Understanding Cropland Carbon Sequestration in North Central Montana Dryland Wheat Systems

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Td10: Final Report on MT Cropland Controlled Test Field Validation Tests

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Executive Summary

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 bi-annually with yields and management monitored annually over the course of six years. It was hoped that six years of consecutive management would result in cumulative changes in soil organic carbon (SOC) which could be detected and used to determine annual rates of terrestrial carbon sequestration resulting from the two treatment factors.

Differences between baseline SOC data and SOC data from the sixth year of the story unfortunately were not consistent with well-established carbon sequestration trends for either of the treatment factors being studied. In fact, these comparisons resulted in suggested changes in SOC which were identified as highly improbable. Substantial variation in baseline SOC data was identified as most likely culprit for the questionable sequestration rates.

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 resulted in differences ($P < 0.05$) 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.

An additional test, the AMBC (active microbial biomass carbon) test, was employed as a means of detecting differences in active microbial biomass carbon pool 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 a 1 year storage time of 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.

1. Overview and Organization of Report

This report summarizes research 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.

Section 2 presents the main component of the study, in which soil samples were collected bi-annually with yields and management monitored annually over the course of six years.

Section 3 summarizes an additional test, the Active Microbial Biomass Carbon (AMBC) test, which researchers employed as a means of detecting differences in active microbial biomass carbon pool as a means of indicating changes that are occurring in the total soil organic carbon (SOC) pool.

2. Measuring On-Farm Soil Carbon Change Due to Tillage and Cropping Intensity in North Central Montana

2.1 *Introduction*

Mounting societal concerns over increasing atmospheric CO_2 concentrations, continued disruptions to the global carbon cycle from land use changes, and increasing fossil fuel consumption have intensified interest in land management practices that sequester carbon in agricultural soils (Bengochea-Morancho et al., 2001; Cochran et al., 2007; Fan et al., 1999; Halvorson et al., 2002; Holtzeakin and Selden, 1995; Houghton, 2003; Keeling et al., 1995; Kleypas et al., 1999; Palmer and Ralsanen, 2002; Pearson and Palmer, 2000; Say and Yucel, 2006; Solomon et al., 2009; Wise et al., 2009). Soil C is one of the five principal global carbon 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 a destruction of soil carbon and release of CO_2 in the atmosphere (Doran, 1980). An estimated 20 - 50% of the native soil organic carbon was lost during the first 20-50 yr of cultivation (Mann, 1986; Rasmussen and Parton, 1994; Tiessen et al., 1982). Many of these soils, now depleted in organic carbon, have the capacity to act as a C sink though a change in management practices that promote carbon sequestration (Lal, 1998).

Terrestrial carbon sequestration research in the Great Plains has focused on the available nitrogen (N) and carbon sequestration relationships (Campbell and Zentner, 1993; Campbell et al., 2000; Halvorson et al., 1999; Halvorson et al., 2002; Nyborg et al., 1999) and how best management practices (BMPs), such as annual cropping and no-till can be adopted to enhance carbon sequestration (Antle et al., 2002; Campbell et al., 2001; Curtin et al., 2000; Eve et al., 2002; Lal, 1998; Liebig et al., 2004; McConkey et al., 2003). Although, BMPs to sequester carbon in agricultural soils have been identified, the sequestration rates remain unknown for some regions of the northern Great Plains due to the absence of long term cropping system studies.

Montana's Golden Triangle represents one such region. This region is defined on the north by the border with Canada, on the west by the eastern front of the Rocky Mountains, and 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 (Watts, 2008). The purpose of this research was to quantify soil carbon sequestration rates in response to agricultural BMP's, including no-till and annual cropping, on six representative well-managed farms in Montana's Golden Triangle.

2.2 *Materials and Methods*

2.2.1 Site Description and Experimental Design

Field studies were conducted at six farmer-managed sites (~32 ha) within Montana's Golden Triangle (Figure 1, Table 1). The six sites were under no till management prior to the inception of this study in 2002. Soil particle size distribution, texture class, and pH for the 0-10, 10-20, and 20-50 cm depths are summarized in Table 1 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 – 56%. Soil pH values are all greater than 7.0, reflecting the occurrence of Ca and Mg carbonates common to the soils of this region.

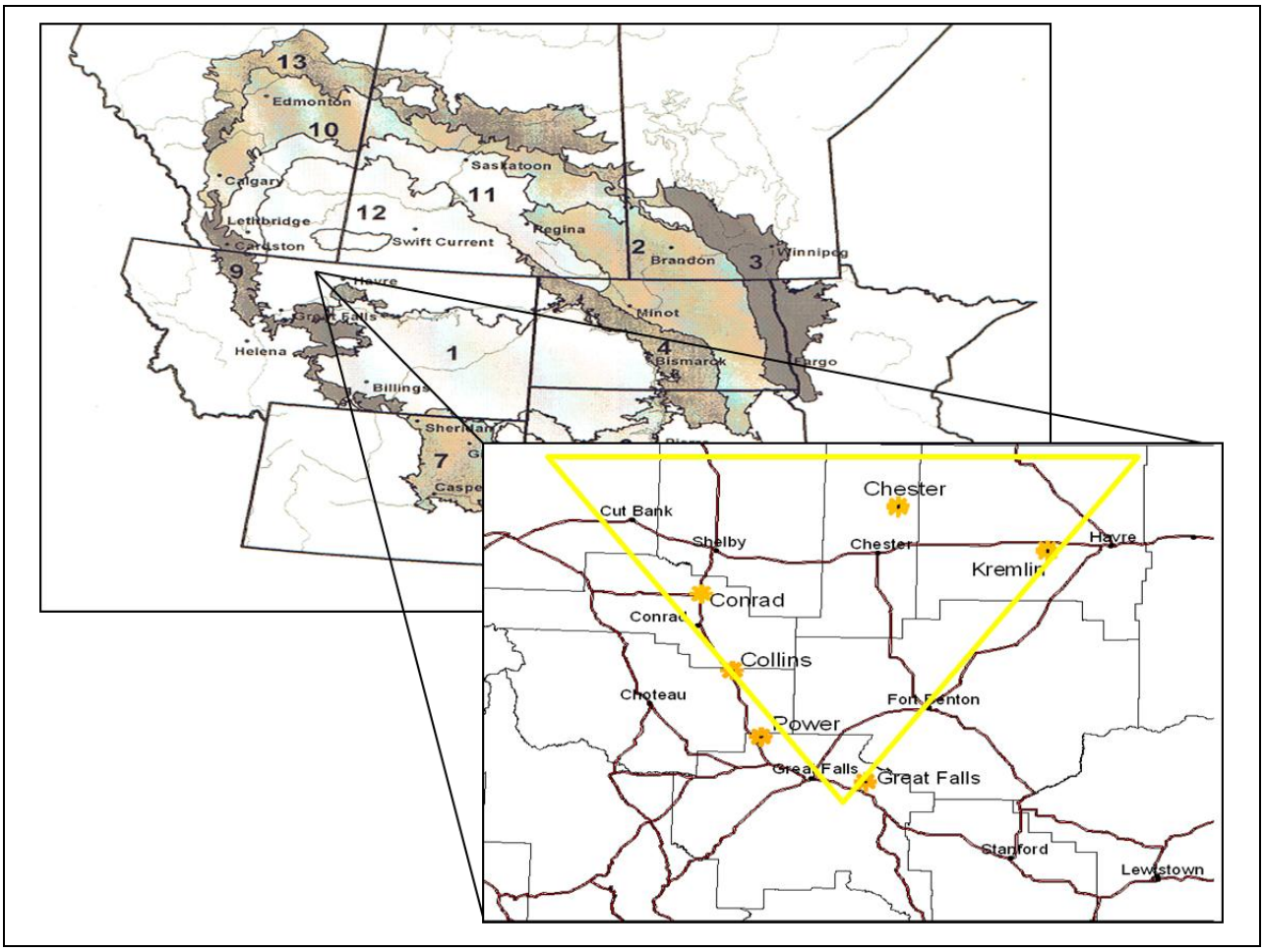

Figure 1: Location of field sites in north central Montana's Golden Triangle (locations marked by asterisks).

Table 1: Site locations and physical and chemical properties, by depth, for the six field sites.

Location	Latitude, Longitude	Depth	% Sand	% Silt	% Clay	Textural Class	pH
Dutton	47°58'37.08"N,	0-10 cm	30	23	46	Clay	7.8
	111°44'42.87"W	10-20 cm	27	21	52	Clay	8.1
		20-50 cm	25	21	54	Clay	8.6
Power	47°40'50.46"N,	0-10 cm	19	38	43	Silty clay	8.2
	111°34'38.47"W	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,	0-10 cm	42	28	30	Clay loam	7.8
	110°51'45.95"W	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,	0-10 cm	34	32	35	Clay loam	7.4
	111°55'50.20"W	10-20 cm	30	29	41	Clay	7.7
		20-50 cm	27	32	41	Clay	8.2
Fife	47°29'13.59"N,	0-10 cm	20	24	56	Clay	7.6
	111°00'17.92"W	10-20 cm	21	22	56	Clay	8.2
		20-50 cm	17	20	63	Clay	8.6
Kremlin	48°31'41.36"N,	0-10 cm	38	34	27	Clay loam	7.8
	110°01'56.91"W	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 to into two cropping intensity subplots, annual cropping (1.0) vs. alternate year cropping (0.5). The plot

arrangements are illustrated in Figure 2. 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. Annual cropped treatments formed the center of the field (except for Chester) to facilitate farming operations. All field plots were sown to wheat during even-numbered years (i.e. 2002, 2004, 2006, and 2008). Class of wheat (hard red winter vs. hard red spring) and cultivar selection were left to the discretion of the producer. Annual legumes were seeded in the annual cropped treatments during odd-numbered years (2003, 2005, 2007). Choice of legume species (pea or lentil), cultivar selection, and management (forage, grain, or green manure) was left to the discretion of the producer.

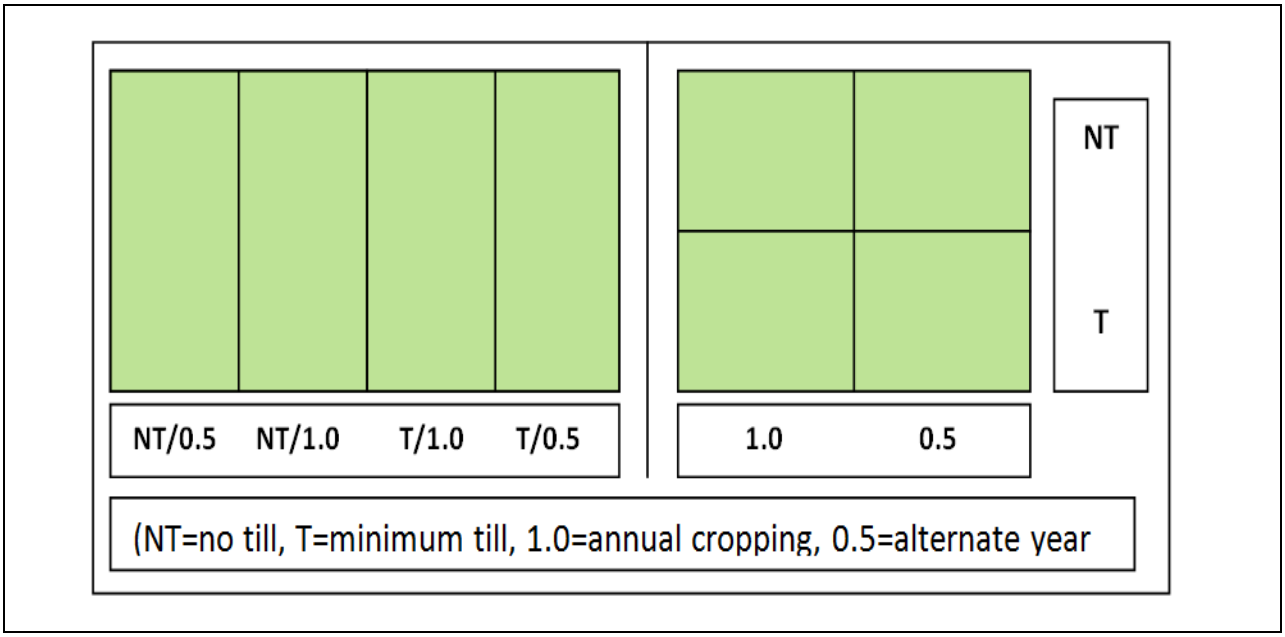

Figure 2: Field plot design at Dutton, Power, Conrad, Fife and Kremlin (left) and Chester (right).

2.2.2 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 (Bricklemyer et al., 2005). Initially a digital grid was superimposed over the entire study area. The grid had a 30-m 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).

A five-pointed star (5 m radius) pattern was created around the center-axis of each microplot to mark soil sampling points (Figure 3). 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 4). 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.

