trend in the grams of carbon per kg of soil estimated by this test as storage time increases. It was observed that after the initial 6 months of storage the response curve appears to become more stable. Unfortunately, due to ongoing soil sample collecting, we were not able to begin data collection at time zero; so any attempt at fitting a response curve is speculative.

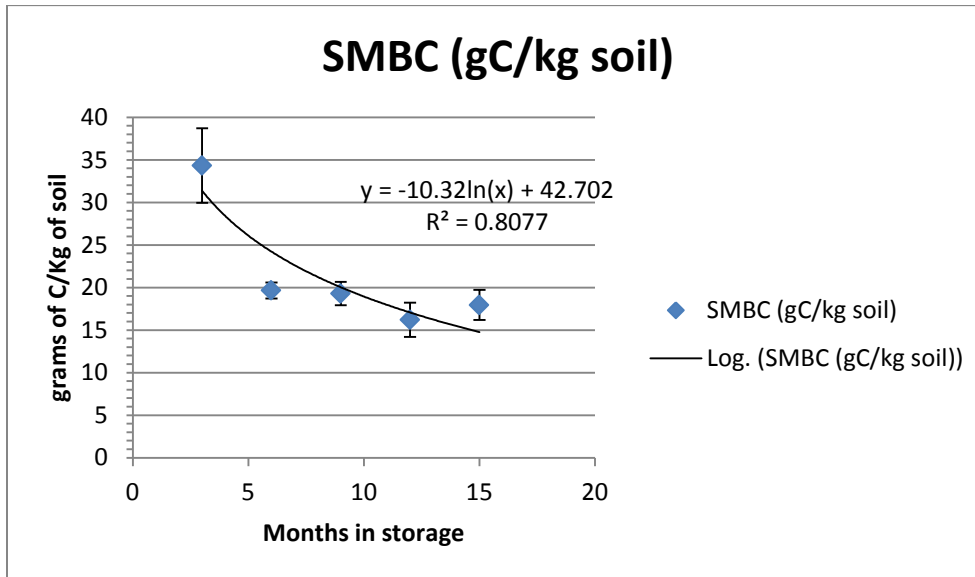


Figure 14. Active microbial biomass carbon test storage response on soil over time. Estimated g C/kg of soil on the vertical axis with the months of storage on the horizontal axis and the standard deviations shown as error bars for the respective tests.

In addition to the repeated AMBC testing, viable plate counts were conducted in parallel (Figure 15). Viable counts (CFUs) decreased significantly as a function of time, potentially stabilizing at the 12- and 15-month time points after a year of storage.

When the results from the repeated plate counts are correlated with the results from the repeated AMBC testing, it is observed that there is a decrease in viable cells in a soil sample with an increase in storage time, which results in a decrease in respired CO₂ during the AMBC test.

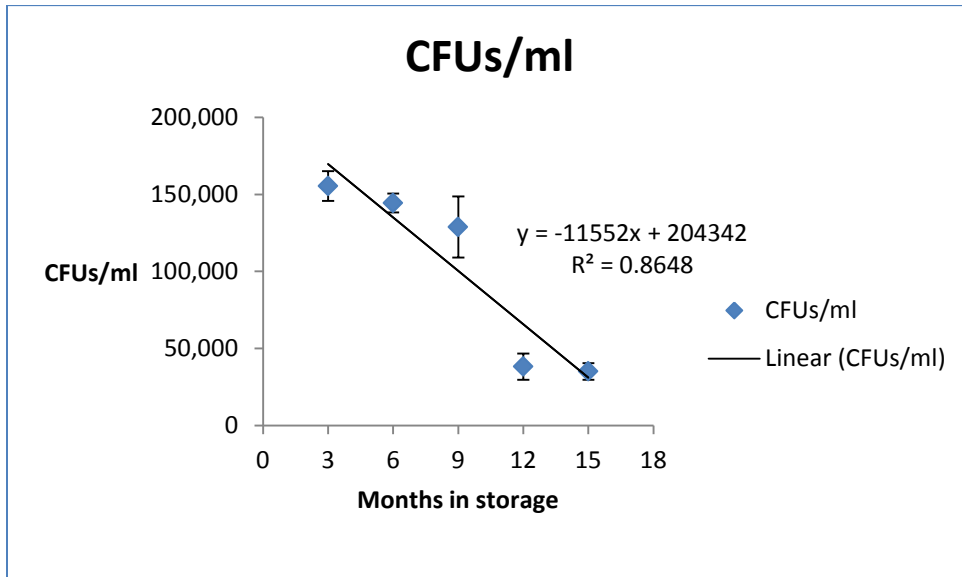


Figure 15. Direct plate count results. This figure displays the CFUs/ml on the vertical axis with the months of storage on the horizontal axis and the standard deviation depicted as error bars.

These plate count results are consistent with data from a previous study in which the viability of bacteria from soil samples was measured over time. That study showed a decrease in viable cells with increased storage time (Sparling, and Cheshire, 1979). A graph created from their published results can be seen in Figure 16.

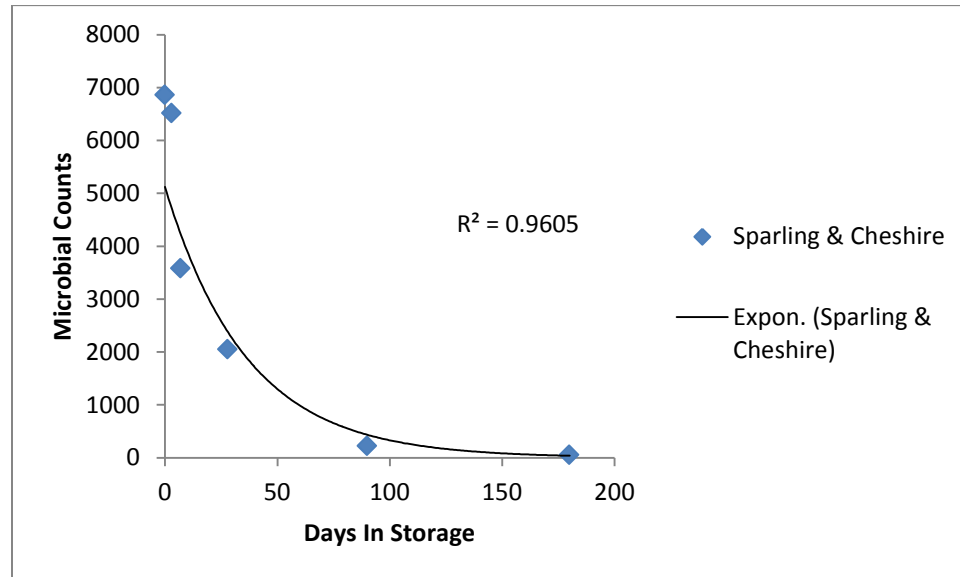


Figure 16. Data plotted from Sparling and Cheshire, 1979, displaying decreased microbial counts related to storage time.

As previously stated, in addition to the fresh soil being tested with the AMBC test, autoclaved samples were included in each run as sterile controls. This was done to determine the ability of the autoclave to sterilize the fresh soil samples and then understand the response from conducting the AMBC test on these autoclaved samples. If the samples could be sterilized, they could then be inoculated with a controlled concentration of microbes as a way to standardize the AMBC test and eliminate any storage effect resulting from differing microbial concentrations in samples with different storage times.

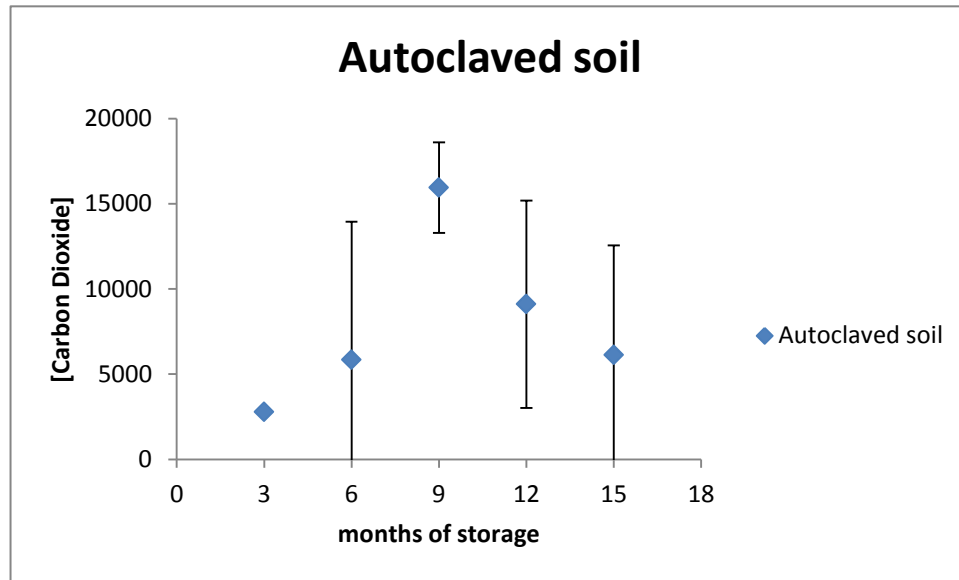


Figure 17. Active microbial biomass carbon test with autoclaved soil. The vertical axis displays the concentration of CO₂ produced during the AMBC test for the autoclaved soil, the horizontal axis describes months of storage with the standard deviations for each.

