

Rainmaker Farm – Baseline Soil Sampling

Final Report

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Introduction

Climate change driven by anthropogenic sources of atmospheric carbon dioxide is one of the most pressing environmental and social issues of our time. Changes to land management practices, such as those described below, have the potential to sequester carbon from the atmosphere, which is essential to slow climate change. Regenerative agriculture seeks to restore soil health and biodiversity through cultivation practices such as conservation tillage, the use of cover crops, and integration of livestock (Newton et al., 2020). The Gulch Environmental Foundation is converting their recently acquired farm, Rainmaker Farm, from a conventional tilled-wheat to no-till, diverse crop model using regenerative agricultural practices. As part of this effort, they are monitoring the changes to soil health in a way that can be evaluated for change to carbon levels in a statistically significant manner and will also meet the requirements of multiple certification programs. Regenerative agriculture certification programs require monitoring of soil carbon sequestration rates. There are also a number of carbon credit programs, such as the Regenerative Organic Certification, Oklahoma Carbon Program, and carbon monitoring protocols by the Food and Agriculture Organization of the United Nations, that evaluate the amount of carbon sequestered in soil.

Whenever possible, it is preferable to sample soils prior to any significant changes in cultivation – this provides a baseline of information. Without an understanding of baseline conditions prior to land use change, it is impossible to attribute any change in soil carbon to the management practices. Soil in a field can vary widely in terms of texture (i.e., the percent of sand, silt, and clay), management history, and other properties that are known to influence soil carbon contents. Prior landowners may have also chosen where they implement cultivation practices based on the observed differences in soil type. In other words, current differences between management areas may have already existed prior to cultivation so it is not possible to say with certainty what is driving differences until the change over time is quantified. The response of carbon to management could be mediated by these pre-existing differences. Thus,

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baseline sampling and analysis aims to characterize the different areas of a farm and capture the spatial variability.

Here, we present results of baseline sampling and analysis of soil at Rainmaker Farm. We collected and analyzed soil samples (Figure 1, Table 1) in a manner that was sufficient to provide data for future assessments of ecosystem service enhancements, particularly carbon sequestration, resulting from implementation of regenerative agriculture principles. Our methodology also met the criteria for multiple carbon assessments, including Regenerative Organic Certification, The Food and Agriculture Organization of the United Nations (FAO UN) Carbon (FAO UN, 2020). In addition to total organic carbon, we also measured the carbon in particulate and mineral-associated organic matter fractions, which are considered the active and slow-cycling pools of carbon, respectively. Mineral-associated organic matter is a more stable, persistent form of carbon. While this measurement is not currently a requirement of certification programs, it could be in the future as there is increasing interest in looking beyond total carbon. From the standpoint of regenerative agriculture, it would be beneficial to increase the quantity of both forms of organic matter, particulate and mineral-associated, as they provide two distinct, but complimentary services. Particulate organic matter is more sensitive to management, and we expect would respond more quickly in the short-term (i.e., within five years) to regenerative agricultural practices. At the same time, this organic matter is just as sensitive to disturbance and could be lost just as quickly as it is gained. Mineral-associated organic matter takes longer to form but is more resilient to disturbance and thus could be seen as a key long-term reservoir of carbon. As such, it may be valuable to monitor the changes in these fractions of organic matter in addition to total organic carbon. In addition to carbon, we also measured additional soil metrics (pH, N, P, and K) to provide guidance on farm management needs.

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Sampling Locations and Soil Mapping Units