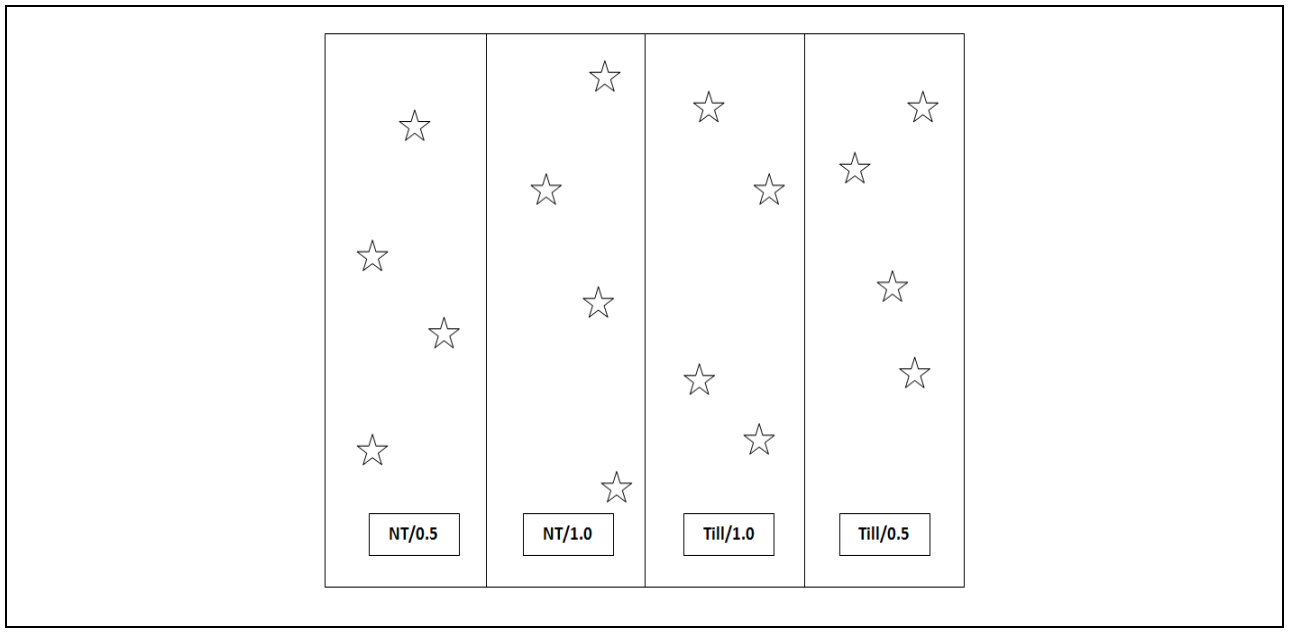

Figure 3: Example of field layout

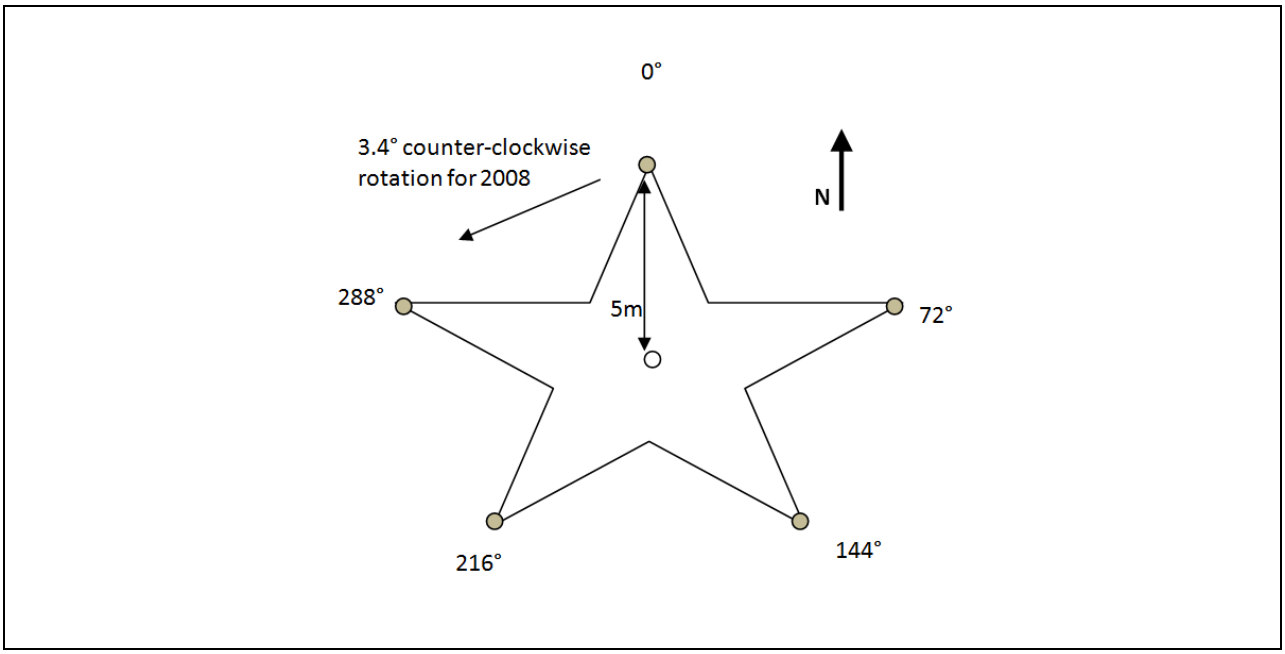

Figure 4: Sample layout and rotation of soil cores around microplots.

A Concord Environmental Equipment hydraulic sampler (Hawley, MN) equipped with a 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 (5.07 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.

2.2.3 Soil Sample Processing, Carbon Analyses, Bulk Density Protocol

Soil samples for all years were oven-dried at 50°C for 4 d and weighed for bulk density determination. Bulk density was calculated by dividing soil dry weights by core volumes minus rock volume.

Soil cores for 2002 and 2008 were processed for soil C analyses according to the schemes outlined in Figure 5 and Figure 6, respectively. 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 time required to do this varied by depth with the surface depths (more fresh surface litter) taking longer than subsequent depths. Another factor that affected the time required to remove fresh biomass was the amount and depth of contamination which fell down the sampling sleeve as the hydraulic hammer was operated on the sampling equipment to get to the 50 cm depth (more hammer time generally resulted in more contamination further down the sleeve from vibration). The rock fraction was calculated by weighing the fragments which would not pass through the screen. From the ground soil, 30.0 g (+/- 0.1 g) from each star point were combined into a single composite sample.

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.

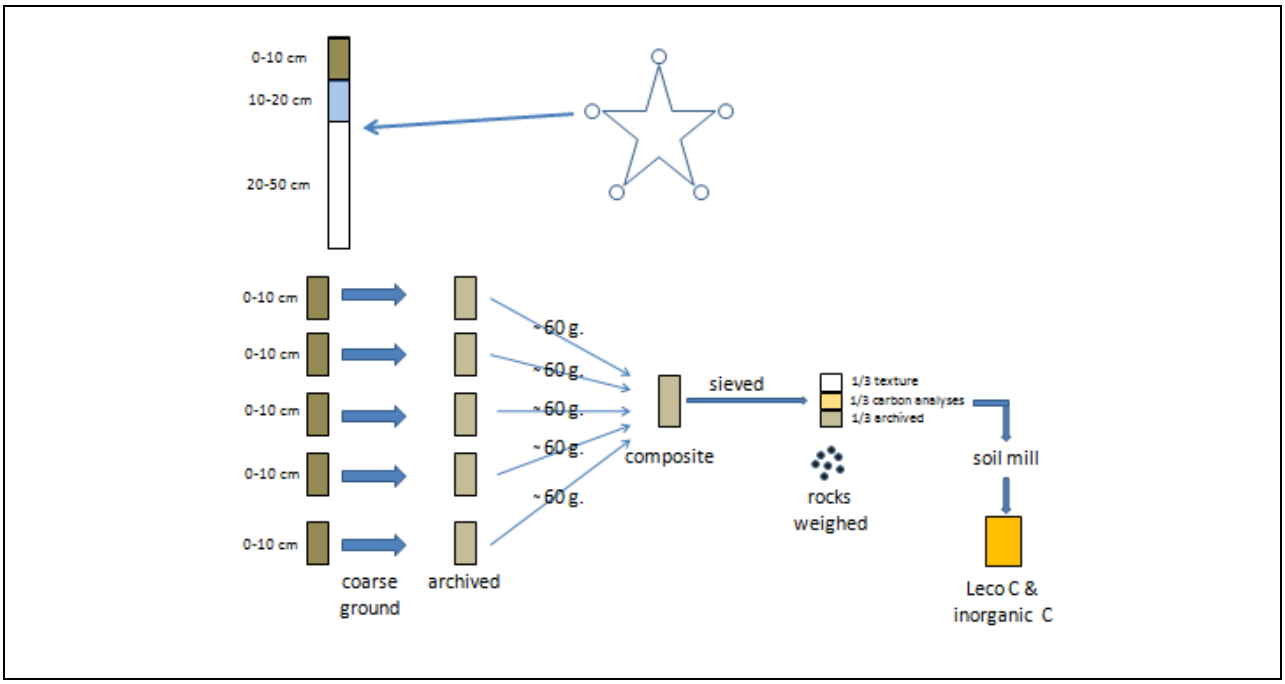

Figure 5: Sample processing scheme used for cores collected in 2002.

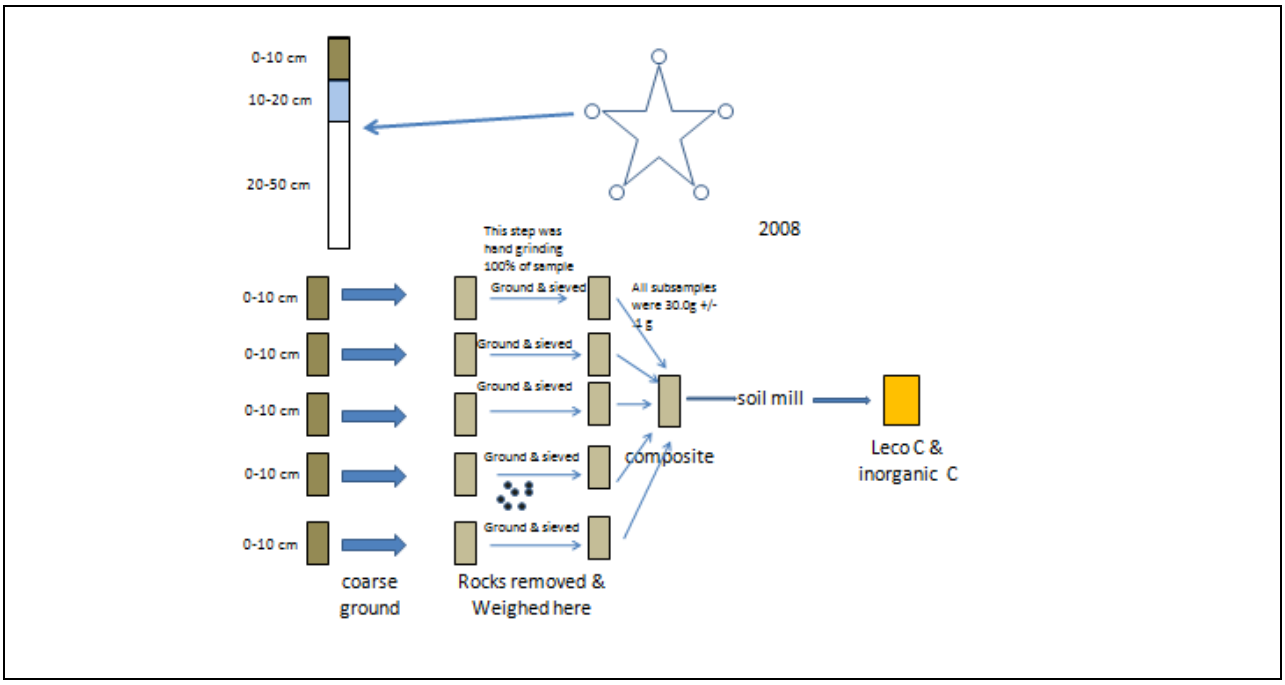

Figure 6: Soil sample processing scheme used for cores collected in 2008.

2.2.4 Biomass Sampling and Plant Carbon Analysis Protocol

Crop biomass for each treatment was estimated by hand-harvesting three areas totaling 2 m^2 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 d 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 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).

2.2.5 Soil Organic Carbon Calculations and Mass Equivalency Adjustment

Soil organic carbon mass per unit area expressed in units of MT 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 many estimates were nonsensical. 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 the MT 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. Mass equivalency adjustments are necessary to compensate for spatial variability across a field site as well as the application of treatments, e.g. tillage, affects 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). This was done by adjusting downward the mass of C per depth increment (i.e. 0-10, 0-20, 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.

Even with the use of 2008 bulk densities and mass equivalency adjustments, there remained considerable variation 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, many of which were nonsensical. Given these uncertain results and the discrepancies in SOC change, we examined the protocols employed from sample collection to sample analysis to identify potential sources of error. These concerns are addressed in the results section below.

2.2.6 Statistical Analysis

Soil C analyses from the six farm sites were 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 mixed procedure (PROC MIXED). The ANOVA model for each site treated tillage, cropping intensity, and tillage by cropping intensity interaction as fixed effects, and pseudo-replication as a random effect.

2.2.7 Site Descriptions

Crop species and cultivar selections seeded at each site are summarized in Table 2. 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.

Table 2: Crop species & cultivar selection seeded at farm sites by year†

Site	Year					
	2003	**2004**	**2005**	**2006**	**2007**	**2008**
Dutton	*Grande* pea	*Fortuna* spring wheat	*Salute* pea	*Fortuna* spring wheat ‡	*Cruiser* pea	*Falcon* winter wheat
Power	*Arvika* pea	*Fortuna* spring /*Vanguard* winter wheat§	*Richlea* lentil	*Pryor* winter wheat	*Richlea* lentil	*Fryer* spring wheat
Chester	*Austrian* winter pea	*Fortuna* spring wheat	*Austrian* winter pea	*Fortuna* spring wheat	*Richlea* lentil	*Fortuna* spring wheat
Conrad	French green lentil	*Ernest* spring wheat	Upright yellow pea	Timer winter wheat	*Salute* pea	*Conan* spring wheat
Fife	*Arvika* pea & *Harrington* barley	*Ernest* spring wheat	*Melrose* winter pea	*Promontory* winter wheat	Pea	*Carlise* winter wheat
Kremlin	*Arvika* pea	*Conan* spring wheat	*Escape* pea	*Choteau* spring wheat	*Cruiser* pea	*Genou* winter wheat

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

‡ Crop planted after winter wheat failed

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

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 d and an annual mean temperature (AMT) of 1 - 8°C (Soil Survey Staff). 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. The soil texture classes for this field were determined experimentally (Table 1). 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. The field management operations and crop inputs at this site are detailed in the appendix.

The Power site is located south of Power, MT, and is described in a web-based soil survey 28 to 36 cm of annual precipitation with a frost free period of 110 to 135 d and AMT of 4 - 8°C (USDA). 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. The soil texture classes were determined experimentally (Table 1). This site was historically in wheat-fallow rotation prior to entering this study with conventional tillage used through 1999. No-till management was initiated in 2000. Management operations and crop inputs at this site are detailed in the appendix.

The Chester site is located 25 km north of Chester. This site is described as receiving 28 to 36 cm of annual precipitation with a frost-free period of 105 to 125 days and AMT of 4 - 8°C (Soil Survey Staff). This survey classifies the map unit of the study area as belonging to the Joplin-Hillon loams series; as a well drained soil with 2 m to the water table or before reaching restrictive features. The soil texture class for this field was determined experimentally (Table 1). 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 began in 1998. Management operations and crop inputs at this site are detailed in the appendix.

The Conrad site is located 12 km north of Conrad MT. This site receives 28 to 36 cm of annual precipitation with a frost free period of 105 to 125 d and AMT of 1 - 8°C (USDA). 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. The soil texture class was determined experimentally (Table 1). 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. Management operations crop inputs at this site are detailed in the appendix.

The Fife site was the wettest of the six sites enrolled in this study, located 20 km east of Great Falls. The area of this site is described as receiving 35 to 46 cm of annual precipitation with a frost-free period of 105 to 130 d and AMT of 3- 8°C (Soil Survey Staff). 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. The soil texture class for this field was determined experimentally (Table 1). This site also has the greatest clay content of the six sites studied with 56% clay dominated by 2:1 expanding type montmorillonite clays. Management operations and crop inputs at this site are detailed in the appendix.

The Kremlin site is located 5 km east of Kremlin, MT and is described as receiving 25 to 33 cm of annual precipitation (making this the driest of the 6 sites) with a frost free period of 105 to 120 d and AMT of 4- 8°C (Soil Survey Staff). This survey lists several soil series names for the sampling area of the study with the Phillips-Elloam complex as being the dominant soil series at this site accounting for 63% of the area. The other two series' making up this site as 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. The soil texture class for this field was determined experimentally (Table 1). This site was historically in a wheat- fallow rotation with no-till management dating back to 1993. Management operations and crop inputs at this site are detailed in the appendix.

2.3 ***Results***

2.3.1 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 **Table 3** and **Table 4** (P. Miller, unpublished data). These tables show grain yield in Mg ha^{-1} which is an important factor for considering the feasibility of an alternative cropping rotation. They also show estimates of fixed carbon inputs over the six years of this study which is an important part of a complex equation for explaining carbon gains and losses. Estimates of carbon inputs are based on percent carbon from measured above ground biomass and estimated root biomass of legume crops based upon reported values for legume root:shoot mass and root C concentration in unrelated studies. The small difference between 'shoot C input' and 'estimated total C' reflects the estimated legume crop root portion, helpful for illustrating more accurately the additional C inputs for the annually cropped treatments (**Table 4**).