While this graph shows considerable concentrations of CO₂ being evolved, it is the variability with time that was most puzzling. Soil extract agar plates spread using a subsample of the autoclaved soil had no growth for all the repeated experiments yet CO₂ was still being produced in the AMBC test with autoclaved soils. Further studies would be required to understand this response and, for that reason, no further modifications to the test using inoculum were investigated in this study.

From the outcomes of the repeated AMBC testing, the direct plate counts, (Figures 14 and 15) and the failed attempts to standardize the test with sterilized soil, it was determined that comparisons made between freshly collected and stored soils would likely result in the storage effect becoming a crucial confounding factor for

determining changes in AMBC over time. Because of this concern, comparisons between soils stored for different lengths of time (i.e., multiple sample years) were not made. Instead comparisons between treatments for the 0–10-cm soil profile from the Fife and Power sites and sample year 2008 were made.

Results from the AMBC test were inconclusive for both the Power and Fife sites. An ANOVA comparing treatment, plot, and treatment x plot interaction showed no significant differences ($P > 0.05$; Tables 19 and 20) despite delta SOC being significant for cropping intensity in the 0–10-cm soil depth at both sites, and tillage at Power (Tables 9 and 15).

Table 2. Summary ANOVA table from AMBC test, Power, 2008.

Source	DF	Sum of Squares	Mean Square	F value	P value
Tillage	1	9552821	9552821	0.470	0.498
Cropping intensity (CI)	1	41314050	41314050	2.040	0.164
Tillage X CI	1	65362461	65362461	3.230	0.0833
Residual	28	567436443	20265587		

Table 20. Summary ANOVA table from AMBC test, Fife, 2008.

Source	DF	Sum of Squares	Mean Square	F value	P value
Tillage	1	450063	450063	0.010	0.906
Cropping intensity (CI)	1	6259607	6259607	0.200	0.661
Tillage X CI	1	85363445	85363445	2.690	0.1124
Residual	28	889867793	31780993		

Conclusions

Soil samples which have been air-dried and stored are subject to a reduction in microbial populations which is inversely related to the storage time of the sample (Sparling and Cheshire, 1979). This reduction in organisms was observed experimentally and resulted in a decrease in the amount of CO₂ evolved over the three-day incubation period of the AMBC test. Because of this result, we were unable to make unbiased comparisons of soil samples from different collection years.

With the peculiar responses from the repeated AMBC test on autoclaved soils, it was determined that any attempts to standardize this test by using autoclaved sterilized soil would have to be further investigated. Because of this, no standardizing modifications to the AMBC test can be recommended at this time for comparing samples with different storage times.

Comparisons between treatments for the Fife and Power sites conducted 1 year after the samples were collected showed no differences in AMBC. Given the above test results demonstrating a storage effect on AMBC, failure to detect AMBC differences between the Fife and Power soils is not conclusive. The results from both trials of the AMBC and the finding from previous studies (Sparling, and Cheshire, 1979) would suggest that detecting differences between AMBC is best done if the test is performed in a timely manner (<3 months) after the sample is collected and air dried. As storage time increases, there is a decrease in the response of the test related to a decrease in

viable soil microorganisms remaining in the sample, making detection of differences more difficult (Sparling and Cheshire, 1979).

4. SUMMARY AND CONCLUSIONS

To determine the rates of terrestrial carbon sequestration resulting from both increased cropping frequencies and decreased tillage in north central Montana's dryland wheat production, soil samples were collected biannually with yields and management monitored annually over the course of six years. It had been hoped that six years of consecutive management would result in cumulative changes in SOC, which could be detected and used to determine annual rates of terrestrial carbon sequestration resulting from the two treatment factors.

Unfortunately, the differences between the baseline SOC data and the SOC data from the sixth year of the study did not result in comprehensive findings consistent with well-established carbon sequestration trends for either of the treatment factors being studied. While three sites were observed to have increased SOC in the continuously cropped treatments, three did not show an increase. One site showed an increase in SOC in the surface depth from no-till management while five showed no effect. Additionally, these comparisons even resulted in a suggested change for SOC in the 20–50-cm soil depth at one site, which was identified as highly improbable. Based on the inconsistent SOC findings with only three out of six sites showing SOC sequestration after six years of continuous management focused on SOC sequestration, it would be suggested that six years of management is an insufficient amount of time to consistently quantify rates of change in SOC related to management practices in Montana's Golden Triangle.

An additional test, the AMBC test, was employed as a means of detecting differences in active microbial biomass carbon pools as a means of indicating changes that are occurring in the total SOC pool. The results of the AMBC test showed no statistical difference between the treatments for the two sites tested; but the signal may have been diminished due to one year of storage time for the soil samples prior to testing.

Attempts to characterize the response of the AMBC test to sterilized soil produced unexpected results which need to be further investigated, so modifications to the AMBC test using sterile soil as a means of standardization across time cannot be recommended at this time.

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APPENDICES

APPENDIX A

POTENTIAL SOURCES OF ERROR

Even with the use of 2008 bulk densities and mass equivalency adjustments, there remained considerable variability in calculated soil organic C levels for 2002. The variability in this 2002 “baseline” likely affected estimates of SOC change over the six years of this study. Given these uncertain results and the potential discrepancies in SOC change, we examined the protocols employed from sample collection to sample analysis to identify potential sources of error. An analysis of potential sources of error or noise was conducted of raw data components; inorganic C, total carbon, and bulk density.

Inorganic Carbon Data Component

To better understand the relationship between the 2002 and 2008 soil inorganic carbon (SIC) values, scatter plots were created to inspect potential error relationships by site and depth. For each of the six sites, the 2002 SIC values are plotted on the vertical axis; the 2008 SIC values on the horizontal axis.

Figure 18 shows the comparisons for the Dutton site. This figure shows a fairly even distribution of values with respect to the 1:1 line. This is consistent with the mean values for this site. The 2002 mean SIC was 5.96 kg IC per mg soil with a standard deviation of 4.95 while the 2008 values were 6.04 and 4.98 respectively. Based on the mean SIC values and the scatter plot distribution, it would seem that the SIC values for this site are fairly consistent from 2002 to 2008.

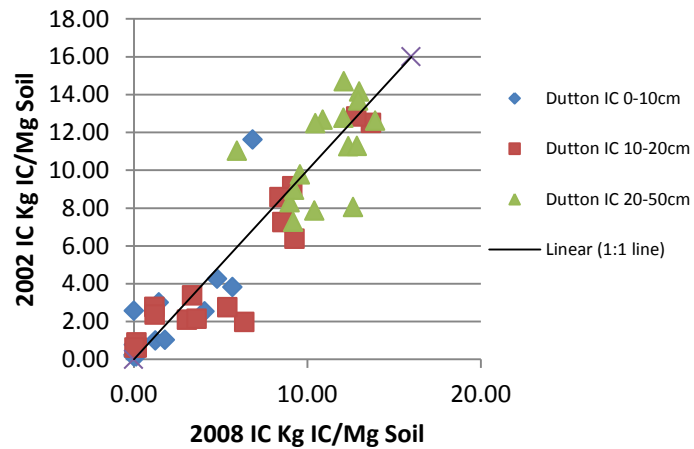


Figure 18. Scatter diagram of 2002 vs. 2008 SIC across three depths (0–10, 10–20, and 20–50 cm) at Dutton, MT.