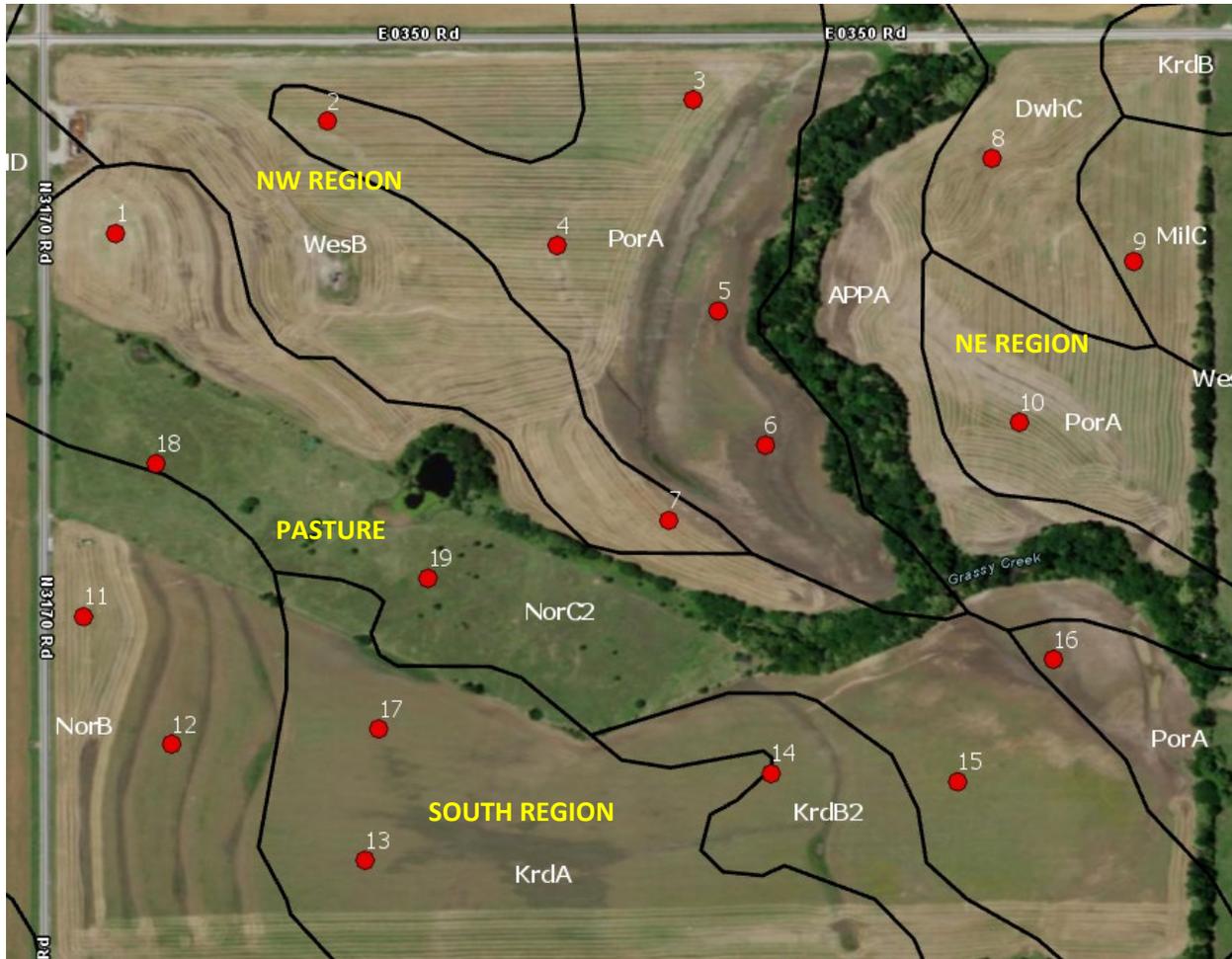


Figure 1. Map of soil sample locations at Rainmaker Farm. The black lines indicate the Natural Resource Conservation Service (NRCS) soil map with the corresponding soil map unit in white text. This is a highly diverse and complex soil system with soils derived from alluvium and shale bedrock. The four management zones are labelled in yellow text.

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Table 1. Assigned region and soil map units for each sampling location.