Table 3 illustrates the site specific production variation that exists across an area the size of the Montana's golden triangle (2 million ha). **Table 3** also shows that after six years of continuous observation, production variation exists from site to site in the total shoot, grain yield, shoot residue and shoot C input categories. The shoot C input column (last column) in **Table 3** shows

that each site has significantly different amounts of carbon inputs being returned to the soil after harvest. This difference is important for it has been shown that for soils which are not carbon saturated, the quality and quantity of crop residue returned to the soil is directly related to changes in SOC (Campbell et al., 2001; Campbell et al., 2007; Cole et al., 1993; Kong et al., 2005; Rasmussen et al., 1980). Based on this relationship, the long history of carbon depleting production agriculture (Lal, 2004) at these sites, the relationship between soil texture and sequestration rates (McConkey et al., 2003), and the shoot C inputs by site (**Table 3**), the Fife site might be expected to be sequestering the most carbon annually with the Dutton and Chester sites sequestering the least if these six sites are not yet carbon saturated. Given soil texture differences (Table 1) and the site-specific weather differences (discussed later), production differences were generally not surprising.

Difference in the tillage comparison (**Table 3**) show greater shoot biomass production and increased grain yields for the no-till treatments averaged across all six sites. This is consistent with work previously report from similar environments under low precipitation (Bonfil et al., 1999).

Table 3: Cumulative shoot biomass, grain yields and carbon inputs over six years (2003-2008) & as affected by site & tillage

Site	Total Shoot	Grain yield	Shoot residue	Shoot C input
Site (Mg ha^{-1})				
Dutton	24.1 BC	6.60 DE	15.8 C	7.11 C
Power	20.5 C	6.87 D	11.8 E	5.35 E
Chester	19.9 C	6.18 E	12.9 D	5.91 D
Conrad	26.0 B	9.18 B	15.7 C	7.12 C
Fife	40.8 A	13.01 A	24.4 A	10.94 A
Kremlin	26.9 B	8.24 C	17.1 B	7.75 B
No-till vs Tilled (Mg ha^{-1})				
No-till	26.9 A	8.57 A	16.5 A	7.48 A
Tilled	25.9 B	8.12 B	16.0 A	7.25 A

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

Table 4 displays cropping intensity comparisons for each site and the production variation that exists when comparing cropping intensities at each site. For all sites except the Power site, the grain (wheat) yield was lower in the continuous cropped treatments (1.0) than in the wheat fallow treatments (0.5). **Table 4** also illustrates that for all sites except the Conrad site, the cumulative total carbon input (last column) is greater for the continuous cropped treatments than for the treatments which included fallow. While more biomass was produced in annual cropping, it is important to realize that the increase in biomass produced, seemed to have a negative effect on the wheat yield (during even years) as seen when comparing the grain yield and total carbon input columns.

Table 4: Cumulative shoot biomass, grain yields and carbon inputs over six years (2003-2008) at six Montana field sites as affected by cropping intensity

Site	Total Shoot	Grain yield	Shoot residue	Shoot C input	Estimated Total C
Cropping Intensity by Site (Mg ha^{-1})					
Dutton - 0.5	23.6 a	7.94 a	15.6 a	7.09 a	7.1 b
Dutton - 1.0	24.7 a	5.27 b	16.0 a	7.13 a	7.9 a
Power - 0.5	16.4 b	6.65 a	9.6 b	4.41 b	4.4 b
Power - 1.0	24.6 a	7.09 a	13.9 a	6.28 a	7.1 a
Chester - 0.5	19.6 a	7.18 a	12.3 b	5.68 a	5.7 b
Chester - 1.0	20.3 a	5.17 b	13.5 a	6.13 a	6.7 a
Conrad - 0.5	27.6 a	10.65 a	16.5 a	7.54 a	7.5 a
Conrad - 1.0	24.5 b	7.71 b	14.8 b	6.70 b	7.3 a
Fife - 0.5	40.8 a	15.72 a	25.0 a	11.23 a	11.2 b
Fife - 1.0	40.8 a	10.31b	23.9 b	10.64 b	12.2 a
Kremlin - 0.5	26.1 a	10.16 a	15.8 b	7.22 b	7.2 b
Kremlin - 1.0	27.7 a	6.32 b	18.4 a	8.29 a	9.1 a

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

**Table Summary: Total shoot biomass, wheat grain yield (even years only), shoot residues, shoot C input, and estimated total C input including root carbon from legume crops for different tillage and cropping intensity regimes at six field sites in north central Montana summed from 2003 thru 2008.

2.3.2 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 ANOVA's conducted using the SAS® software (Proc Mixed) can be seen in Tables 5 through 16 below. These tables are grouped by site and show the estimated changes in organic carbon by site and depth over the 6 yr of this study with the corresponding ANOVA results from on the 0 -10, 0-20, and 0 -50 cm depths.

Dutton

Table 6 displays the SOC values for 2002, 2008, and δC by individual and composited depths. The analysis of the data from the Dutton site shows no differences ($P < 0.05$) in δC for any depths (**Table 5**). After examining the delta SOC values in **Table 6**, it can be seen that there are no obvious sequestration trends being detected at this site for any depth.

Table 5: 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	0.738	0.738	0.830	0.381
	CI	1	0.001	0.001	0.000	0.969
	Tillage X CI	1	1.684	1.684	1.900	0.195
	Residual	11	9.750	0.886		.
10-20cm						
	Tillage	1	0.056	0.056	0.120	0.732
	CI	1	0.328	0.328	0.710	0.414
	Tillage X CI	1	0.162	0.162	0.350	0.563
	Residual	12	5.502	0.459		
20-50cm						
	Tillage	1	6.812	6.812	0.370	0.554
	CI	1	0.731	0.731	0.040	0.845
	Tillage X CI	1	0.168	0.168	0.010	0.925
	Residual	12	220.583	18.382		
0-20cm						
	Tillage	1	0.403	0.403	0.250	0.628
	CI	1	0.362	0.362	0.220	0.646
	Tillage X CI	1	2.808	2.808	1.730	0.215
	Residual	11	17.805	1.619		
0-50cm						
	Tillage	1	9.850	9.850	0.720	0.414
	CI	1	0.052	0.052	0.000	0.952
	Tillage X CI	1	4.320	4.320	0.320	0.585
	Residual	11	150.493	13.681		

CI = Cropping Intensity

Table 6: SOC 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**	**0-20**	**0-50**
		---------- MT C ha^{-1} ----------				
2002	NT 0.5	13.31	10.56	24.66	23.87	48.53
	NT 1.0	11.68	9.61	23.05	21.29	44.34
	TILL 1.0	14.13	10.05	22.68	24.18	46.86
	TILL 0.5	12.34	10.51	22.78	22.85	45.63
2008	NT 0.5	12.20	10.22	25.28	22.43	47.70
	NT 1.0	11.27	9.76	23.44	21.03	44.47
	TILL 1.0	11.60	9.89	24.18	21.49	45.66
	TILL 0.5	12.35	10.26	24.91	22.61	47.52
δC	NT	-0.76	-0.09	0.50	-0.85	-0.35
	Till	-1.26	-0.21	1.82	-1.47	0.35
	0.5	-0.55	-0.30	1.38	-0.84	0.53
	1.0	-1.47	-0.01	0.95	-1.48	-0.54

Power

Table 7 displays the SOC values for 2002, 2008, and δC by individual and composited depths. Tillage significantly affected delta SOC in the 0 – 10 cm depth (**Table 7**, **Table 8**) with a net gain of 0.87 MT C ha^{-1} from no-till, and a net loss of 0.77 MT C ha^{-1} from tillage. Cropping intensity significantly ($P = 0.046$) affected delta SOC in the 0 – 20 cm depth (**Table 7**) with a net gain of 0.35 MT C ha^{-1} in the continuous cropped (1.0) treatments and a net loss of 1.74 MT C ha^{-1} from the alternate year cropping (0.5). The 0 – 50 cm depth showed no detectable ($P < 0.05$) differences (**Table 7**).

Table 7: 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	10.791	10.791	7.380	0.019
	CI	1	4.928	4.928	3.370	0.091
	Tillage X CI	1	2.592	2.592	1.770	0.208
	Residual	12	17.542	1.462		
10-20cm						
	Tillage	1	1.392	1.392	0.470	0.505
	CI	1	3.901	3.901	1.330	0.272
	Tillage X CI	1	0.462	0.462	0.160	0.699
	Residual	12	35.309	2.942		
20-50cm						
	Tillage	1	17.851	17.851	0.420	0.527
	CI	1	3.979	3.979	0.760	0.400
	Tillage X CI	1	0.053	0.053	0.000	0.972
	Residual	12	504.603	42.050		
0-20cm						
	Tillage	1	4.399	4.399	1.250	0.286
	CI	1	17.535	17.535	4.970	0.046
	Tillage X CI	1	0.860	0.860	0.240	0.630
	Residual	12	42.325	3.527		
0-50cm						
	Tillage	1	4.494	4.494	0.090	0.772
	CI	1	2.161	2.161	0.040	0.841
	Tillage X CI	1	0.476	0.476	0.010	0.925
	Residual	12	615.599	51.300		

CI = Crop Intensity

Table 8: SOC 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**	**0-20**	**0-50**
		---------- MT C ha^{-1} ----------				
2002	NT 0.5	16.40	13.99	29.00	30.38	59.39
	NT 1.0	14.38	13.36	32.35	27.75	60.10
	TILL 1.0	17.47	14.54	32.29	32.01	64.30
	TILL 0.5	18.28	15.30	27.73	33.58	61.31
2008	NT 0.5	16.31	12.63	27.09	28.94	56.02
	NT 1.0	16.21	12.65	27.49	28.86	56.35
	TILL 1.0	16.84	14.76	29.66	31.60	61.26
	TILL 0.5	17.36	14.18	27.81	31.54	59.35
δC	NT	0.87 a	-1.04	-3.39	-0.17	-3.56
	Till	-0.77 b	-0.45	-1.28	-1.23	-2.50
	0.5	-0.50	-1.24	-0.92	-1.74 b	-2.67
	1.0	0.60	-0.24	-3.75	0.35 a	-3.40

Chester

Table 10 displays the SOC values for 2002, 2008, and δC by individual and composited depths. The analysis of the delta SOC data from the Chester site shows a significant cropping intensity effect on delta SOC in the 0 – 10, 10 – 20, and 0 – 20 cm depths. Higher cropping intensities resulted in accretion of SOC (Table 9). Under continuous cropping, SOC increased 0.91, 0.70 and 1.61 MT ha^{-1} for the 0 – 10, 10 – 20, and 0 – 20 cm depths, respectively. Conversely, a net SOC loss of 0.77, 0.77 and 1.54 MT ha^{-1} was observed in the 0 – 10, 10 – 20, and 0 – 20 cm depths, respectively, within plots that included fallow in rotation . Soil organic C in the 0 – 50 cm depth was not affected by tillage, cropping intensity, or their interaction (Table 9).

Table 9: 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	0.017	0.017	0.120	0.737
	CI	1	2.739	2.739	18.670	0.001
	Tillage X CI	1	0.272	0.272	1.850	0.201
	Residual	11	1.614	0.147		
10-20cm						
	Tillage	1	0.898	0.898	1.470	0.249
	CI	1	8.600	8.600	14.090	0.003
	Tillage X CI	1	2.333	2.333	3.820	0.074
	Residual	12	7.323	0.610		
20-50cm						
	Tillage	1	20.954	20.954	1.920	0.191
	CI	1	10.874	10.874	1.000	0.338
	Tillage X CI	1	47.576	47.576	4.360	0.059
	Residual	12	130.896	10.908		
0-20cm						
	Tillage	1	1.120	1.120	1.080	0.321
	CI	1	20.189	20.189	10.430	0.001
	Tillage X CI	1	3.873	3.873	3.730	0.080
	Residual	11	11.432	1.039		
0-50cm						
	Tillage	1	4.277	4.277	0.290	0.603
	CI	1	29.597	29.597	1.990	0.186
	Tillage X CI	1	53.703	53.703	3.610	0.084
	Residual	11	163.590	14.872		

CI = Cropping Intensity

Table 10: SOC 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**	**0-20**	**0-50**
		---------- MT C ha^{-1} ----------				
2002	NT 0.5	12.13	10.93	24.77	23.06	47.83
	NT 1.0	10.28	11.11	25.99	21.40	47.38
	TILL 1.0	11.62	11.40	24.30	23.02	47.31
	TILL 0.5	11.94	12.30	26.91	24.24	51.15
2008	NT 0.5	11.53	10.79	23.33	22.32	45.65
	NT 1.0	11.90	11.67	22.75	23.57	46.32
	TILL 1.0	11.81	12.25	24.98	24.06	49.03
	TILL 0.5	11.00	10.92	24.31	21.92	46.22
δC	NT	0.51	0.20	-2.34	0.71	-1.63
	Till	-0.38	-0.27	-0.96	-0.65	-1.61
	0.5	-0.77 b	-0.77 b	-2.02	-1.54 b	-3.56
	1.0	0.91 a	0.70 a	-2.56	1.61 a	0.33

Conrad

Table 12 displays the SOC values for 2002, 2008, and δC by individual and composited depths for the Conrad site. The analysis of the delta SOC data from the Conrad site shows no differences ($P < 0.05$) in SOC for the 0 – 10 or 0 – 20 cm depths (Table 11) but Table 23 suggests detectable interaction between tillage and intensity for the 20 – 50, and 0 – 50 cm depths.

Table 11: 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	0.405	0.405	0.240	0.634
	CI	1	4.042	4.042	2.390	0.151
	Tillage X CI	1	4.061	4.061	2.400	0.150
	Residual	11	18.619	1.693		
10-20cm						
	Tillage	1	0.426	0.426	0.130	0.728
	CI	1	0.400	0.400	0.120	0.736
	Tillage X CI	1	2.410	2.410	0.720	0.414
	Residual	12	40.310	3.359		
20-50cm						
	Tillage	1	22.208	22.208	1.860	0.197
	CI	1	113.050	113.050	9.480	0.010
	Tillage X CI	1	148.779	148.779	12.470	0.004
	Residual	11	143.120	13.011		
0-20cm						
	Tillage	1	5.018	5.018	0.420	0.530
	CI	1	13.764	13.764	1.150	0.305
	Tillage X CI	1	2.907	2.907	0.240	0.632
	Residual	11	144.039	13.094		
0-50cm						
	Tillage	1	48.476	48.476	2.300	0.155
	CI	1	47.852	47.852	2.270	0.157
	Tillage X CI	1	193.141	193.141	9.180	0.011
	Residual	11	252.505	22.955		

CI = Cropping Intensity

Table 12: SOC 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	0-20	0-50
		---------- MT C ha^{-1} ----------				
2002	NT 0.5	16.09	13.76	33.15	29.85	62.99
	NT 1.0	17.56	12.86	37.19	30.57	69.23
	TILL 1.0	13.88	12.99	42.34	26.87	69.21
	TILL 0.5	18.39	14.52	33.45	32.91	66.37
2008	NT 0.5	15.98	13.64	33.83	29.62	63.45
	NT 1.0	17.37	13.20	38.66	30.57	69.23
	TILL 1.0	15.14	12.24	35.35	27.38	62.73
	TILL 0.5	17.55	14.86	37.88	32.41	70.29
δC	NT 0.5	-0.12	-0.11	0.68 a	-0.23	0.45
	NT 1.0	-0.12	0.35	1.46 a	0.44	2.88
	TILL 1.0	1.26	-0.75	-6.99 b	0.51	-6.49
	TILL 0.5	-0.84	0.34	4.42 a	-0.50	3.92

Fife

Table 14 displays the SOC values for 2002, 2008, and δC by individual and composited depths for the Fife site. Cropping intensity significantly affected delta SOC in the 0 – 10, 0-20, and 0-50 cm depths (Table 13). Under continuous cropping there was a net SOC gain of 2.30, 4.01, and 12.7 MT ha^{-1} for the 0-10, 0-20, and 0-50 cm depths, respectively. Under alternate year cropping, SOC gains were equivalent to 0.41, 1.03, and 7.6 MT C ha-1, for the 0-10, 0-20, and 0-50 cm depths, respectively.