Figure 19 shows the SIC values for the Power site. This figure shows that the SIC values from the Power site for the 0–10 and 10–20-cm depths were more tightly distributed (had less variation) than the values for the subsequent depth (20–50 cm). It can be observed that many SIC values are slightly greater in 2008 than in 2002, as determined by their relationship to the 1:1 line. This is consistent with the mean SIC values for this site which were measured from 2002 to be 23.27 kg IC per mg soil with a standard deviation of 16.83, and for 2008, the values were 26.24 and 18.20 respectively. Much of the variability in the 20–50-cm depth would likely be due to the extremely high SIC values found at this site.

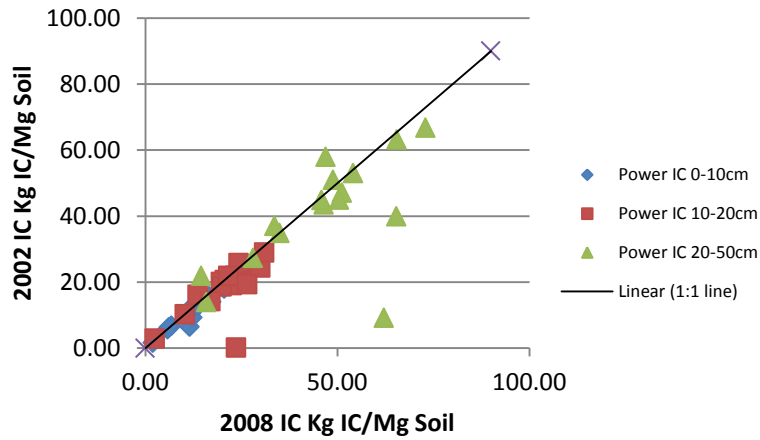


Figure 2. Scatter diagram of 2002 vs. 2008 SIC across three depths (0–10, 10–20, and 20–50 cm) at Power, MT.

Figure 20 displays the results from the SIC comparison at the Chester site. This figure shows SIC values for this site are fairly uniform between sample years 2002 and 2008. This is consistent with the mean SIC values measured at this site. The 2002 samples had a mean SIC of 9.14 kg IC per mg soil with a standard deviation of 8.04, while the 2008 values were 8.74 and 8.21 respectively.

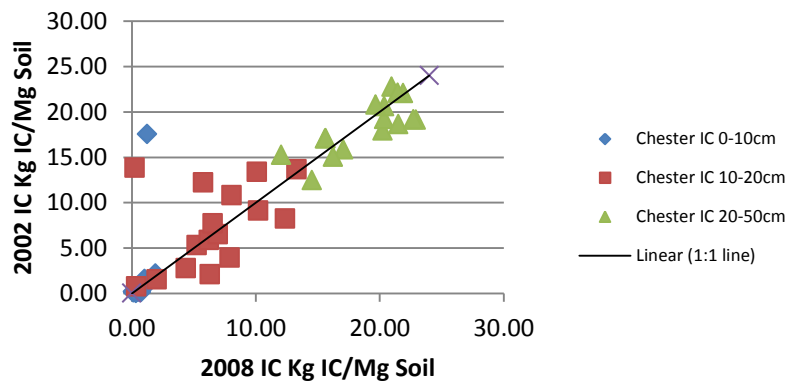


Figure 20. Scatter diagram of 2002 vs. 2008 SIC across three depths (0–10, 10–20, and 20–50 cm) at Chester, MT.

Figure 21 shows the SIC comparisons for the Conrad site. Examination of this SIC data from these two years and their relationships to the 1:1 line reveals extreme variability for all depths resulting in a decrease in the signal-to-noise ratio. The mean SIC values for this site would suggest slightly greater 2002 SIC values with the 2002 mean value being 4.52 kg IC per mg soil and having a standard deviation of 5.39 compared to the 2008 values of 3.97 and 5.25 respectively.

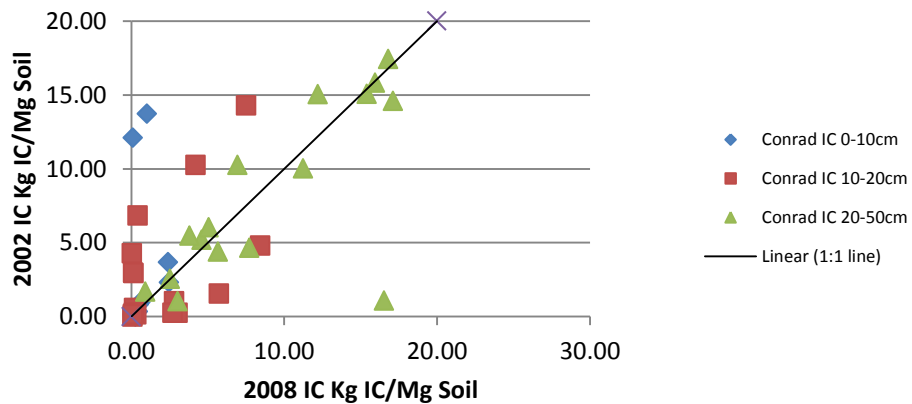


Figure 3. Scatter diagram of 2002 vs. 2008 SIC across three depths (0–10, 10–20, and 20–50 cm) at Conrad, MT.

Figure 22 displays the comparisons for SIC values at the Fife site for the 2002 and 2008 sample years. This figure reveals most of the data points are evenly distributed with tight groupings and only slightly greater values in 2008 than in 2002 at Fife. One obvious outlier does exist in the data set from this site making the distribution seen in this figure artificially tight. The mean SIC values for this site were measured to be 5.78 kg IC per mg soil in 2002 with a standard deviation of 7.91, while in 2008 these values were 3.94 and 3.73 respectively.

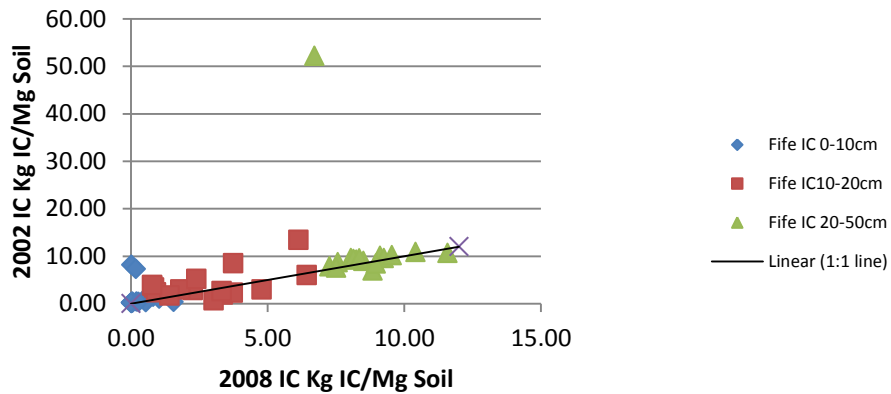


Figure 4. Scatter diagram of 2002 vs. 2008 SIC across three depths (0–10, 10–20, and 20–50 cm) at Fife, MT.

Figure 23 displays the SIC comparisons for the Kremlin site. This figure reveals that the SIC values have a great deal of variability for all depths. While a great deal of variability exists within the groupings, the mean SIC value at this site remained steady over the sample years. This SIC value in 2002 was 4.60 kg IC per mg soil with a standard deviation of 5.44 compared to 2008 with values of 4.55 and 4.71 respectively.

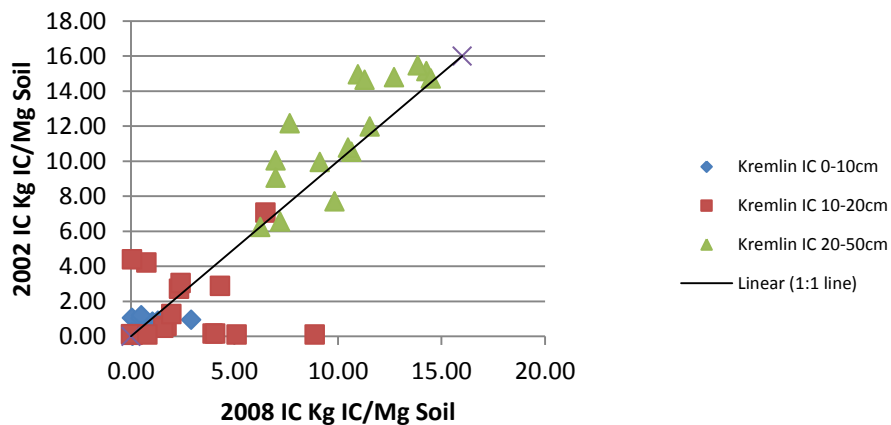


Figure 5. Scatter diagram of 2002 vs. 2008 SIC across three depths (0–10, 10–20, and 20–50 cm). Kremlin, MT.