Location	Region of field	Soil map unit
1	NW Region	Norge Silt Loam 3-5% slope eroded
2	NW Region	Port Silt Loam 0-1 % slope
3	NW Region	Port Silt Loam 0-1 % slope
4	NW Region	Port Silt Loam 0-1 % slope
5	NW Region	Port Silt Loam 0-1 % slope
6	NW Region	Port Silt Loam 0-1 % slope
7	NW Region	Westsum Silty Clay loam 1-3% slope
8	NE Region	Dilworth Silty Clay Loam 3-5 % slope
9	NE Region	Milan Loam 3-5% slope
10	NE Region	Port Silt Loam 0-1 % slope
11	South Region	Norge Silt Loam 1-3% slope
12	South Region	Norge Silt Loam 1-3% slope
13	South Region	Kirkland Silt loam 0-1% slope
14	South Region	Kirkland Silt loam 1-3% slope eroded
15	South Region	Norge Silt Loam 3-5% slope eroded
16	South Region	Port Silt Loam 0-1 % slope
17	South Region	Kirkland Silt loam 0-1% slope
18	Pasture	Norge Silt Loam 3-5% slope eroded
19	Pasture	Norge Silt Loam 3-5% slope eroded

Sampling Methodology

We collected soil samples to meet the requirements of the Regenerative Organic Certified program and the CSOC MRV Protocol published by the FAO UN. The sampling and analysis requirements for each program is detailed in Table 2. After reviewing the requirements of each program, we decided to conduct an additional, more extensive round of sampling that we believed was necessary to accurately monitor changes in carbon stock.

On October 26, 2021, Jason Warren, Andrew Whitaker, and two Oklahoma State University (OSU) graduate students conducted this extensive sampling campaign. Five cores were collected within 30 feet of each of the sample locations indicated in Figure 1 and Appendix 1

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(spatial data files) with a hydraulic 2-inch diameter soil probe to a depth of 36 inches. Each core was cut to 0-4, 4-8, 8-12, 12-18, 18-24, and 24-36 inches. A “sample” refers to an individual depth range from a single core. Each sample was placed in separate Ziploc bags in the field at sampling. The samples were weighed, and a subsample removed to determine field moisture for each depth of all cores. The subsamples were weighed then dried at 230 degrees F and weighed again to determine moisture content of the soil at sampling. This was used to calculate the bulk density of each segment collected. The remaining sample was air-dried. The data reported for these samples on the whole soil basis include bulk density, total nitrogen, total carbon, and carbon/nitrogen ratio. The bulk density was applied to carbon concentrations to determine the carbon stock in terms of Mg of CO₂ equivalent per acre. The air-dried samples were further processed to isolate particulate organic matter (POM) and mineral-associated organic matter (MAOM) fractions, which represent active and slower-cycling forms of organic matter, respectively. We determined the mass distribution across fractions (i.e., percent POM or MAOM by weight) in addition to the carbon and nitrogen contents of each fraction.

Sampling for baseline nutrient analysis: In addition to the soil cores for carbon sequestration monitoring, we collected composite samples on October 30, 2021 for routine nutrient analysis from each management zone to a depth of 0-4 inches with 0.75-inch push probes. In each management zone, 25 subsamples were collected and mixed to create 1 composite sample for each management zone. These samples were taken to provide fertilizer recommendations for the farm.

Sampling to meet requirements of ROC and FAO UN certification programs: On August 1, 2022, Jason Warren conducted sampling with a 0.75-inch hand probe to satisfy the requirements of the Regenerative Organic Certification and FAO UN carbon monitoring programs (Table 2). For the Regenerative Organic Certification, two composite samples were taken from each

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management region. For the FAO UN program, five composite samples were taken from each region. Note in Table 2 that there are different depth requirements for each program.

Soil samples were submitted to Soil, Water, and Forage Analytical Lab at OSU for analysis of electrical conductivity, pH, and texture. The methods for analyzing pH, electrical conductivity are standardized across accredited soil analysis labs and so the specific protocol is not provided here. The samples were additionally analyzed, as described above, for organic carbon and particulate organic matter carbon. Bulk density data from the extensive sampling conducted with the hydraulic probe were used to calculate carbon stocks.