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

Year	Treatment	Soil depth layer (cm)				
		0-10	**10-20**	**20-50**	**0-20**	**0-50**
		---------- MT C ha^{-1} ----------				
2002	NT 0.5	16.09	13.76	33.15	29.85	62.99
	NT 1.0	17.56	12.86	37.19	30.57	69.23
	TILL 1.0	13.88	12.99	42.34	26.87	69.21
	TILL 0.5	18.39	14.52	33.45	32.91	66.37
2008	NT 0.5	15.98	13.64	33.83	29.62	63.45
	NT 1.0	17.37	13.20	38.66	30.57	69.23
	TILL 1.0	15.14	12.24	35.35	27.38	62.73
	TILL 0.5	17.55	14.86	37.88	32.41	70.29
δC	NT 0.5	-0.12	-0.11	0.68 a	-0.23	0.45
	NT 1.0	-0.12	0.35	1.46 a	0.44	2.88
	TILL 1.0	1.26	-0.75	-6.99 b	0.51	-6.49
	TILL 0.5	-0.84	0.34	4.42 a	-0.50	3.92

Table 14: SOC 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**	**0-20**	**0-50**
		---------- MT C ha^{-1} ----------				
2002	NT 0.5	19.18	13.90	27.30	33.08	60.38
	NT 1.0	19.45	13.37	27.00	32.82	59.82
	TILL 1.0	19.25	14.84	25.78	34.09	59.87
	TILL 0.5	19.48	13.66	26.50	33.14	59.64
2008	NT 0.5	20.16	14.89	33.19	35.05	68.24
	NT 1.0	21.79	15.67	35.28	37.46	72.73
	TILL 1.0	21.51	15.96	34.93	37.47	72.40
	TILL 0.5	19.31	14.73	32.84	34.04	66.88
δC	NT	1.66	1.65	7.09	3.30	10.90
	Till	1.05	1.10	7.75	2.15	9.89
	0.5	0.41 b	1.03	6.12	1.44 b	7.55 b
	1.0	2.30 a	1.71	8.72	4.01 a	12.72 a

Kremlin

Table 16 displays the SOC values for 2002, 2008, and δC by individual and composited depths for the Kremlin site. The analysis of the delta SOC data from all depths shows no detectable treatment effect (*P* = 0.05*)* for cropping intensity or tillage (Table 15). There is a detectable interaction on SOC between tillage and cropping intensity in the 0 – 20 cm depth (Table 15) where tillage can reduce the rates of sequestration under continuous cropping (1.0).

Table 15: 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	0.001	0.001	0.000	0.989
	CI	1	7.840	7.840	2.380	0.149
	Tillage X CI	1	3.115	3.115	0.950	0.350
	Residual	12	39.536	3.295		
10-20cm						
	Tillage	1	0.381	0.381	0.240	0.630
	CI	1	4.633	4.633	2.970	0.110
	Tillage X CI	1	4.763	4.763	3.060	0.106
	Residual	12	18.690	1.558		
20-50cm						
	Tillage	1	2.473	2.473	0.050	0.831
	CI	1	1.697	1.697	0.030	0.860
	Tillage X CI	1	62.055	62.055	1.190	0.297
	Residual	12	625.699	52.142		
0-20cm						
	Tillage	1	0.357	0.357	0.140	0.715
	CI	1	0.426	0.426	0.170	0.690
	Tillage X CI	1	15.622	15.622	6.130	0.029
	Residual	12	30.568	2.547		
0-50cm						
	Tillage	1	0.946	0.946	0.020	0.891
	CI	1	3.851	3.851	0.080	0.783
	Tillage X CI	1	15.347	15.347	0.320	0.585
	Residual	12	584.010	48.667		

CI = Cropping Intensity

Table 16: SOC 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	0-20	0-50
		---------- MT C ha^{-1} ----------				
2002	NT 0.5	9.74	9.21	22.48	18.95	41.43
	NT 1.0	10.75	9.43	22.22	20.18	42.39
	TILL 1.0	9.75	9.26	24.45	19.00	43.46
	TILL 0.5	10.31	9.97	24.47	20.28	44.75
2008	NT 0.5	9.21	9.74	28.24	18.95	47.19
	NT 1.0	7.93	9.95	31.26	17.88	49.14
	TILL 1.0	7.80	11.17	28.77	18.98	47.75
	TILL 0.5	8.89	9.71	33.38	18.60	51.98
δC	NT 0.5	-0.53	0.53	5.76	0.00	5.76
	NT 1.0	-2.81	0.51	9.04	-2.30	6.75
	TILL 1.0	-1.94	1.92	4.32	-0.03	4.29
	TILL 0.5	-1.42	-0.25	8.91	-1.67	7.24

2.3.3 Error Analyses

Given the inconsistent SOC results listed in Tables 5-16, from sample years 2002, 2008, and the resulting estimated SOC changes among treatments from 2002 to 2008, it was determined that further investigation into the SOC values (and their individual data components) would be required to identify the source(s) of the improbable values of change in SOC seen in many of these tables. Some examples of concerns with the data include, but are not limited to; the apparent loss of carbon in all treatment combinations for the 0 – 50 cm depth at the Power site, apparent gains in SOC under tillage but losses on no-till at the Conrad site, and an apparent loss of 2.8 MT OC ha-1 under the no-till, cropping intensity 1.0 at the Kremlin site.

To understand the source of questionable results, we examined comparisons of the individual data components from 2002 and 2008 (inorganic carbon, total carbon, organic carbon, and bulk density).

Inorganic Carbon Data Component

The mean inorganic carbon values measured for sample years 2002 and 2008 are summarized in Table 17 below. This table shows that there is only a slight variation in the inorganic carbon (IC) values for years 2002 and 2008.

Table 17: Mean inorganic carbon (IC) by site in (0 – 50 cm) 2002 and 2008. Standard deviation 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. Table 17 would suggest that there has been an increase in inorganic carbon at Power and a decrease at Fife. 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. The amount of variation seen in the Table 17 is likely not the major source of the noise observed in the 2002 to 2008 SOC comparisons but is likely contributing to it.

Total Carbon Data Component

The next data component analyzed was 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. With the inorganic carbon expected to remain relatively constant over the short time frame of this study, these differences can be interpreted as gains of losses in SOC. However, since we would not likely predict net losses of SOC across these fields over the time of this study, any difference in

total carbon which appears to suggest a loss of carbon would need further investigation. The means for the total carbon concentrations can be seen in Table 18.

Table 18: 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 18 would suggest that for most of the sites the mean total carbon concentration has apparently 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 4 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 axes, the 2008 SOC values on the horizontal axes.

Figure 7 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).

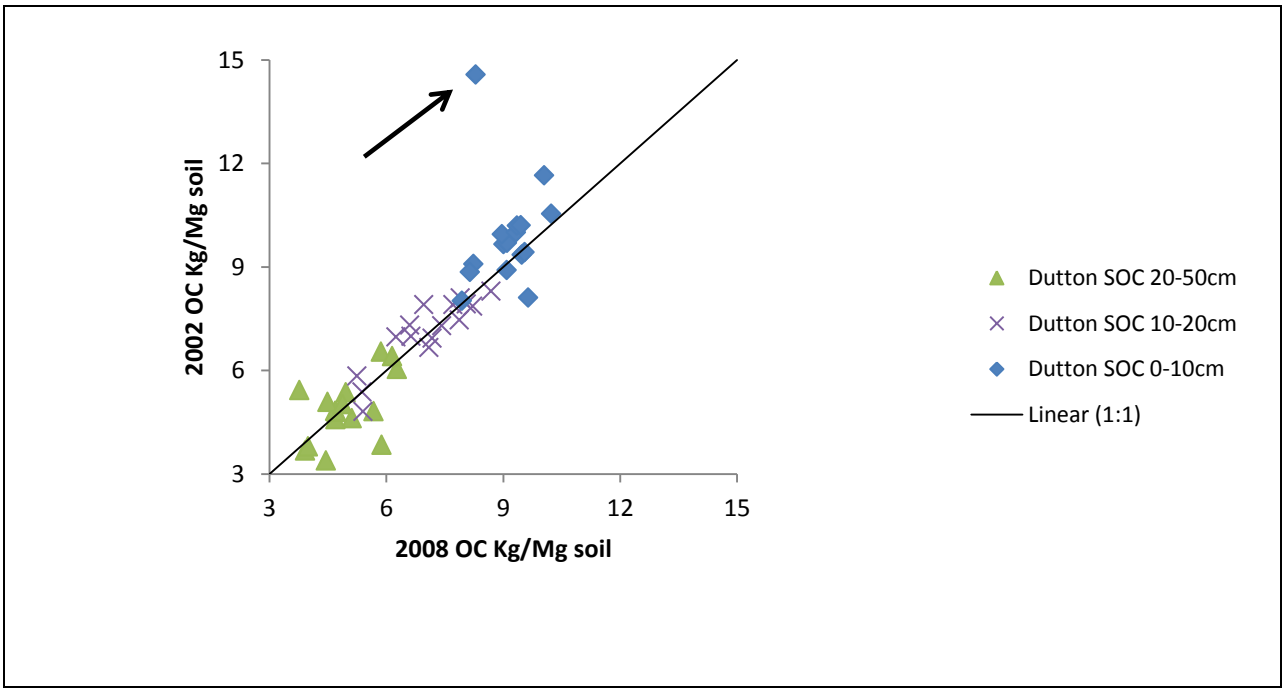

Figure 7: Scatter diagram of 2002 vs. 2008 SOC across three depths (0 – 10, 10 – 20, and 20 – 50 cm) at Dutton, MT. Arrow indicates outlier.

Figure 8 displays comparison 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 for 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 sampled.

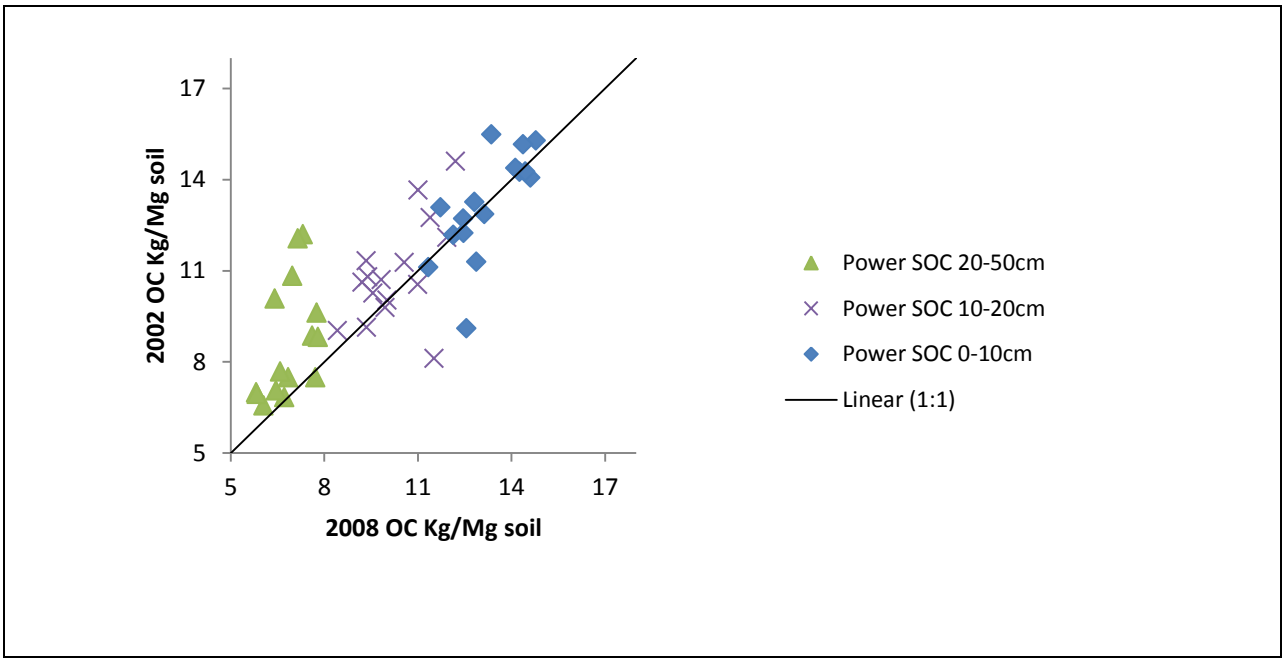

Figure 8: Scatter diagram of 2002 vs. 2008 SOC across three depths (0 – 10, 10 – 20, and 20 – 50 cm) at Power, MT.

Figure 9 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 that 2008, again either indicating losses of carbon or a bias in the sampling or analysis procedures. The variability of these values again seems to increases 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 14 that the apparent bias in the SOC values increased with sample depth.