The mean inorganic carbon values, measured for sample years 2002 and 2008 and listed by site, are summarized in Table 21 below. This table shows that there is only a slight variation in the SIC values for years 2002 and 2008.

Table 21. Mean inorganic carbon (IC) by site in (0–50 cm) 2002 and 2008. Standard deviation appears in parentheses.

Site	2002 Kg IC Mg soil	2008 Kg IC Mg soil
Dutton	5.96 (4.95)	6.04 (4.98)
Power	23.27 (16.83)	26.24 (18.20)
Chester	9.14 (8.04)	8.74 (8.21)
Conrad	4.52 (5.39)	3.97 (5.25)
Fife	5.78 (7.91)	3.94 (3.73)
Kremlin	4.60 (5.44)	4.55 (4.71)

We would expect the inorganic carbon concentrations to remain relatively constant over the course of this study. The amount of variation seen in Table 19 and Figures 18–23, is likely not the major source of the noise observed in the 2002 to 2008 SOC comparisons, but is likely contributing to it. It is also important to recognize that the values (2002 vs. 2008) seen at the Power and Fife sites might warrant further investigation to determine why these two sites, which have the least amount of topographical variation of the six sites, and are very different in virtually all physical aspects. Both have inorganic carbon values that differ from the other four sites in both mean IC concentrations and the standard deviations of those values by the amounts

they do for the two years compared. Table 21 would suggest that there has been an increase in inorganic carbon at the Power site and a decrease at the Fife site.

Total Carbon Data Component

The next data component we analyzed was the total carbon values as measured by dry combustion analysis (described in materials and methods section). Understanding the origination of the variability within this data can be quite useful for identifying the possible source of noise in our 2002–2008 SOC comparisons due to the high degree of analytical certainty in the total carbon values as determined by dry combustion. When these values show a difference between the two years compared, it would suggest an increase or decrease in total carbon in the system or a sample processing issue. The means for the total carbon concentrations can be seen in Table 22.

Table 22. Mean total carbon concentration by site in (0–50 cm) 2002 and 2008. Standard deviation appears in parentheses.

	2002 Kg C/Mg soil	2008 Kg C/Mg soil
Dutton	13.26 (15.57)	13.08 (3.63)
Power	34.14 (15.04)	36.33 (16.23)
Chester	16.60 (6.03)	16.00 (6.96)
Conrad	14.76 (6.27)	14.22 (4.63)
Fife	16.44 (6.69)	16.09 (1.80)
Kremlin	11.24 (4.70)	11.38 (4.06)

The results seen in Table 22 would suggest that for most of the sites listed, the mean total carbon concentration has slightly decreased or remained constant over the

six years of the study. It is interesting to note the decreased variability in the 2008 total carbon numbers for four of the six sites. Because of the decreases in both mean total carbon concentrations and their respective standard deviations, the sample collection and preparation procedures were studied in depth.

Soil Organic Carbon Data Component

To better understand the relationship between the 2002 and 2008 SOC values, scatter plots were created to inspect potential error relationships by site and depth. For each of the six sites, the 2002 SOC values are plotted on the vertical axis; the 2008 SOC values on the horizontal axis.

Figure 24 shows the comparisons for the Dutton site and displays an obvious outlier at this site (marked with an arrow), which was removed from the data set for the final 2002–2008 delta C analysis. It can be observed that many of the SOC values for the 0–10 and 10–20-cm depths were greater in 2002 than in 2008 (as defined by their location above the 1:1 line).

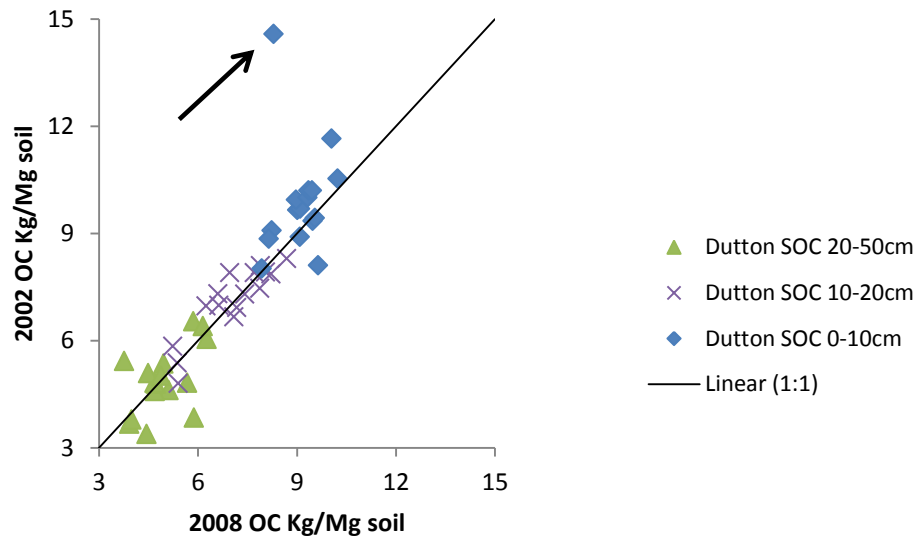


Figure 6. Scatter diagram of 2002 vs. 2008 SOC across three depths (0–10, 10–20, and 20–50 cm) at Dutton, MT. Arrow indicates outlier.

Figure 25 displays comparisons of SOC for the Power site. This figure shows that the SOC carbon values for the 0–10-cm depth were more tightly distributed (had less variation) than the values for the subsequent depths (10–20 and 20–50 cm) at Power. It can also be observed that the 10–20 and 20–50-cm depths have SOC values which are greater in 2002 than in 2008, which would indicate either true losses of carbon in these depths or a concern in the sample collection and analysis protocols for these two years' samples.

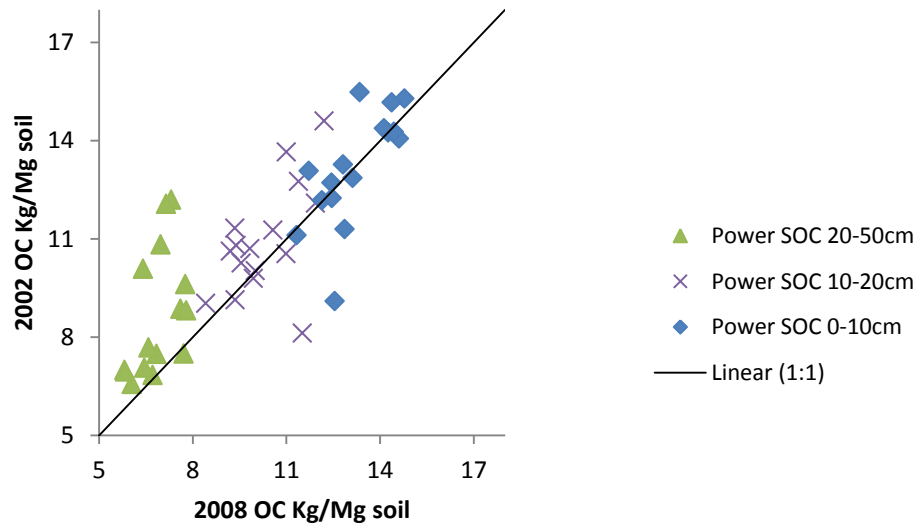


Figure 7. Scatter diagram of 2002 vs. 2008 SOC across three depths (0–10, 10–20, and 20–50 cm) at Power, MT.

Figure 26 displays the results from the SOC comparison at the Chester site. This figure reveals an obvious outlier (marked with arrow) that was removed from the data set for the 2002–2008 delta SOC analysis at Chester. The 2002 SOC values generally lie above the 1:1 line indicating greater measured SOC in 2002 than 2008; again either indicating losses of carbon or a bias in the sampling or analysis procedures. The variability of these values again seems to increase with depth, resulting in a decreased signal-to-noise ratio with increased depth making detecting differences in SOC more difficult. It can also be observed from examining Figure 26 that the apparent bias in the SOC values increased with sample depth.