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Table 2. Description of the three approaches to baseline sampling as recommended by ROC, FAO, and Jason Warren

Recommending program or individuals	Sampling tool and depth	Sampling date	Sampling design/location	Lab tests conducted	Recommended time for subsequent sampling
Regenerative Organic Certification	Hand probe (0.75-in diameter); 0-6 inch depth	August 1, 2022	Two composite samples per management region	pH, electrical conductivity, soil organic carbon (SOC), bulk density and soil texture*	August 1, 2025
FAO UN	Hand probe (0.75-in diameter); 0-10 cm and 10-30 cm depths	August 1, 2022	Five composite samples per management region	Soil organic carbon, bulk density*, and particulate organic matter carbon (POM-C) for 0-10cm depth (required); pH and EC (reported here, but not required by FAO)	SOC and bulk density measured no earlier than August 1, 2026 (required); POC measured August 1, 2024 (optional analysis)
Jason Warren and Andrea Jilling at OSU	Tractor-mounted hydraulic probe; 0-4, 4-8, 8-12, 12-24, and 24-36 inch depths	October 26, 2021	Five cores per sampling location, which were selected to capture the variability in soil type within each management region (See Figure 1 and Table 1)	Soil organic carbon, bulk density, POM-C and mineral-associated organic matter carbon (MAOM-C)	October 26, 2026 (See Interpretation of Results section)

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Lab Methods

Nutrient analyses, EC, pH, and texture were analyzed by the Soil Water Forage Analytical Lab at OSU. These methods are standardized across accredited soil testing labs.

Total soil carbon: Total carbon of dried, ground soil samples was analyzed on a dry combustion analyzer (Leco Corp., Saint Joseph, MI).

Soil organic matter fractions: Many methods exist for fractionating soil into POM and MAOM. This project used the method derived from Cambardella and Elliott (1993) and Gale and Cambardella (2000). First, the soil is sieved using a 2mm mesh sieve then oven dried to remove water weight. 10 ± 0.1 g of dry soil is then weighed into a 50 mL centrifuge tube and shaken overnight with 30 mL of 0.5% sodium hexametaphosphate. Using an automatic wet sieve shaker (Analysette 3 Pro, Fritsch) with a 53- μ m sieve, the soil suspension is then sieved. The suspended soils that pass through the sieve are collected in 500 mL centrifuge bottles and centrifuged to assist in the retrieval of the MAOM soil pellet. Once centrifuged, the soil pellet is transferred to a clean, pre-weighed and labelled aluminum drying pan using a squirt bottle of DI water. The POM soils retained on the 53- μ m sieve were similarly transferred to separate drying pans. Both sets of pans were placed in an oven set at 105°C no longer than 24 hours to evaporate the water. After reaching a constant weight, the pans were weighed to calculate the amount of soil collected and the dried soils were ground and then stored until further analysis. Each fraction was tested for TC as described above.

Acidification of select samples to remove inorganic carbon: Inorganic carbon was measured using a modified pressure calcimeter-method as described by Sherrod et al. (2002). Briefly, 1 ± 0.01 g of soil was placed into a 100 mL glass Wheaton bottle. A 2 mL (0.5 dram) vial with 2 mL of 6M HCl acid containing 3% by weight of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ was carefully placed into each Wheaton bottle. A gray butyl rubber stopper was then placed onto each Wheaton bottle and sealed by

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hand-crimping an aluminum tear off cap. The samples were gently swirled to completely mix the acid and soil without splashing the solution up the side walls of the bottle. After sitting for 18 hours, we measured the samples and standards pressure (in mbars) produced via CO₂ evolution from the reaction by inserting the pressure-calcmeter needle into the septum and recording the digital output. Using a standard curve generated the same day, we calculated the mass of CaCO₃ and %IC in each sample. The resulting inorganic carbon concentrations were subtracted from total carbon to obtain organic carbon.

Results

Table 3 shows results of nutrient analysis of composite samples collected on November 30, 2021. The pH for this soil is adequate, as are all other nutrients except for nitrogen and phosphorus. If a 40 bushel per acre yield goal is desired for wheat the OSU fertilizer recommendation calls for 75 lbs N/acre and 22 lbs P₂O₅/acre.