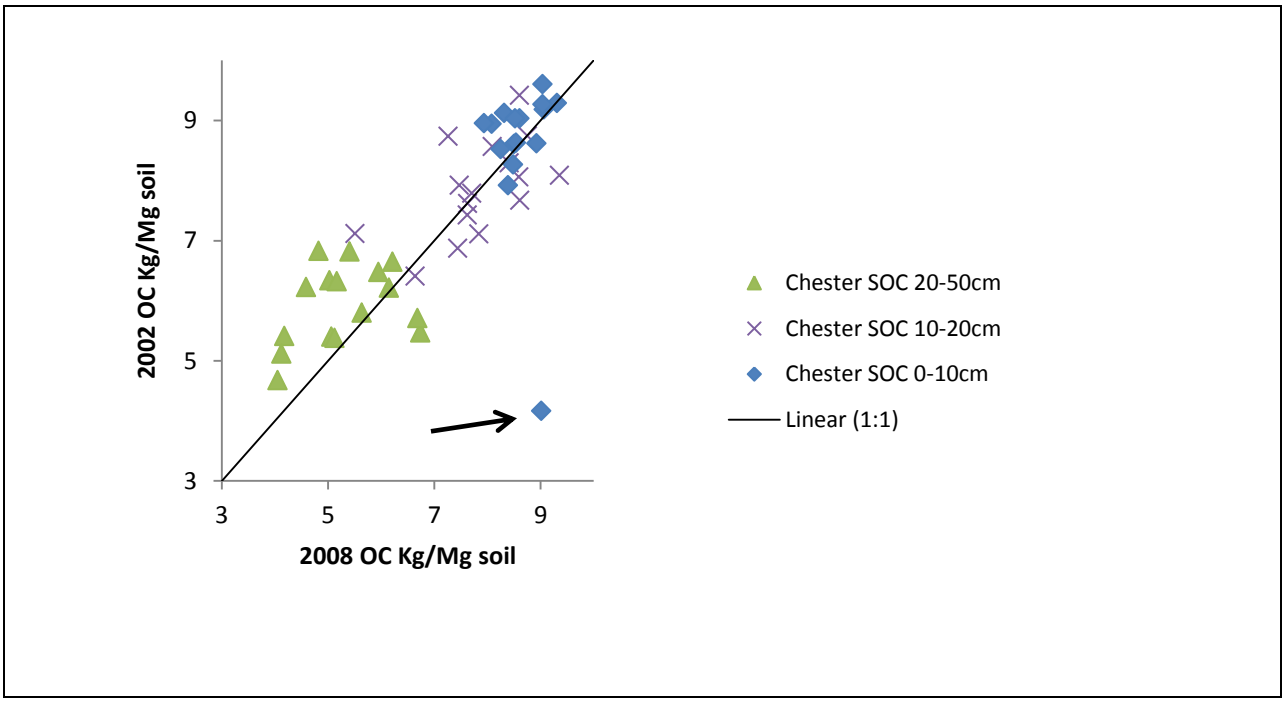

Figure 9: Scatter diagram of 2002 vs. 2008 SOC across three depths (0 – 10, 10 – 20, and 20 – 50 cm) at Chester, MT. Arrow indicates outlier.

Figure 10 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.

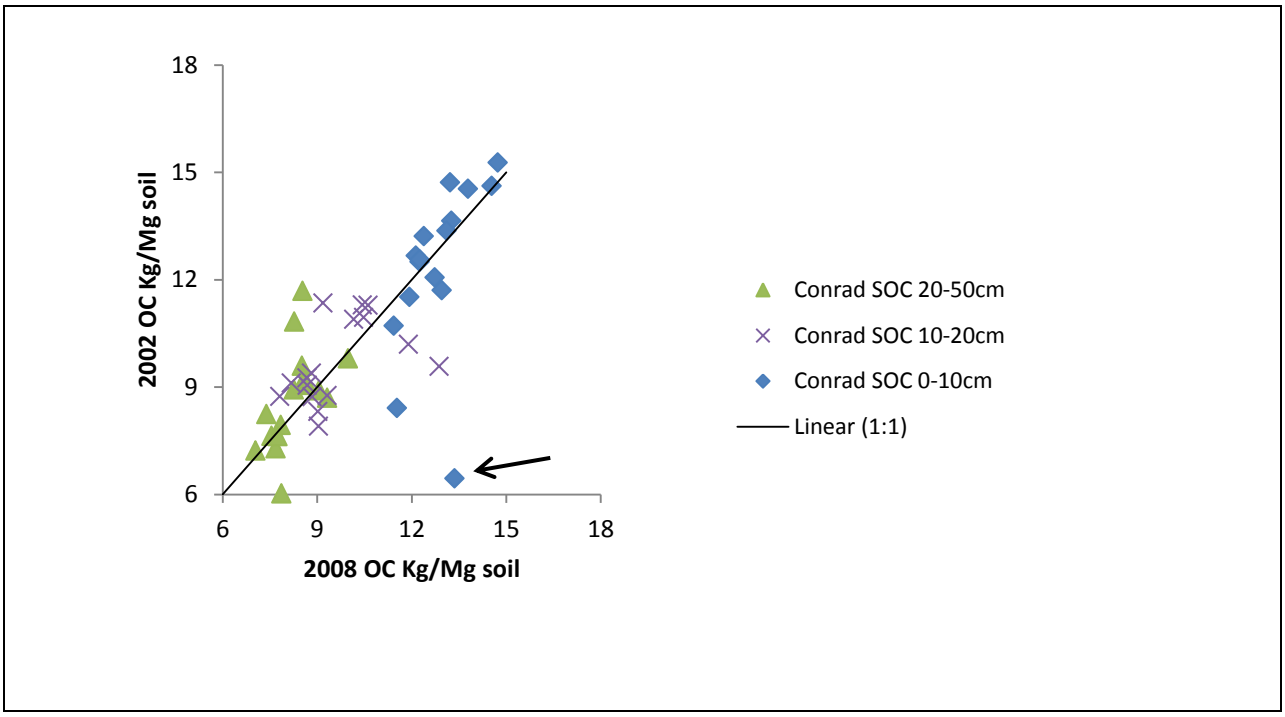

Figure 10: Scatter diagram of 2002 vs. 2008 SOC across three depths (0 – 10, 10 – 20, and 20 – 50 cm) at Conrad, MT.

Figure 11 displays the comparisons for SOC values at the Fife site. This figure reveals 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 which 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.

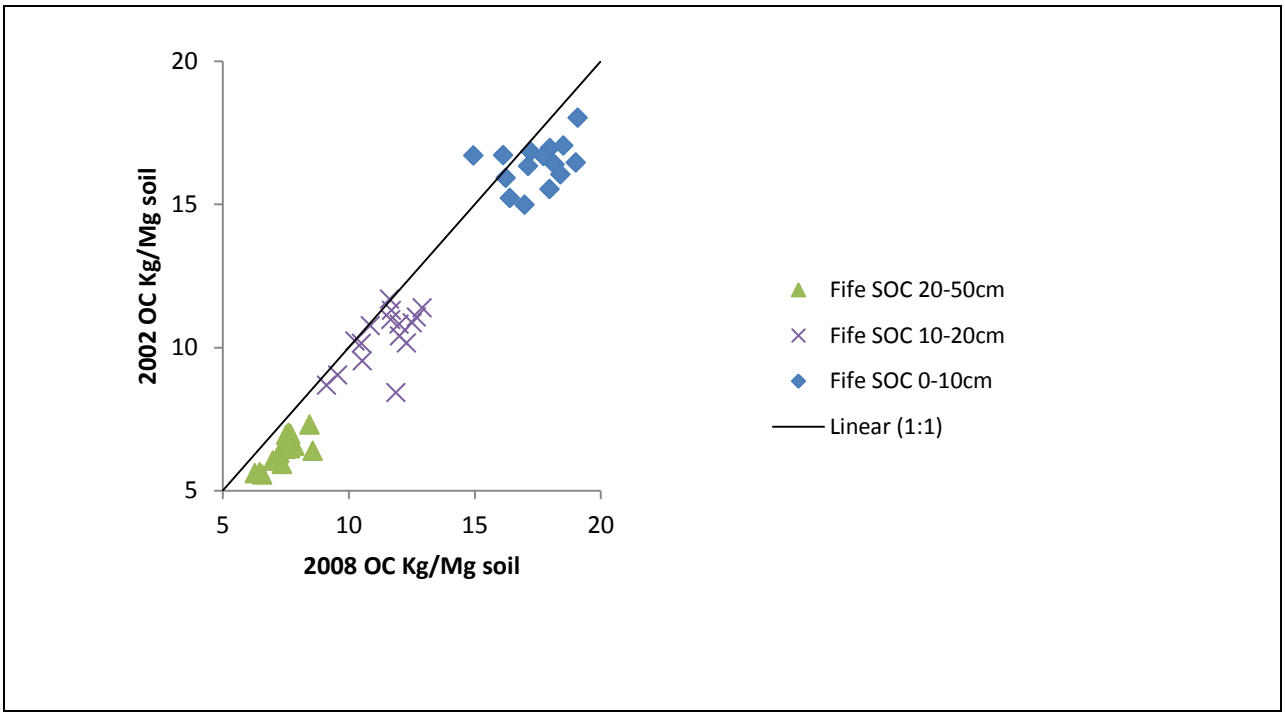

Figure 11: Scatter diagram of 2002 vs. 2008 SOC across three depths (0 – 10, 10 – 20, and 20 – 50 cm) at Fife, MT.

Figure 12 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.

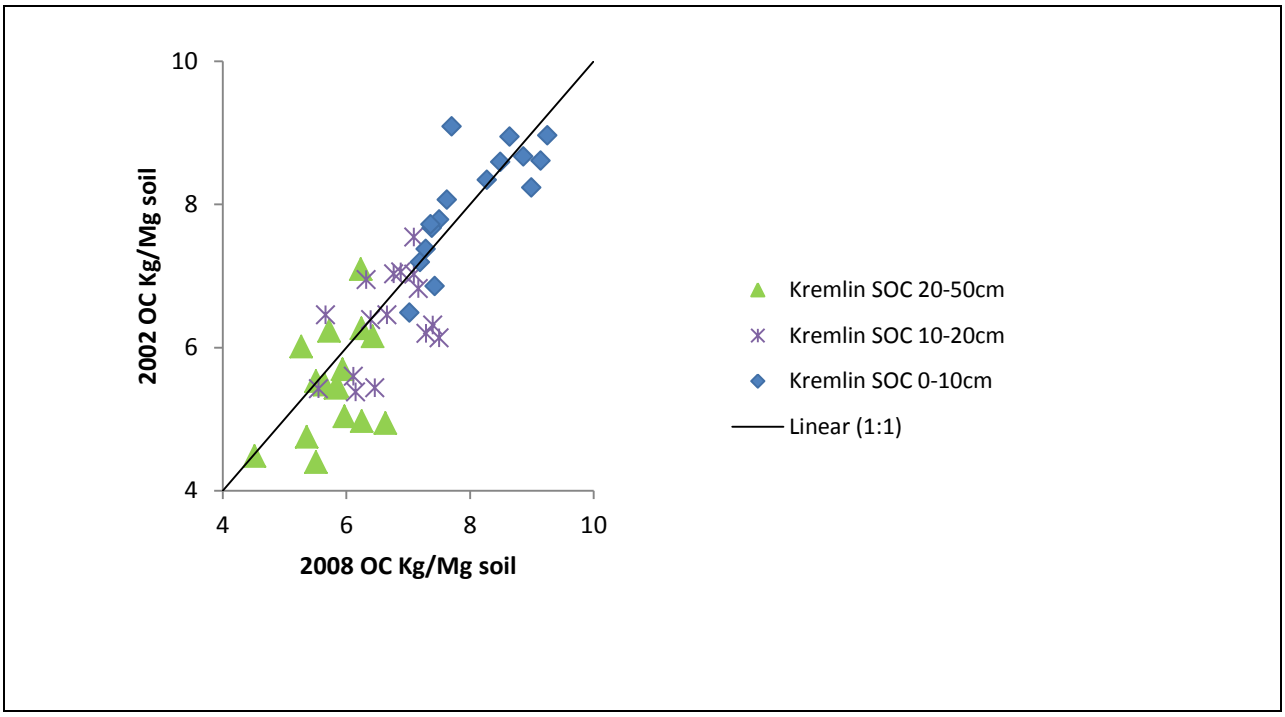

Figure 12: Scatter diagram of 2002 vs. 2008 SOC across three depths (0 – 10, 10 – 20, and 20 – 50 cm). Kremlin.

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 19 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 19: 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 potentially 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 a scatter diagrams with the 2002 values on the vertical axis and the 2008 values on the horizontal axis. A 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 (13-18).

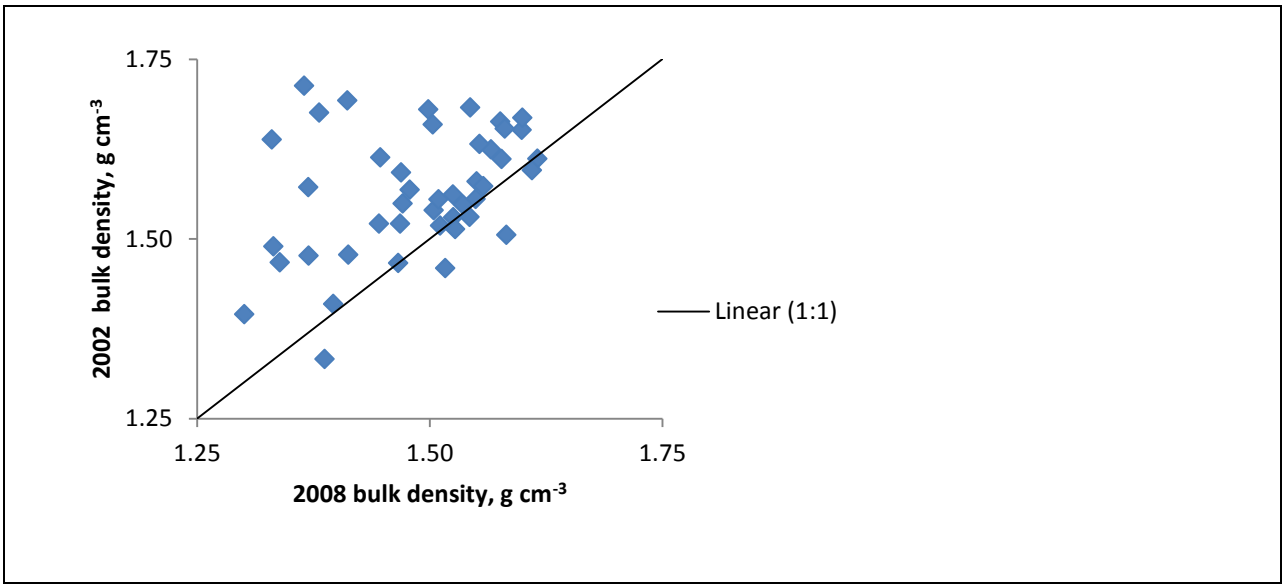

Figure 13: Scatter plot of 2002 vs. 2008 bulk density for the Dutton site.

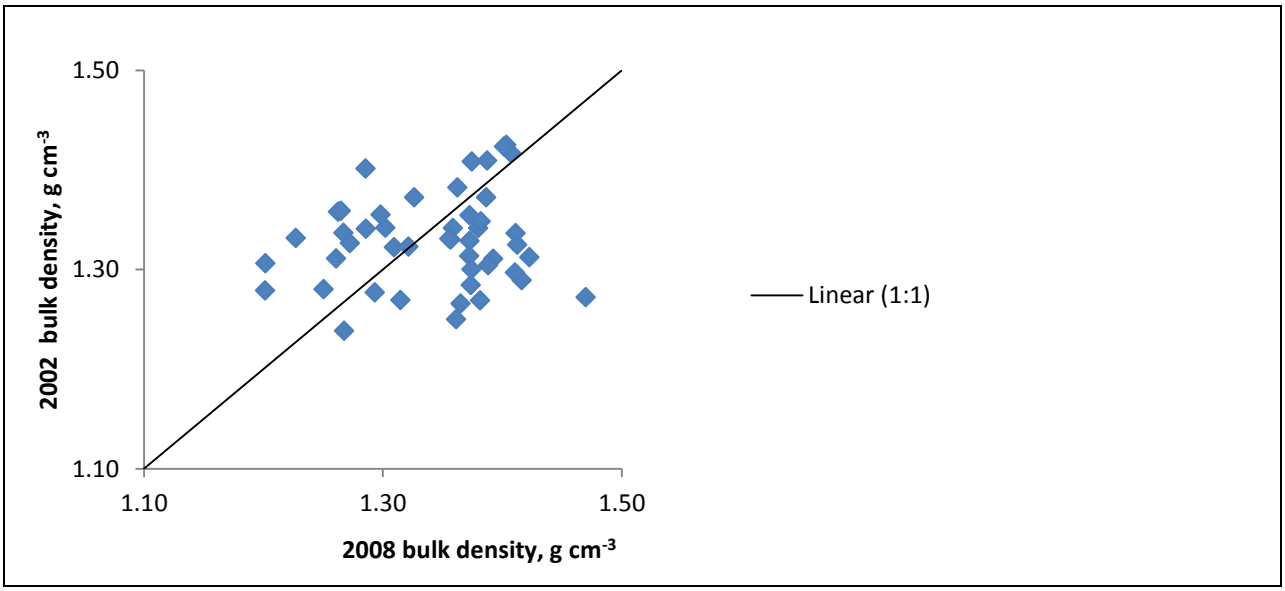

Figure 14: Scatter plot of 2002 vs. 2008 bulk density for the Power site.