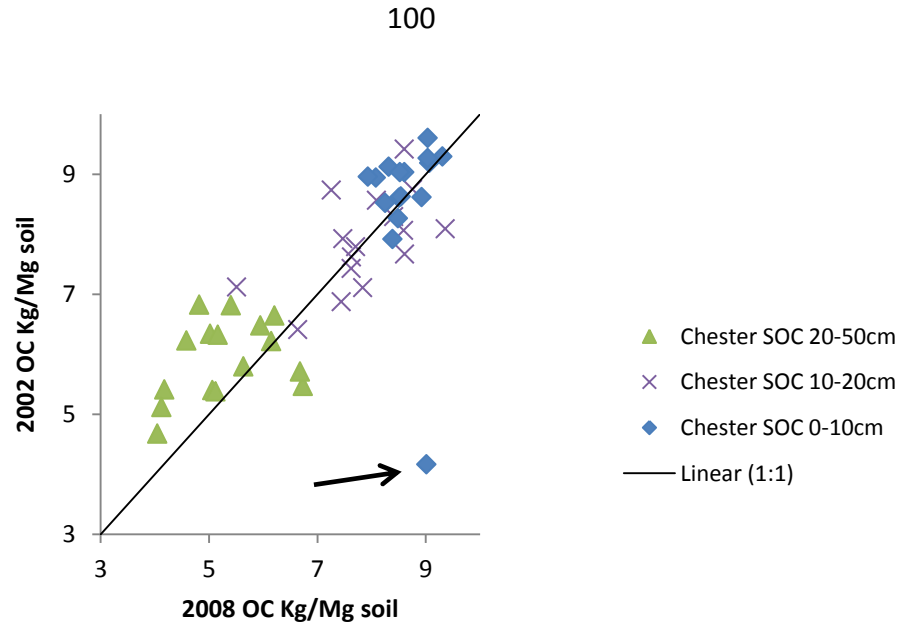


Figure 8. Scatter diagram of 2002 vs. 2008 SOC across three depths (0–10, 10–20, and 20–50 cm) at Chester, MT. Arrow indicates outlier.

Figure 27 shows the comparisons for the Conrad site. This figure revealed an obvious outlier (marked with arrow) that was removed from the data set for the 2002–2008 delta SOC analysis at Conrad. Examination of the SOC data from these two years and their relationships to the 1:1 line reveals that, for all depths (0–10, 10–20, and 20–50 cm), the SOC values were generally greater in 2002 than in 2008. It can also be observed, based on the relationships of the SOC values to the 1:1 line, the variability in the SOC values increased with increased depth from the surface, again resulting in a decrease in the signal-to-noise ratio making detection of differences in SOC more difficult with depth.

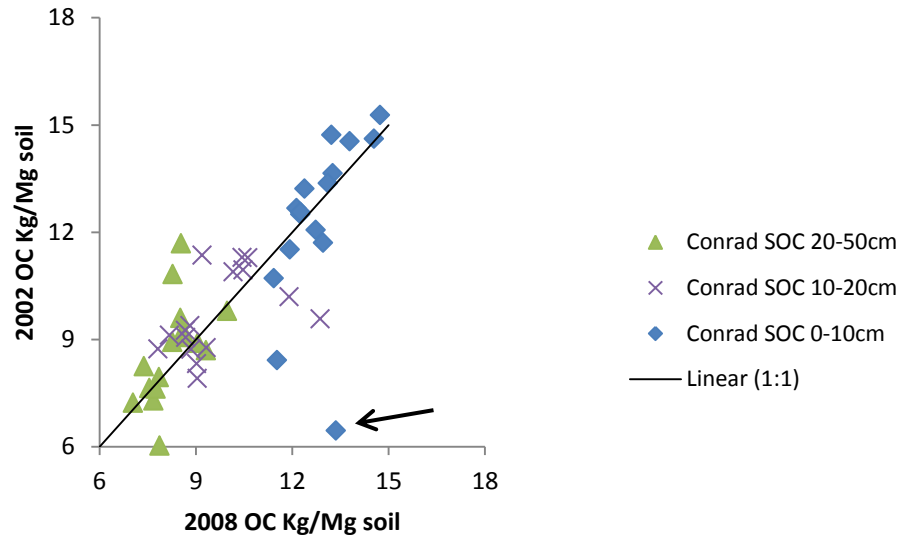


Figure 9. Scatter diagram of 2002 vs. 2008 SOC across three depths (0–10, 10–20, and 20–50 cm) at Conrad, MT.

Figure 28 displays the comparisons for SOC values at the Fife site. This figure reveals that most of the data points fall below the 1:1 line, indicating SOC estimates were greater in 2008 than 2002 at Fife. This is contrary to the results from Dutton, Power, and Chester. This data alone might suggest carbon is actively being sequestered at this site. However, given the results at the Dutton, Power, and Chester sites these gains in SOC are subject to some uncertainty. It is also interesting to note the distinct separation in the SOC values by depth that occurs at this site for both years sampled indicating a large gradient (a decreasing trend) in SOC from the surface to the 50-cm depth.

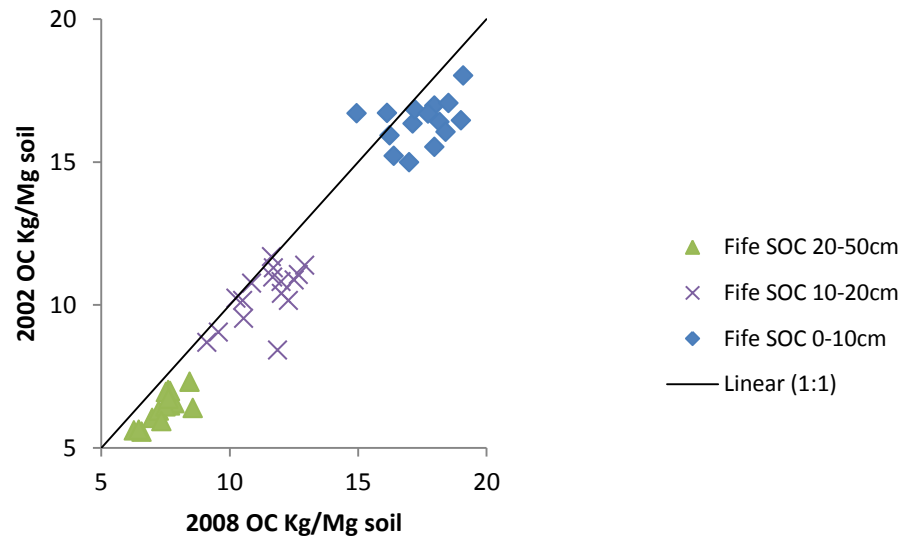


Figure 10. Scatter diagram of 2002 vs. 2008 SOC across three depths (0–10, 10–20, and 20–50 cm) at Fife, MT.

Figure 29 displays the SOC comparisons for the Kremlin site. This figure reveals that the SOC values again have greater variability with depth, consistent with the results seen at Conrad, Chester, and Power. For the 20–50-cm depth, SOC values appear greater in 2008 than in 2002, which would suggest a very unusual (and unlikely) pattern in SOC gain at this site. This may simply be the result of underestimated carbon values for the 2002 samples resulting from protocol concerns to be discussed later.

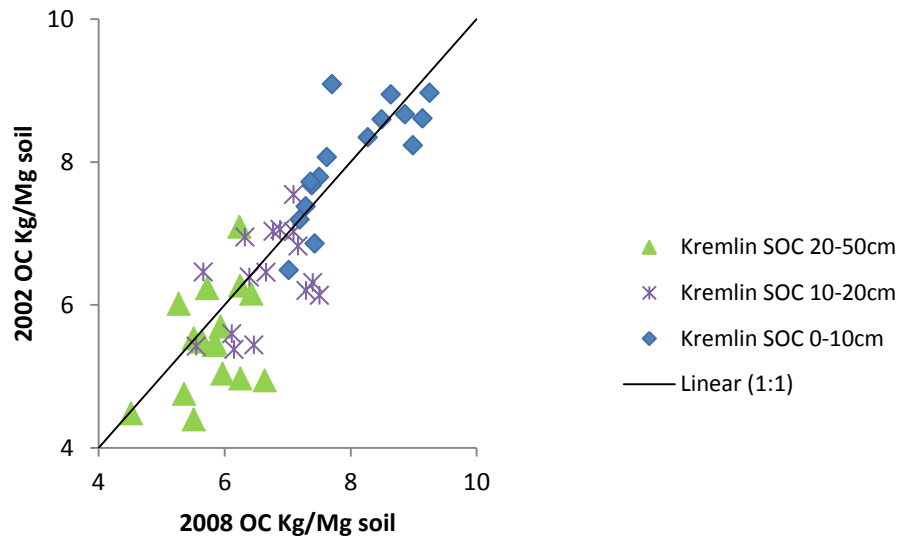


Figure 11. Scatter diagram of 2002 vs. 2008 SOC across three depths (0–10, 10–20, and 20–50 cm). Kremlin, MT.