Table 3. Results from 0-4 inch composite soil sampling of management regions

Region	pH	TopN	P	K	TopSO ₄	Ca	Mg	Fe	Zn	B	Cu	OM	Organic Carbon
						(ppm)						(%)	%
NW field	6.5	2.5	18.5	215	1.4	1639	520	24.4	0.6	0.21	0.9	1.88	0.94
South field	6.2	1	20.5	160	2.16	1107	337	46.3	0.5	0.069	1	1.68	0.84
Pasture	6.5	1	3	262	3.64	1296	480	33.8	1.6	0.138	0.9	2.48	1.24
NE field	6.4	1	25	188	1.08	1339	423	32.4	0.5	0.083	0.9	1.58	0.79

Tables 4, 5 and 6 are presented as separate tabs in Appendix 2. Table 4 are the results from soil analyses as required by the ROC and FAO UN carbon monitoring programs. Note, these are the raw values, not the averages and standard errors. The program did not specify how data should be summarized.

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Table 5 summarizes the bulk density, total N, total C, Inorganic C (IC), Organic C, and C/N ratio and carbon stocks for the spatially extensive sampling conducted with the hydraulic probe.

Table 6 summarizes the POM and MAOM mass and carbon distribution for the spatially extensive sampling conducted with the hydraulic probe. POM, by mass, was a minimal component of the whole soil profile and ranged from 3.9% to 33% of the soil mass. The POM carbon had a much wider range of 17-79% of the total soil carbon. This fraction of organic matter carbon is usually elevated near the surface and declines rapidly with depth. Inversely, MAOM carbon tends to increase with depth as a percentage of the total soil carbon.

Table 7 shows the organic carbon stocks calculated as Mg CO₂eq/acre found in each depth and location. It is important to note that the near surface depth increments are only 4 inches, in contrast to the 24-36 inch increment, which contains more than 3 times the soil mass and can therefore contain extensive organic stocks. It is noteworthy that in the 0-4 inch depth the carbon stocks for the historically cultivated locations ranged from 19-26 Mg/acre as compared to the pasture areas that contained 35-42 Mg/acre. In contrast, when the total profile is utilized to compare carbon stocks among sample locations, we see a wide range in carbon stocks ranging from 201 to as little as 83 Mg CO₂eq/acre.

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Table 7. Organic carbon stocks calculated as Mg of CO₂eq/acre and presented for all depths across all 19 sites

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Depth (in)	Mg CO₂eq/acre																		
0-4	22	21	24	23	24	26	22	19	19	25	20	23	23	22	18	22	22	42	35
4-8	14	23	22	21	20	23	17	16	19	22	20	20	20	21	17	19	21	26	21
8-12	13	17	20	20	17	21	15	19	18	22	17	19	22	20	11	16	23	22	15
12-18	9	29	29	33	25	30	21	26	19	27	21	23	29	23	12	20	30	25	18
18-24	6	28	31	32	26	29	18	5	21	22	17	21	24	19	9	16	24	21	11
24-36	20	31	53	73	45	52	27	24	40	37	40	37	41	37	18	21	34	29	21
Profile total	83	148	179	201	157	181	119	110	135	155	135	142	158	143	85	114	154	164	121

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Interpretation of Results

Baseline soil sampling was conducted to assess the initial soil properties prior to making changes in management. The bulk density data presented in Table 3 ranged from 1.1 to as high as 1.76 which is within the range of expected values. The coefficients of variation for bulk density data were less than 10% at all sample locations, which is a very low value for this type of data and shows that this measurement is very precise and does not vary greatly at a spatial scale within a sample location. In contrast, the variability was generally much higher for the total C, inorganic C, organic C and carbon stock estimates. This is particularly true for locations 1, 4, 8, 9, 11, 15, and 19 which all had average coefficients of variation for total C greater than 20%. In fact, a coefficient of 94% was found in the 24-36 inch depth of location 19. The high variability in the subsoil can be expected due to small scale differences in soil forming processes that resulted in large variability in the vertical distribution of carbon in the soil profile. These variations in total carbon concentrations in turn result in high variation in the carbon stocks measured in each soil depth at these locations.