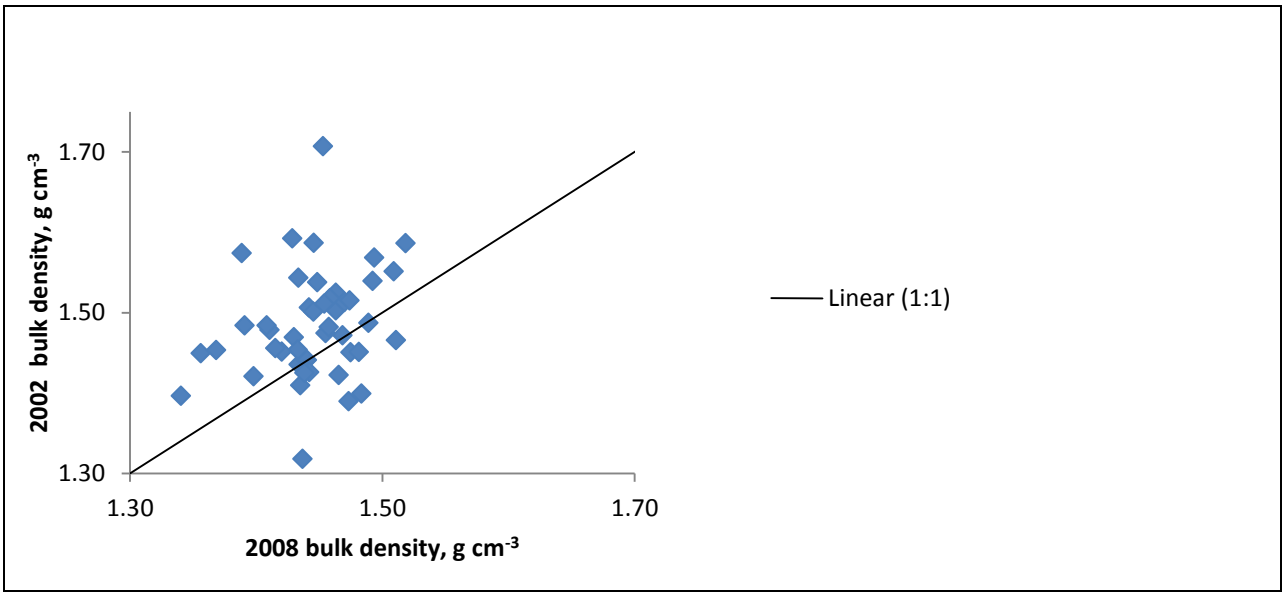

Figure 15: Scatter plot of 2002 vs. 2008 bulk density for the Chester site.

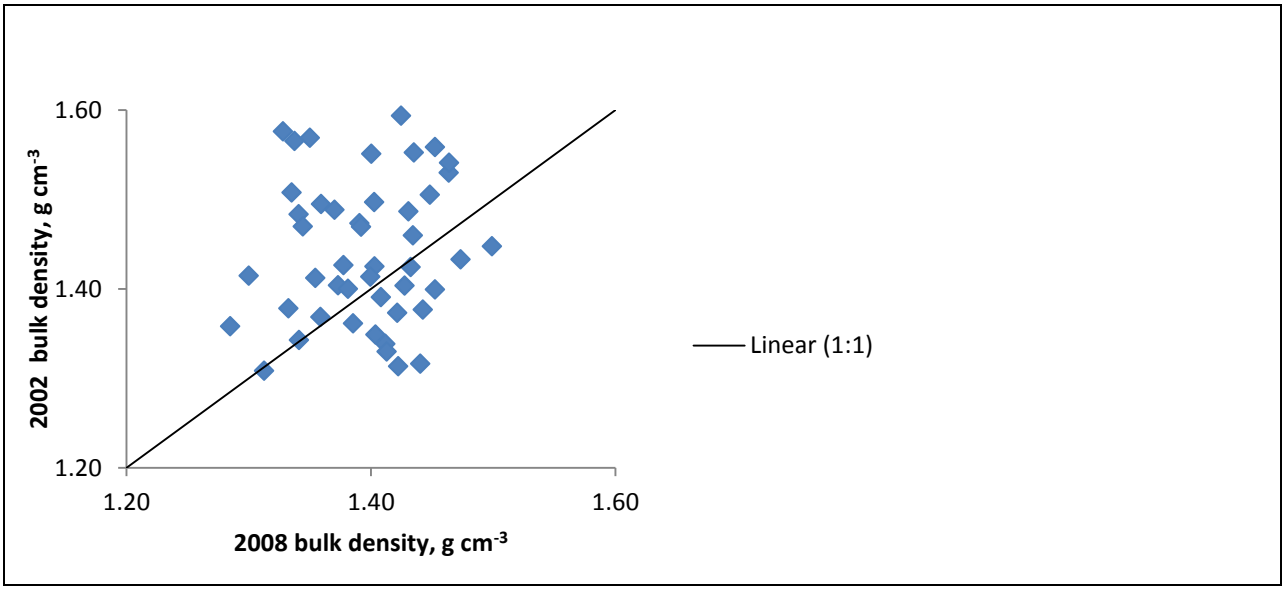

Figure 16: Scatter plot of 2002 vs. 2008 bulk density for the Conrad site.

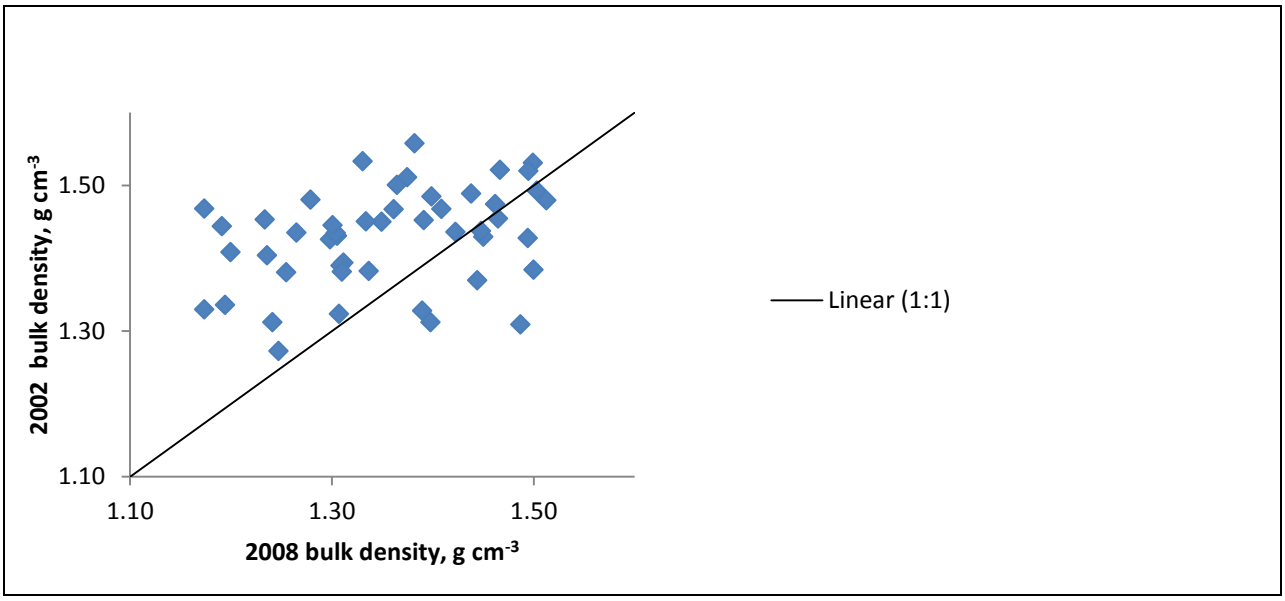

Figure 17: Scatter plot of 2002 vs. 2008 bulk density for the Fife site.

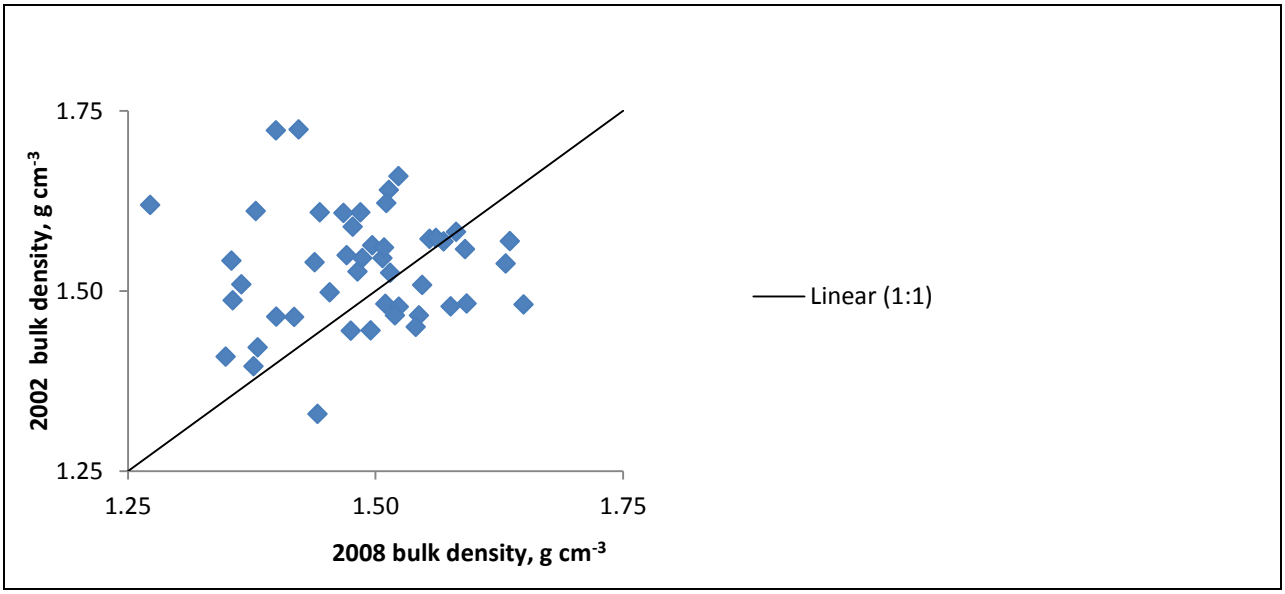

Figure 18: Scatter plot of 2002 vs. 2008 bulk density for the Kremlin site.

In Figures 13 – 18, 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 which 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 to determine the mass of the carbon within the soil profile. 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 sub samples 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 sub sampling procedure. To address this issue in 2008, composite samples were made using 30.0 g +/- 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.

2.3.4 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 dataset. This determination is based on the fact that comparisons of SOC from 2002 – 2008 result in values of SOC change which contradict previously reported responses (Campbell et al., 2000; Campbell et al., 2005; Halvorson et al., 2002; McConkey et al., 2003). These unusual results lead to our investigation of the data and the source of the values which have ultimately resulted in questions about the 2002 soil processing procedures. Unfortunately, the assumption of an unknown common baseline greatly reduces our ability to detect changes in SOC, due to within-field soil variability. With this assumption, we may be able to detect differences in SOC between treatments however we cannot definitively describe the rates of SOC change associated with management treatments.

Dutton

SOC was not significantly ($P < 0.05$) affected by treatments for any depth increment (Table 20).

Table 20: 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	0.230	0.230	0.330	0.574
	CI	1	2.865	2.856	4.130	0.065
	Tillage X CI	1	0.034	0.034	0.050	0.828
	Residual	12	8.294	0.691		
10-20cm						
	Tillage	1	0.024	0.024	0.010	0.926
	CI	1	0.689	0.689	0.250	0.625
	Tillage X CI	1	0.008	0.008	0.000	0.958
	Residual	12	32.817	2.735		
20-50cm						
	Tillage	1	0.139	0.139	0.010	0.929
	CI	1	6.566	6.566	0.400	0.541
	Tillage X CI	1	1.216	1.216	0.070	0.791
	Residual	12	199.132	16.594		
0-20cm						
	Tillage	1	0.400	0.400	0.080	0.785
	CI	1	6.363	6.363	1.240	0.287
	Tillage X CI	1	0.072	0.072	0.010	0.908
	Residual	12	61.585	5.132		
0-50cm						
	Tillage	1	1.005	1.005	0.030	0.861
	CI	1	25.093	25.093	0.820	0.383
	Tillage X CI	1	1.898	1.898	0.060	0.811
	Residual	12	379.578	31.632		

CI = Cropping Intensity

Power

Summary results from the ANOVA's run on data from Power are shown in Table 21. SOC in the 0-10 cm depth was not significantly affected by cropping intensity, tillage, or their interaction. This table would suggest Tillage increased SOC in the 10 – 20 cm depth (14.48 MT C ha-1) compared with no till (12.64 MT C ha-1). This difference detected assuming a common baseline could be simple the result of a truly non common baseline in 2002 or the result of dissolved organic carbon elluviation resulting in illuvial deposits in the 10 – 20 cm depth. No other significant affects were detected (Table 21) for either the 20 – 50 cm depth or over the whole profile (0 – 50 cm).

Table 21: 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	2.831	2.831	1.340	0.269
	CI	1	0.369	0.369	0.180	0.683
	Tillage X CI	1	0.170	0.170	0.080	0.781
	Residual	12	25.295	2.108		
10-20cm						
	Tillage	1	13.451	13.451	10.760	0.007
	CI	1	0.351	0.351	0.280	0.606
	Tillage X CI	1	0.311	0.311	0.250	0.627
	Residual	12	15.007	1.251		
20-50cm						
	Tillage	1	8.338	8.338	0.970	0.344
	CI	1	5.051	5.051	0.590	0.458
	Tillage X CI	1	2.081	2.081	0.240	0.631
	Residual	12	102.988	8.582		
0-20cm						
	Tillage	1	28.569	28.569	4.700	0.051
	CI	1	0.000	0.000	0.000	0.994
	Tillage X CI	1	0.020	0.020	0.000	0.956
	Residual	12	72.993	6.083		
0-50cm						
	Tillage	1	67.980	67.980	2.810	0.120
	CI	1	4.973	4.973	0.210	0.658
	Tillage X CI	1	2.496	2.496	0.100	0.754
	Residual	12	290.384	24.199		

CI = Cropping Intensity

Chester

Summary results from the ANOVA's run on data collected from this site are displayed in Table 22. Results suggest SOC in the 0-10 cm depth was significantly affected 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}). No significant treatment effects on SOC were observed in the 10-20 cm depth. Table 22 indicates a detectable difference (P=0.018) by cropping intensity when the whole profile is studied (0 – 50 cm). Since there were no differences in any depth other than the 0 – 10 cm depth, the effect of cropping intensity on SOC, 0 – 50 cm depth, was likely limited to the upper 10 cm.