Bulk Density Data Component

The last data component comparison that was investigated was that between bulk density values by site for years 2002 and 2008. While the 2008 bulk densities were used to determine carbon values associated with the 2002 data set, this discussion will outline why this was done. Table 23 summarizes the mean values and the respective standard deviations for the 2002 and the 2008 raw bulk densities (not adjusted for rocks). These raw bulk densities were used for this comparison because the percent rock fraction by microplot was incomplete for the 2002 soil samples.

Table 23. Mean bulk density and standard deviations by site in 2002 and 2008.

	2002	2008
	----- gm cm ⁻³ -----	
Dutton	1.57 (0.12)	1.48 (0.09)
Power	1.33 (0.05)	1.34 (0.07)
Chester	1.48 (0.08)	1.44 (0.04)
Conrad	1.44 (0.09)	1.40 (0.05)
Fife	1.43 (0.07)	1.36 (0.10)
Kremlin	1.54 (0.09)	1.48 (0.09)

This table indicates that, for most sites (Dutton, Chester, Conrad, Fife, and Kremlin), the bulk densities were greater in 2002 than in 2008 while the standard deviations remained relatively constant. Since these numbers are the result of weights measured immediately after removal from the oven (no physical or mathematical manipulation conducted on the samples), this would suggest that there was a systematic problem associated with sample collection which was constant for all sites sampled within a sampling year. The most likely problem identified is the requisite blind soil sampling (soil sampling sleeve without observation slot) conducted in 2002 may have resulted in unobserved compaction of the 2002 soil samples. To better understand the relationships between the 2002 and 2008 samples, the raw bulk density values for each site were plotted in scatter diagrams with the 2002 values on the vertical axis and the 2008 values on the horizontal axis. A simple 1:1 line has been added to better illustrate the distribution relationships between the bulk densities of the soil samples for these two sampling years. These plots can be seen in Figures 30–35.

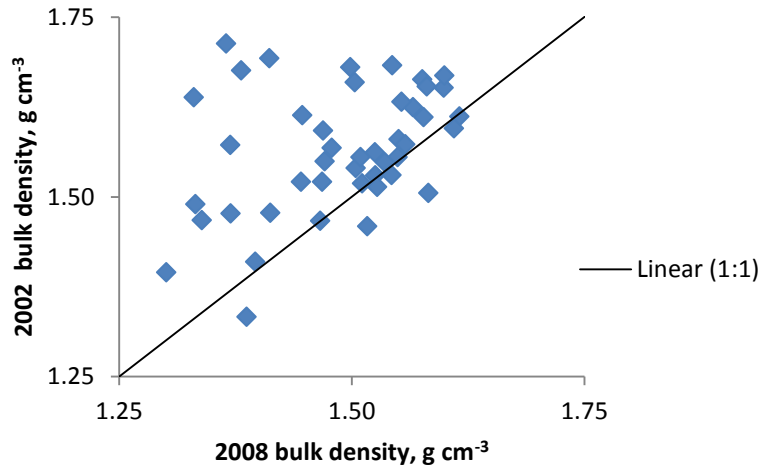


Figure 30. Scatter plot of 2002 vs. 2008 bulk density for the Dutton site.

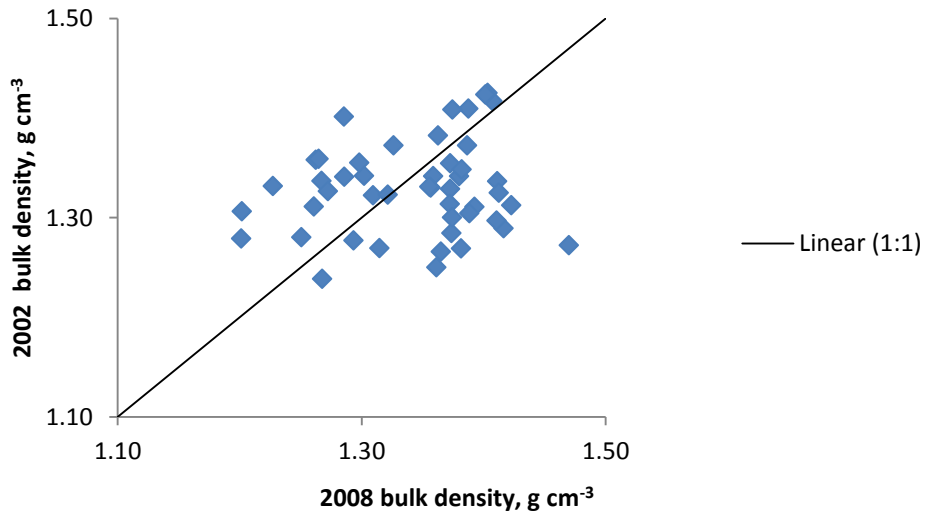


Figure 31. Scatter plot of 2002 vs. 2008 bulk density for the Power site.

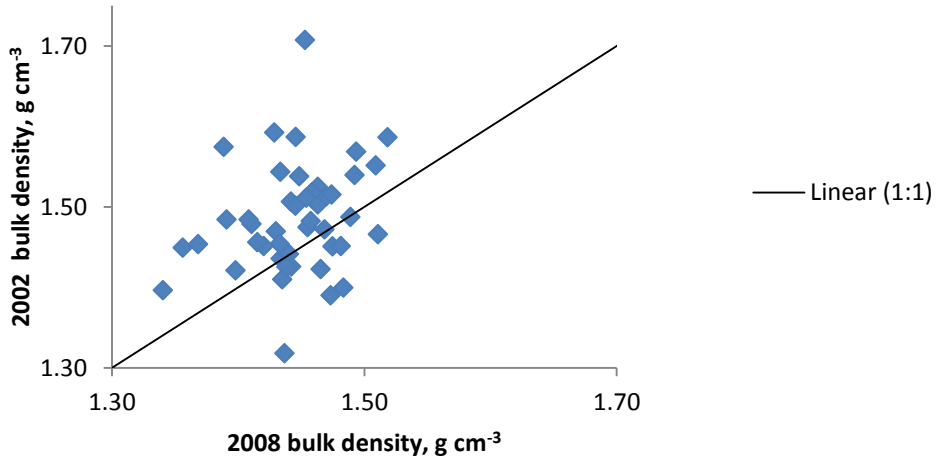


Figure 32. Scatter plot of 2002 vs. 2008 bulk density for the Chester site.

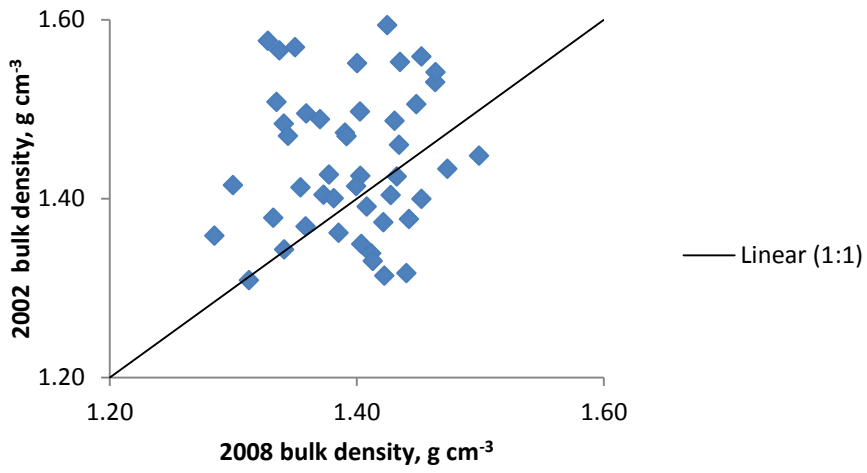


Figure 33. Scatter plot of 2002 vs. 2008 bulk density for the Conrad site.