Table 22: 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	0.384	0.384	2.020	0.180
	CI	1	1.392	1.392	7.330	0.019
	Tillage X CI	1	0.194	0.194	1.020	0.333
	Residual	12	2.280	0.190		
10-20cm						
	Tillage	1	0.494	0.494	0.320	0.583
	CI	1	4.873	4.873	3.140	0.102
	Tillage X CI	1	0.196	0.196	0.130	0.729
	Residual	12	18.640	1.553		
20-50cm						
	Tillage	1	10.256	10.256	0.730	0.410
	CI	1	0.009	0.009	0.000	0.981
	Tillage X CI	1	1.556	1.556	0.110	0.745
	Residual	12	168.545	14.045		
0-20cm						
	Tillage	1	0.008	0.008	0.000	0.946
	CI	1	1.475	1.475	7.440	0.018
	Tillage X CI	1	0.788	0.788	0.510	0.489
	Residual	12	18.505	1.542		
0-50cm						
	Tillage	1	10.841	10.841	1.010	0.336
	CI	1	12.093	12.093	1.120	0.311
	Tillage X CI	1	4.569	4.569	0.420	0.527
	Residual	12	129.436	10.786		

CI = Cropping Intensity

Conrad

Summary ANOVA results from the Conrad site are displayed in Table 23. These tables suggest a significant difference for the interaction of tillage and CI for all depths except 10 – 20 cm. Table 23 illustrates comparisons of SOC (MTOC/ha, mass adjusted) between the alternate year cropping treatment (0.5) and the annual cropping treatment (1.0) and between conservation tillage (T) and non tillage (NT) for this site. Results indicates a significant cropping intensity and tillage interaction in the 20-50 and 0 – 50 cm depths as a result of the increase in SOC with

cropping intensity under NT and decrease in SOC under till (Table 23). Field topography at Conrad is characterized by rolling terrain. Locations of the four microplots within the tilled annual crop treatment were concentrated in an upslope position (Figure 19). The SOC levels in this area would be expected to be lower than in toe slope or depressional areas. Soil samples (0 – 10 cm) from this upland location had the three lowest SOC values at this site. Hence, the significant interaction effect observed when assuming a common unknown baseline may not truly be an interaction, but rather natural variation resulting from the extensive topographical relief at this site.

Table 23: 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	0.423	0.423	0.710	0.417
	CI	1	1.051	1.051	1.760	0.209
	Tillage X CI	1	14.516	14.516	24.330	0.0003
	Residual	12	7.159	0.597		
10-20cm						
	Tillage	1	0.064	0.064	0.020	0.888
	CI	1	9.410	9.410	3.050	0.106
	Tillage X CI	1	4.742	4.742	1.540	0.239
	Residual	12	37.052	3.088		
20-50cm						
	Tillage	1	0.544	0.544	0.050	0.823
	CI	1	5.256	5.256	0.510	0.491
	Tillage X CI	1	54.206	54.206	5.210	0.042
	Residual	12	124.818	10.401		
0-20cm						
	Tillage	1	0.158	0.158	0.030	0.869
	CI	1	16.626	16.626	3.010	0.109
	Tillage X CI	1	35.790	35.790	6.460	0.026
	Residual	12	66.372	5.531		
0-50cm						
	Tillage	1	0.112	0.230	0.010	0.943
	CI	1	3.204	2.856	0.150	0.702
	Tillage X CI	1	177.956	0.034	8.560	0.013
	Residual	12	249.375	0.691		

CI = Cropping Intensity

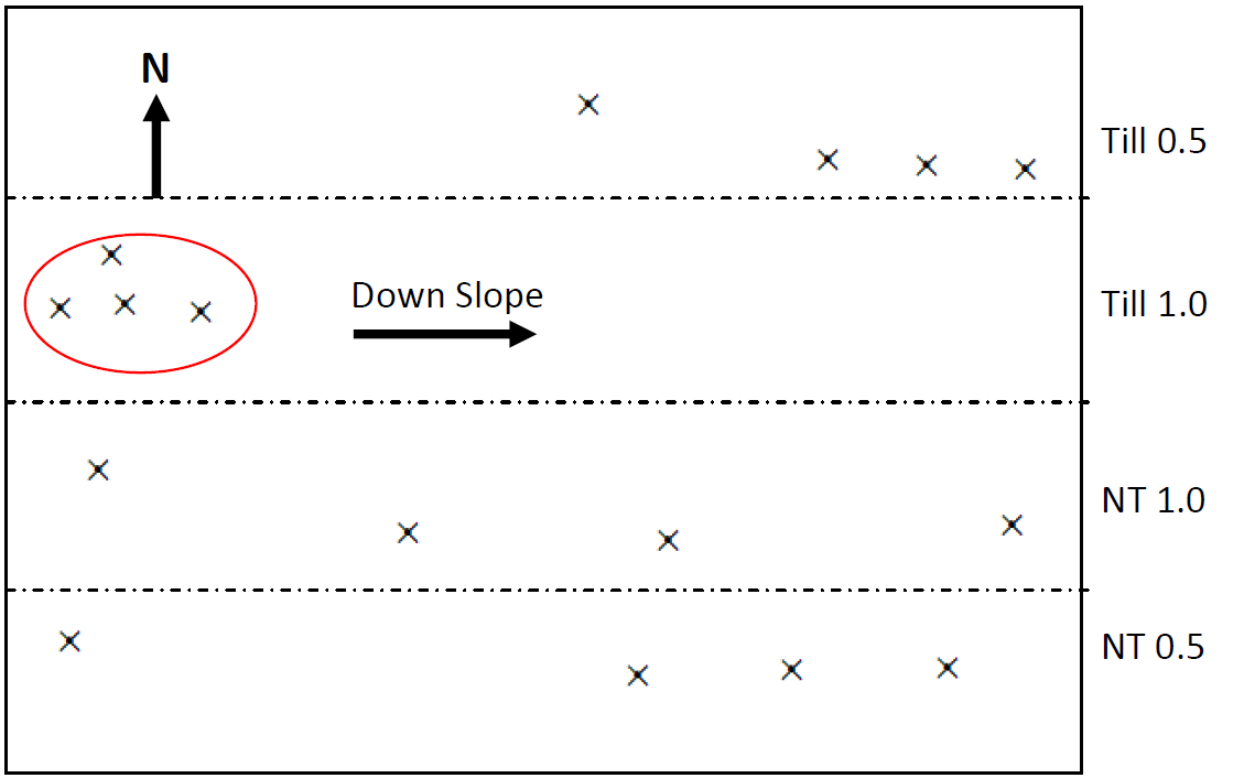

Figure 19: Conrad microsite locations. Treatment boarders notated with dashed lines.

Fife

Summary results from the ANOVA's run on data collected from the Fife site are displayed in Table 24. Results indicate 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, and 20-50 cm depths (Table 24). However, a detectable difference by cropping intensity for the 0 – 50 cm profile, caused by the 0-10 cm depth.

Table 24: 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	1.271	1.271	0.760	0.401
	CI	1	14.650	14.650	8.720	0.012
	Tillage X CI	1	0.328	0.328	0.200	0.6666
	Residual	12	20.159	1.680		
10-20cm						
	Tillage	1	0.018	0.018	0.010	0.938
	CI	1	4.060	4.060	1.430	0.255
	Tillage X CI	1	0.221	0.221	0.080	0.785
	Residual	12	34.154	2.846		
20-50cm						
	Tillage	1	0.483	0.483	0.040	0.853
	CI	1	17.431	17.431	1.290	0.278
	Tillage X CI	1	0.000	0.000	0.000	0.999
	Residual	12	161.778	13.481		
0-20cm						
	Tillage	1	0.990	0.990	0.150	0.708
	CI	1	34.164	34.164	5.060	0.044
	Tillage X CI	1	1.071	1.071	0.160	0.697
	Residual	12	80.945	6.745		
0-50cm						
	Tillage	1	2.848	2.848	0.080	0.783
	CI	1	100.451	100.451	2.800	0.120
	Tillage X CI	1	1.056	1.056	0.030	0.867
	Residual	12	430.100	35.842		

CI = Cropping Intensity

Kremlin

Summary results from the ANOVA's run on data from the Kremlin site (Table 25) show there are no statistical differences ($P < 0.05$) seen between either pair of treatment comparisons for any depth at this site.

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	0.205	0.230	0.080	0.778
	CI	1	5.581	2.856	2.270	0.158
	Tillage X CI	1	0.039	0.034	0.020	0.9018
	Residual	12	29.046	2.421		
10-20cm						
	Tillage	1	1.434	1.434	0.770	0.397
	CI	1	2.764	2.764	1.490	0.246
	Tillage X CI	1	1.581	1.581	0.850	0.374
	Residual	12	22.262	1.855		
20-50cm						
	Tillage	1	7.009	7.009	0.240	0.630
	CI	1	2.536	2.536	0.090	0.771
	Tillage X CI	1	58.179	58.179	2.030	0.180
	Residual	12	343.791	28.649		
0-20cm						
	Tillage	1	0.566	0.566	0.220	0.646
	CI	1	0.487	0.487	0.190	0.670
	Tillage X CI	1	2.095	2.095	0.820	0.382
	Residual	12	30.559	2.547		
0-50cm						
	Tillage	1	11.577	11.577	0.660	0.432
	CI	1	5.233	5.233	0.300	0.595
	Tillage X CI	1	38.100	38.100	2.180	0.166
	Residual	12	210.137	17.511		

CI = Cropping Intensity

2.4 ***Discussion***

Recently, studies in the northern Great Plains have identified a range of SOC sequestration rates based on site-specific factors. One study conducted in a 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 0.027 to 0.430 Mg ha^{-1} yr^{-1} (McConkey et al., 2003) The same study found rates of SOC carbon sequestration associated with no-till management ranged from 0.067 Mg ha^{-1} yr^{-1} to

0.512 Mg ha^{-1} yr^{-1} (McConkey et al., 2003). They attributed the variability in SOC sequestration rates from this study largely to the durations of the studies (8 – 25 yrs) and the soil texture differences at each site studied (28 – 63 % clay). A second study conducted near Mandan, ND, over 12 yr 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 0.233, 0.025, and -0.141 Mg ha^{-1} 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. While we did not observe any significant increases in SOC from decreased tillage alone, we did observe a significant difference in SOC related to increased cropping intensity at two sites, Chester and Fife, in the 0 – 10 cm depth, when assuming a common unknown baseline. It is possible that had we been able to make more precise 2002 – 2008 SOC comparisons, more sites might have had detectable SOC differences.

A more recent review study conducted (Campbell et al., 2005), examined carbon sequestration rates related to cropping frequencies and tillage practices for soils in the semiarid North American Great Plains. The authors of this review were able to identify gains in SOC under no-till management that were 0.20 Mg ha^{-1} yr^{-1} to 0.25 Mg ha^{-1} yr^{-1} greater than rates associated with tilled systems, regardless of cropping frequencies.

While our finding are not consistent with those reported by Campbell et al. (2005), again it is possible that had we been able to make competent 2002 – 2008 delta SOC comparisons, or had the duration of the study been longer, we may have seen results similar to those from Campbell (2005). 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 and 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 +/- 0.14 Mg C ha^{-1} yr^{-1}.

The site to site variation in SOC seen in Tables 5, 9, 13, 17, and 23 is, in part, a cumulative response to site-specific factors such as clay content and onsite management. The relationship between %clay and SOC, predicts greater SOC accumulations in soils with elevated clay concentrations (McConkey et al., 2003). While clay content plays a major role in SOC sequestration, there are also a variety of other site-specific factors such as nutrient management, weed pressure, local weather, and actual farmer operations which have resulted in site-specific nuances that affect the rates of sequestration. When looking at each site as an individual site rather than reps, these differences become more apparent.

Our concerns with sample processing would be the second major factor affecting the variation seen in SOC values from Tables 5, 9, 13, 17, and 23. If the concerns with the baseline 2002 data can be addressed, it is possible the result would be reduced variability in the 2002 – 2008 delta SOC values. A reduction in variability in the delta SOC values could result in an increased signal to noise ratio adequate to detect differences in SOC and true rates of change in SOC for more sites and depths than just the detection of differences for the 0 – 10 cm depth at the Chester and Fife sites and the 20 – 50 cm depth at the Conrad site.

2.5 *Conclusions*

Given the inconsistent delta SOC values in the 2002 – 2008 SOC comparisons, the only comparisons that could be made with confidence are those between treatment combinations by site (assuming a common baseline). Under the assumption of a common unknown baseline, only two sites showed a difference at the shallowest depth sampled (0 – 10 cm), and a third site showed an interaction between the treatments in the 20 -50 cm depth. It is important that this study continues in order to determine SOC sequestration rates for this region. This study is truly in its infancy. Most studies reporting significant management impacts on carbon sequestration rates in similar agro-climatic regions have had more years of continuous management (minimum of 8) when C sequestration rate determinations were made. If determining rates of change continues to be the focus of this study it is also important that future processing continues to be done using the adjusted protocols employed during sample year 2008. The adjustments to the original protocol will result in a higher quality dataset and potentially increased signal to noise ratios necessary for accurate determination of significant management-induced SOC change.

3. Active Microbial Biomass Carbon Testing

3.1 *Introduction*

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; Bremer et al., 2008; Bricklemyer et al., 2005; McConkey et al., 2003) and still the amount of natural variation among soil samples can confound detection of SOC changes (Al-Kaisi et al., 2005; Bricklemyer et al., 2005; Munoz et al., 2007; Robertson et al., 1997). Because of these difficulties, researchers have experimented with alternative methods for detecting or predicting changes in soil organic carbon pools (Campbell et al., 2005; Franzluebbers et al., 1996; Powlson et al., 1987; Sparling, 1992). 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 sub pool 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 yrs. 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 yrs. This slow pool is comprised of structural plant compounds and physically stabilized carbon (Cochran et al., 2007). The active organic C (AOC) pools make up <5% of SOC and has a mean turnover time ranging from hours to months (Burke et al., 1997). 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 (Cochran et al., 2007). 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., 2000). 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 CO_2 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 (Appel, 1998; Franzluebbers et al., 2000; Jenkinson and Powlson, 1976; Wu and Brookes, 2005).

One specific test which employs this concept, the Active Microbial Biomass Carbon (AMBC) Test (Campbell et al., 2005; Franzluebbers et al., 1996; Franzluebbers et al., 2000) 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 CO_2. The differences in CO_2 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) 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 airdried 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 CO_2 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:

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

3.2 *Material and Methods*

Soil microbial biomass carbon was analyzed using the AMBC test and methods described by L. A. Sherrod (Campbell et al., 2005). This involved incubating 20g soil samples in sealed 1L canning jars at 30°C and 50% water–filled pore space for 3 d. Pore space percentage was calculated by weighing samples into 45mm Wheaton screw cap jars with a line indicating a bulk density of 1.0 and using a particle density of 2.65 g cm^{-3}. Respired CO_2 was measured after 3 d 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 (Ellert and Bettany, 1995).

$$Y = 337 + 2.4\ x$$

This analysis was done on soil samples collected at the Power and Fife sites, during the fall of 2008. If deemed appropriate, 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 a 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. (Campbell et al., 2005), with modifications to address the effect of storage time on the outcome of the test. To ensure adequate soil for repeated testing, 20 kg of fresh soil 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° for 4 d. After complete drying, the samples were cooled and processed for analysis by removing plant litter and grinding the sample to ensure it was a homogeneous mixture.

The AMBC test was repeated every three months using the soil collected from Amsterdam during June of 2008. Additional controls were added to the experiment. These controls consisted of 3 incubation chambers which contained autoclaved soil from the Amsterdam site rather than fresh soil. The soil for these controls was autoclaved for 1 hr then allowed to cool overnight. This was repeated for three consecutive days on the same soil. After the third cycle, the 20g sample was weighed out and placed into the incubation chamber and the incubation container and soil was autoclaved once more for 1 hr to ensure minimal chances of contamination of the soil and incubation chamber. Additionally, the de-ionized water use 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 counts procedures were conducted using site-specific soil extract agar and the procedures outline 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 micro-organisms present in the soil are as described in the Soil Science Society of America (SSSA) handbook (Weaver and Soil Science Society of America., 1994), with the following modifications. Soil extract was prepared by autoclaving 1kg of soil in 1L of de-ionized water for 30 min and then allowed to cool at 4°C. Soil used to make the extract was soil collected in the area of samples being tested (i.e. site specific). Once cool, 0.5 g of $CaCO_3$ is mixed into the extract to induce flocculation of the clay particles and then the mixture is allowed to settle for 12 h. The liquid and was decanted and then centrifuged in large Oakridge bottles for 30 min at 8000g 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 min and refrigerated at 4°C until used.

The soil extract agar was prepared using 17 g of agar in 1L of site-specific liquid soil extract. Cooled plates were refrigerated at 4°C until used. Diluent for dilution series was prepared by mixing 1L of liquid site-specific soil extract with 1g of sodium pyrophosphate (soil-extract-phosphate solution). The mixture was then autoclaved for 45 min 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 sec and then on a horizontal shaker for 30 min. Bottles were allowed to settle for 30 sec and then 1 ml 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 d, after which colony counts were recorded. This was done for both fresh soil and soil which had been autoclaved for 3 consecutive days. The autoclaved soil was weighed into the milk dilution bottles and autoclaved once more for 1hr 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 airdrying.

3.3 *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. The results from the repeated AMBC testing can be seen in Figure 20. This figure displays the 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. This figure shows a decreasing trend in the number of 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 we were not able to begin data collection at time zero so any attempt at fitting a response curve is speculative.

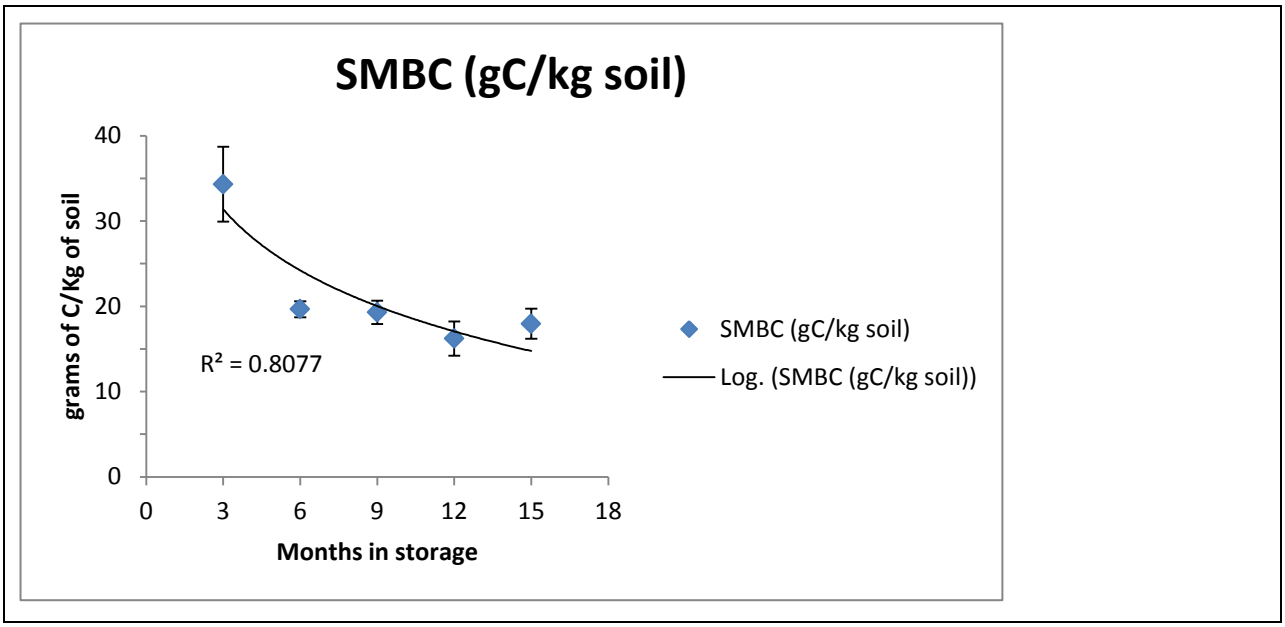

Figure 20: AMBC test storage response.

In addition to the repeated AMBC testing, viable plate counts were conducted in parallel (Figure 21). This figure displays the CFU's/ml on the vertical axis with the months of storage on the horizontal axis and the standard deviation depicted as error bars. 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.

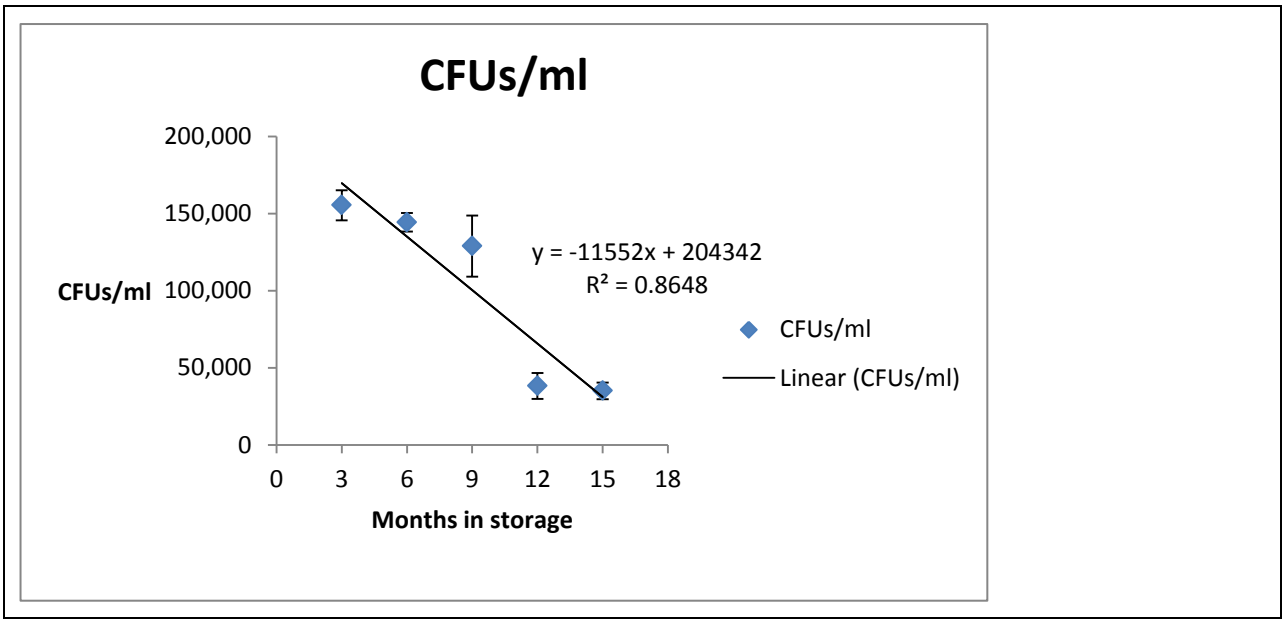

Figure 21: Direct plate count results

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 CO_2 during the AMBC test.

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 22.

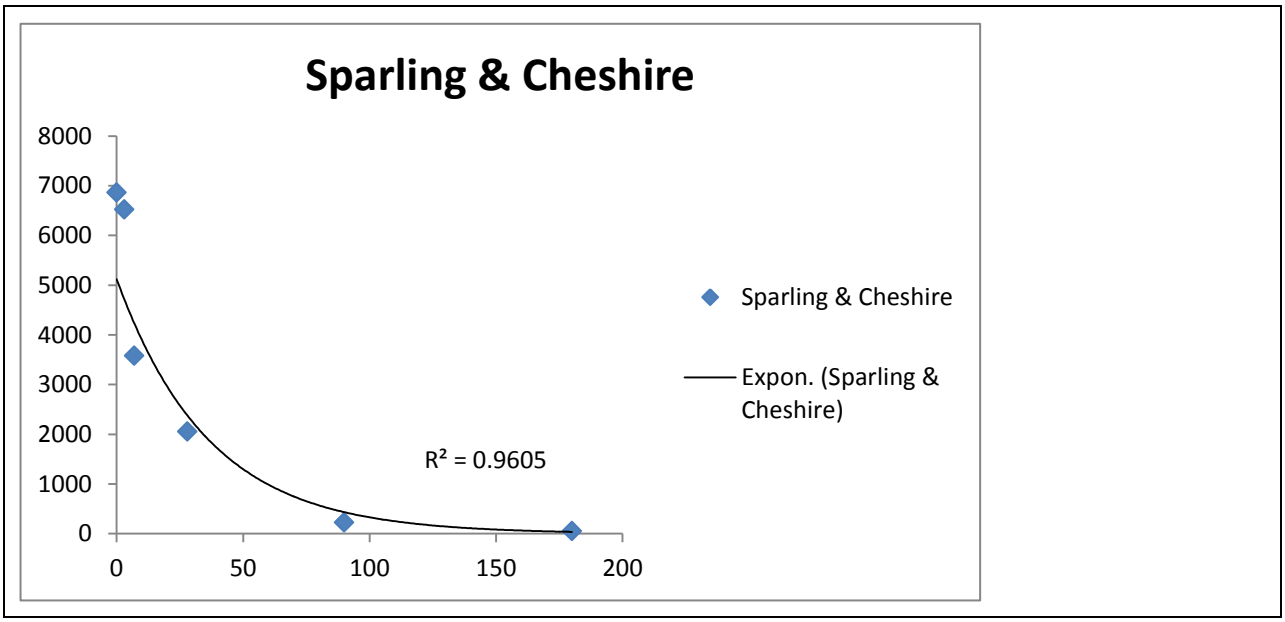

Figure 22: Data plotted from Sparling & Chelshire, 1979.

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 affect resulting from differing microbial concentrations in samples with different storage times. The results from the repeated AMBC testing on the autoclaved soil samples can be seen in Figure 23. This figure shows the concentration of CO_2 produced during the AMBC test on the vertical axis with the months of storage on the horizontal axis and the standard deviations for each round of testing shown as error bars. For the first point at 3 months, the error bars are so small they cannot be seen at this scale.

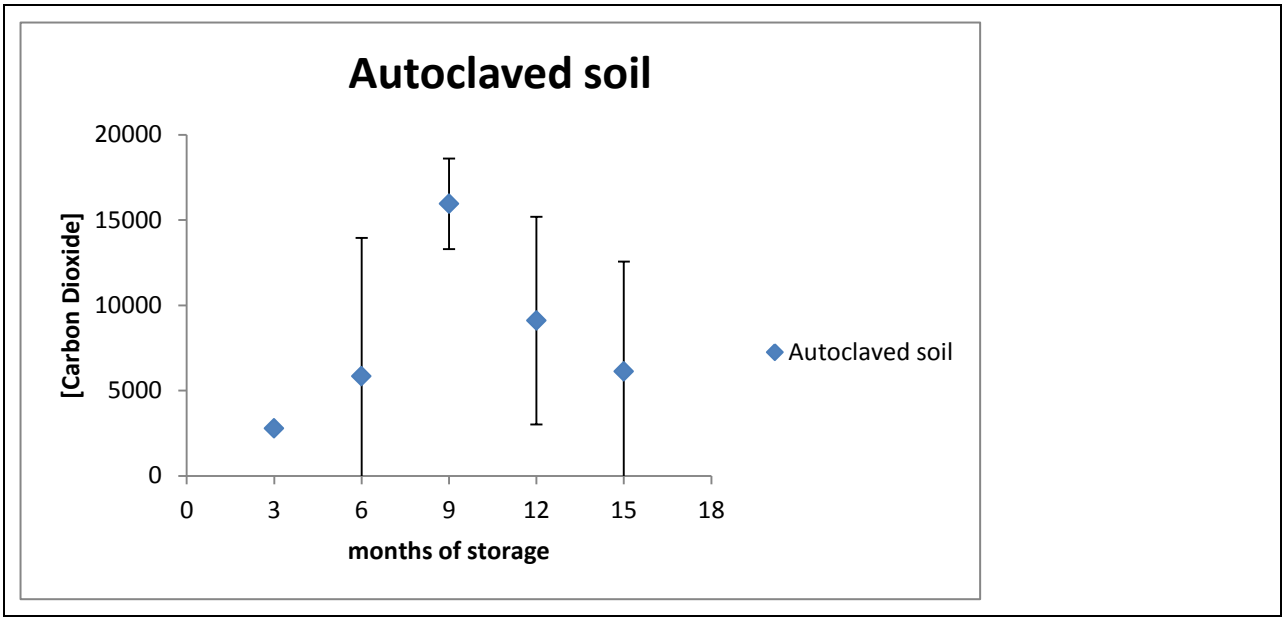

Figure 23: AMBC test with autoclaved soil.

While this graph shows considerable concentrations of CO_2 being evolved, it is the variability that was most puzzling. Soil extract agar plates spread using a sub sample of the autoclaved soil had no growth for all the repeated experiments yet CO_2 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.

From the outcomes of the repeated AMBC testing, the direct plate counts and the failed attempts to standardize the test with sterilized soil, it was determined comparisons made between freshly collected and stored soils would likely result in the storage effect becoming a confounding factor for determining accurate changes in AMBC over time. Because of this concern, comparisons between soils stored for various lengths of time 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 site and the Fife site. An ANOVA conducted using the SAS® software to make comparisons by treatment, plot and a treatment x plot interaction showed no significant differences ($p < 0.05$). These results can be seen in Tables 26 and 27.

Table 26: ANOVA summary 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 27: ANOVA summary 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		

3.3.1 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 CO_2 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 without a modification to the methods of the AMBC test.

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.

Comparisons between treatments for the Fife and Power sites, conducted 1 yr 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 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 making detection of differences more difficult.

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