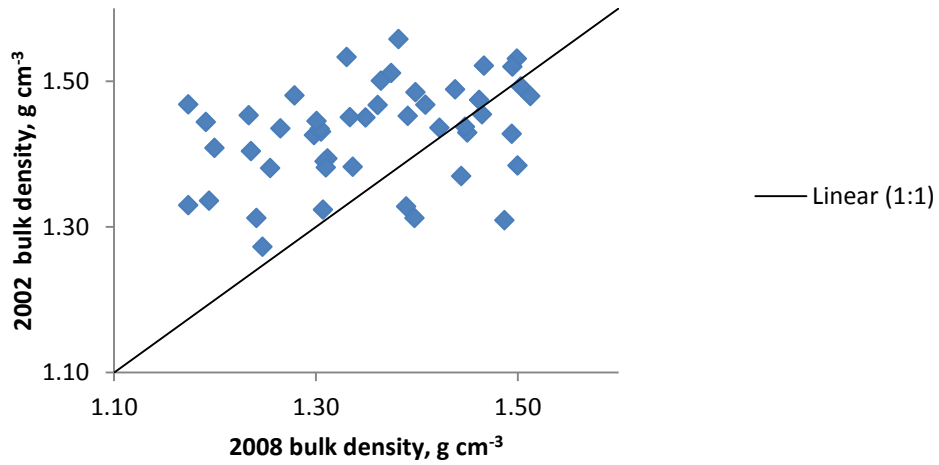


Figure 34. Scatter plot of 2002 vs. 2008 bulk density for the Fife site.

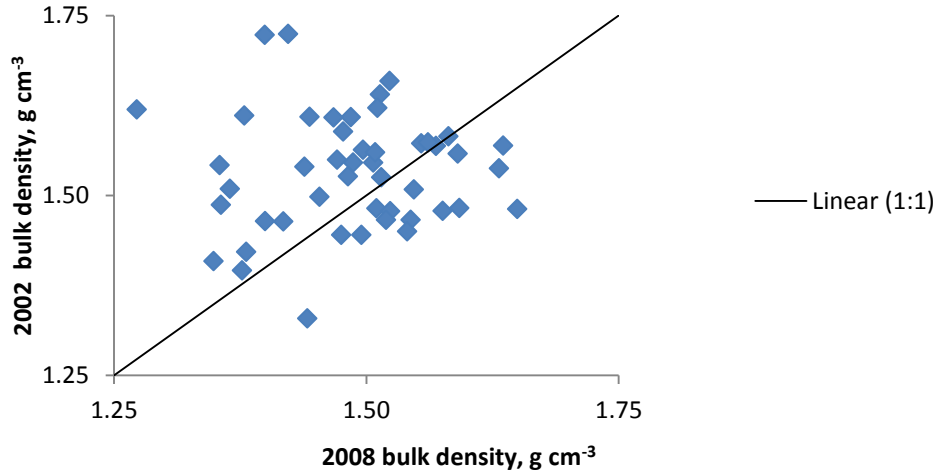


Figure 35. Scatter plot of 2002 vs. 2008 bulk density for the Kremlin site.

In Figures 30–35, the bulk densities measured in 2002 for most sites are greater than in 2008, as indicated by the greater number of points plotted above the 1:1 line.

This concern with the bulk density is a major one because the bulk density value is first

used to determine the mass of carbon in the profile and secondly to mathematically adjust the mass of cores for mass equivalency adjustments. For this reason, and because this concern was identified early, all SOC calculations in this document were made using the 2008 bulk density values.

Had we used the bulk densities from 2002, the resulting SOC values would have been even greater for 2002 suggesting even greater (and more unlikely) losses of carbon for the 2002–2008 comparisons. This increase in SOC for the 2002 samples would be the result of two separate steps in calculating SOC. First, when the SOC concentration is multiplied by the soil mass (derived from the bulk density) in the depth being calculated. If the bulk density is artificially high, the resulting mass of SOC in the profile will be as well. These numbers can become even more skewed when the SOC mass-per-unit-area values are mass adjusted for equivalency at each site based on the mass (again derived from the bulk density) of the lightest core. This mass adjustment again involves calculations involving soil mass, so any soil mass values which are artificially high (from compaction) would result in increased estimates of SOC.

Identified Concerns

The results seen in the total and organic carbon numbers are either the result of true losses of carbon from the sites being tested or the result of errors in the various steps involved with collecting, processing, and analyzing the samples. Since we have no way to determine if there were true losses of carbon from these sites, we spent a great

deal of time looking at the protocols and procedures employed in both years 2002 and 2008 as a way of explaining the decreases in total carbon.

We were able to identify two specific points of concern related to processing of 2002 soils cores. In 2002, soil samples were first ground through a 2-mm screen using a flail mill. Soils with high clay contents, such as found at the field sites, will form hard aggregates that do not easily break after drying. Hence, the flail mill ground only a portion of the total sample, and only the fraction that passed through the screen was used to make composite samples. The composite samples for each microplot were comprised of ~ 60-g subsamples from each of the five star-points. There are two potential problems associated with this step.

The first concern with the method previously described is that the composites were not made from a homogenous mixture of the soil core but rather a fractional portion of the sample. The portion of the samples which would have been most friable and passed through the 2-mm screen would most likely have been the fraction of the cores with the greatest concentrations of SOC. To correct this problem in 2008, the entire sample was ground to pass through a 2-mm screen and then thoroughly mixed before composite samples were prepared. This also ensured that all rock fragments were accurately weighed for each of the segmented soil cores resulting in a more accurate estimate of rock corrected bulk density.

The second concern with the original composite preparation is the amount of soil from each core used to make composites. Originally composites were made using

approximately 60 g of ground soil. This procedure may have resulted in one of the five cores being over- or under-represented (more or less than 60 g) from the non-representative subsample from the core in the eventual composites. This alone may not result in large deviations in the resulting data; however, these bulk samples from which subsamples were taken, were not a homogeneous mixture of all the soil from the soil core for a given depth, which could further skew any problems associated with this approximate subsampling procedure. To address this issue in 2008, composite samples were made using 30.0 g \pm 0.1 g of soil from the homogenous mixture for each soil core depth segment to ensure equal representation of all five cores forming the composite.

APPENDIX B

ANALYSIS OF SOC ASSUMING A CONSTANT BASELINE
SOC AS AFFECTED BY CROPPING INTENSITY
AND TILLAGE IN 2008

Because of the concerns with the bulk densities and the SOC values related to the 2002 data set, the only treatment comparisons that could be made with confidence are from the 2008 data set. This determination is based on the fact that comparisons of SOC from 2002 to 2008 result in values of SOC change which contradict previously reported responses (Mcconkey, et al., 2003). These unusual results lead to our investigation of the data and the potential source of error related to the values which have ultimately resulted in questions about the 2002 soil processing procedures.

Dutton Site

Results from the ANOVA's run on the 2008 SOC data from Dutton in 2008 are displayed in Table 24.

Table 24. Summary SOC ANOVA table by depth (2008) at Dutton.

Depth	Source	DF	Sum of Squares	Mean Square	F value	P value
0–10cm	Tillage	1	0.23	0.23	0.33	0.57
	Cropping intensity (CI)	1	2.86	2.86	4.13	0.07
	Tillage X CI	1	0.03	0.03	0.05	0.83
	Residual	12	8.29	8.29		
10–20cm	Tillage	1	0.02	0.02	0.01	0.93
	Cropping intensity (CI)	1	0.69	0.69	0.25	0.63
	Tillage X CI	1	0.01	0.01	0.00	0.96
	Residual	12	32.82	32.82		
20–50cm	Tillage	1	0.14	0.14	0.01	0.93
	Cropping intensity (CI)	1	6.57	6.57	0.40	0.54
	Tillage X CI	1	1.22	1.22	0.07	0.79
	Residual	12	199.13	16.59		

It would seem cropping intensity moderately affected SOC for the 0–10-cm depth ($P=0.07$). Annual cropping systems averaged 11.44 MT ha^{-1} and alternate cropping systems averaged 12.28 MT ha^{-1} . However, the difference in SOC would suggest that more carbon was accreted with decreased cropping intensity. No other SOC differences were significantly affected by treatments for any other depth increment.

Power, MT

Summary results from the ANOVA's run on data from Power in 2008 are shown in Table 25. SOC was not affected by cropping intensity, tillage, and cropping intensity x tillage in the 0–10-cm and 20–50-cm depths. Tillage significantly affected SOC in the 10–20-cm depth with SOC averaging 14.48 and 12.64 MT ha^{-1} for the till and no-till treatments respectively. No other significant effects were detected for the 10–20-cm depth.

Table 25. Summary SOC ANOVA table by depth (2008) at Power.

Depth	Source	DF	Sum of Squares	Mean Square	F value	P value
0–10cm	Tillage	1	2.83	2.83	1.34	0.27
	Cropping intensity (CI)	1	0.37	0.37	0.18	0.68
	Tillage X CI	1	0.17	0.17	0.08	0.78
	Residual	12	25.3	25.3		
10–20cm	Tillage	1	13.45	13.45	10.76	<0.01
	Cropping intensity (CI)	1	0.35	0.35	0.28	0.61
	Tillage X CI	1	0.31	0.31	0.25	0.63
	Residual	12	15.01	15.01		
20–50cm	Tillage	1	8.34	8.34	0.97	0.34
	Cropping intensity (CI)	1	5.05	5.05	0.59	0.46
	Tillage X CI	1	2.08	2.08	0.24	0.63
	Residual	12	102.99	8.582		

Chester, MT

Summary results from the ANOVA's run on data collected in 2008 from this site are presented in Table 26. SOC in the 0–10-cm depth was significantly affected ($P = 0.02$) by cropping intensity with greater SOC in the continuous cropping systems (11.85 MT C ha⁻¹) compared to the alternate year crop systems (11.27 MT C ha⁻¹). Additionally, a moderately significant ($P = 0.10$) difference would be suggested for cropping intensity in the 10–20-cm depth resulting again in increase C under increased cropping intensity. No significant treatment effects on SOC were observed in the 20–50-cm depth.

Table 26. Summary SOC ANOVA table by depth (2008) at Chester.

Depth	Source	DF	Sum of Squares	Mean Square	F value	P value
0–10cm	Tillage	1	0.38	0.38	2.02	0.18
	Cropping intensity (CI)	1	1.39	1.39	7.33	0.02
	Tillage X CI	1	0.19	0.19	1.02	0.33
	Residual	12	2.28	2.28		
10–20cm	Tillage	1	0.49	0.49	0.32	0.58
	Cropping intensity (CI)	1	4.87	4.87	3.14	0.10
	Tillage X CI	1	0.20	0.20	0.13	0.73
	Residual	12	18.64	18.64		
20–50cm	Tillage	1	10.26	10.26	0.73	0.41
	Cropping intensity (CI)	1	0.01	0.01	0.00	0.98
	Tillage X CI	1	1.56	1.56	0.11	0.75
	Residual	12	168.55	14.05		

Conrad, MT

Summary results from the ANOVA's run on data collected from the Conrad site in 2008 are presented in Table 27. SOC in the 0–10 and 20–50-cm depths was significantly affected by cropping intensity x tillage. The ANOVA's indicates a significant cropping intensity and tillage interaction in the 0–10 and 20–50-cm depths as a result of the increase in SOC with cropping intensity under no-till (NT) and decrease in SOC under till. The significant interaction may have a result of biases created by the field topography and the lack of randomness in location of the star locations. The four star locations used for soil sampling were concentrated in an upslope position (Fig 36). It was observed that

three of lowest SOC values found at this site occurred in the four soil samples (0–10 cm) from this upslope position. Given that SOC in upslope positions is often lower than in toe slope or depression areas, the significant cropping system x tillage interaction was not unexpected.

Table 27. Summary SOC ANOVA table by depth (2008) at Conrad.

Depth	Source	DF	Sum of Squares	Mean Square	F value	P value
0–10cm						
	Tillage	1	0.42	0.42	0.71	0.42
	Cropping intensity (CI)	1	1.05	1.05	1.76	0.21
	Tillage X CI	1	14.52	14.52	24.33	<0.01
	Residual	12	7.16	7.16		
10–20cm						
	Tillage	1	0.06	0.06	0.02	0.89
	Cropping intensity (CI)	1	9.41	9.41	3.05	0.11
	Tillage X CI	1	4.74	4.74	1.54	0.24
	Residual	12	37.05	37.05		
20–50cm						
	Tillage	1	0.54	0.54	0.05	0.82
	Cropping intensity (CI)	1	5.26	5.26	0.51	0.49
	Tillage X CI	1	54.21	54.21	5.21	0.04
	Residual	12	124.82	10.40		

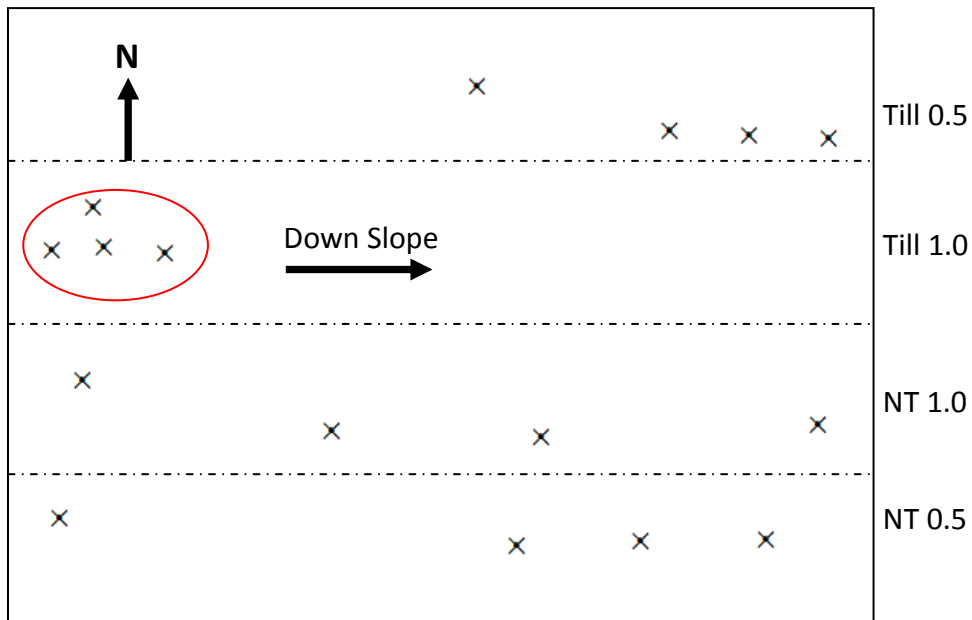


Figure 36. Conrad microsite locations. Treatment borders notated with dashed lines.

Fife, MT

Summary results from the ANOVA's run on data collected from the Fife site are presented in Table 28.

This table shows SOC in the 0–10-cm depth was significantly affected by cropping intensity with greater SOC in the continuous cropping systems (21.69 MT C ha⁻¹) compared to the alternate-year crop systems (19.74 MT C ha⁻¹). No significant treatment effects on SOC were observed in the 10–20 or 20–50-cm depths.

Table 28. Summary SOC ANOVA table by depth (2008) at Fife.

Depth	Source	DF	Sum of Squares	Mean Square	F value	P value
0–10cm	Tillage	1	1.27	1.27	0.76	0.40
	Cropping intensity (CI)	1	14.65	14.65	8.72	0.01
	Tillage X CI	1	0.33	0.33	0.20	0.67
	Residual	12	20.16	20.16		
10–20cm	Tillage	1	0.02	0.02	0.01	0.94
	Cropping intensity (CI)	1	4.06	4.06	1.43	0.26
	Tillage X CI	1	0.22	0.22	0.08	0.79
	Residual	12	34.15	34.15		
20–50cm	Tillage	1	0.48	0.48	0.04	0.85
	Cropping intensity (CI)	1	17.43	17.43	1.29	0.28
	Tillage X CI	1	0.000	0.000	0.00	1.00
	Residual	12	161.78	13.481		

Kremlin, MT

Summary results from the ANOVA's run on data from the Kremlin site (Table 29) show there are no significant effects of tillage, cropping intensity, and cropping system x intensity on SOC in the depth layers samples.

Because of the concerns with the baseline data, comparisons among treatments at each of the six field sites were made assuming a common unknown baseline. The comparisons seen in the previous figures and tables resulted in differences ($P < 0.10$) which were consistent with the established carbon sequestration trends being detected in the 0–10-cm soil profile for two of the six sites tested, which was less than we detected when not assuming a common baseline.

Table 29. Summary SOC ANOVA table by depth (2008) at Kremlin.

Depth	Source	DF	Sum of Squares	Mean Square	F value	P value
0–10cm						
	Tillage	1	0.21	0.21	0.08	0.78
	Cropping intensity (CI)	1	5.58	5.58	2.27	0.16
	Tillage X CI	1	0.04	0.04	0.02	0.90
	Residual	12	29.05	29.05		
10–20cm						
	Tillage	1	1.43	1.43	0.77	0.407
	Cropping intensity (CI)	1	2.76	2.76	1.49	0.25
	Tillage X CI	1	1.58	1.58	0.85	0.37
	Residual	12	22.26	22.26		
20–50cm						
	Tillage	1	7.01	7.01	0.24	0.63
	Cropping intensity (CI)	1	2.54	2.54	0.09	0.77
	Tillage X CI	1	58.18	58.18	2.03	0.18
	Residual	12	343.79	28.65		