All sampled soils have relatively low organic carbon contents, regardless of the soil type, management area, or depth. Organic carbon contents less than 2% would be considered degraded for agricultural soils and for all sites, organic carbon was below 1% below the 4-inch depth in all locations. However, there are some notable differences between management areas. Locations 18 and 19 are the grass pasture, which is comprised of many native species and has not been tilled for many years (possibly decades). It is noteworthy that the samples from the grass pasture contain the highest carbon levels in the surface 4 inches, which is expected in a warm season grass pasture as compared to tilled soils. Despite these pastures being relatively undisturbed for many years, carbon contents below the 4-inch depth quickly decline. Only Location 1 has higher total carbon and much of that was in the form of carbonate from the parent material. In fact, at this location the inorganic C represented 74% of the total carbon found to 3 feet. In general, these observations suggest that there is potential to increase

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organic carbon storage across all management areas by using practices that minimize disturbance to the soil profile and increase organic inputs. Practices such as conversion to perennial systems, implementation of cover crops, increase of manure inputs, and conservation tillage all have potential to increase carbon contents in these soils.

As expected based on prior research, the carbon content of these soils at Rainmaker farmer are highly variable in space across the landscape with sample locations containing as much as 201 Mg CO₂eq/acre as organic carbon to as little as 83 Mg CO₂eq/acre (Table 7). Furthermore, within a sample location the variability in carbon stocks within a depth can be very high due to small scale variability in carbon distribution within the profile. This variability will limit the frequency with which samples are collected and is important to consider for the quantification of carbon sequestration over time, which is discussed below in the recommendations section. It is also apparent based on results that historic management has much less impact on the total profile stocks which are controlled largely by soil type (i.e., parent material) and landscape position.

The results for POM and MAOM demonstrate the majority of carbon is stored as MAOM, but there are differences with depth. POM-C concentrations are generally highest at the surface and decrease with depth. This was expected as POM-C will generally reflect recent plant inputs; with litter inputs entering soil at surface, it follows that the quantity of soil POM-C would be greatest at surface. With increasing depth, litter is more decomposed and increasingly more carbon is stored in association with mineral particles—hence, the increasing concentration of MAOM-C with depth.

The POM values in the subsoils at locations 1, are moist likely elevated due to sand sized inorganic carbon particles within this fraction. This is based on visual observations that the profile was comprised of unconsolidated shale rock that apparently contained carbonates. The POM may have been influenced by the presence of sand-sized carbonates in the other 5 locations with elevated carbonates in the subsoil, but remaining locations are not influenced by carbonates and can therefore be regarded as organic carbon. It is important to note that the

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POM and MAOM carbon data in Table 7 are total carbon, not organic carbon. Removing the carbonates from all isolated POM and MAOM fractions was outside the scope of the proposal and the budget. However, based on whole soil carbon data, we were able to identify those sampling locations with appreciable carbonate concentrations. These include locations 1, 2, 8, 9, 12, and 17.

Recommendations

Table 8 shows the organic carbon stocks in the top 1 foot of soil and the top three feet of soil. The table also includes the coefficient of variation (CV) for each location. The average CV for the top one foot of soil is 7.6%. In contrast, including the subsoil down to three feet increased the CV to 12.5%. The increase in CV with increasing depth of observation is due to an increase in the variability in carbon concentrations at depth. Fortunately, the variability in bulk density within a sample location is very low and so future efforts to quantify organic carbon stocks may opt out of analyzing all samples for bulk density and focus simply on quantifying carbon pools and stocks.

In prior research conducted in 2013, the Oklahoma Carbon Program soil sampling protocol found the average CV across 46 sample sites was 12.1% for a sampling depth of one foot (Appendix 3). That research also demonstrated that based on this level of variation, a total of 127 core samples would be required to measure a change of 1.6 Mg CO₂eq/acre expected after four years of no till management or 82 core samples after five years. In the Rainmaker project we collected 95 core samples so it would be prudent to wait five years to ensure that measurable accumulations of carbon may be observed. However, given the lower variability as compared to the analysis conducted in 2013, a four-year sample interval may be sufficient. However, if a sample depth of three feet is desired to quantify carbon sequestration it is advised to wait the full five years to overcome the variability in carbon stocks in the subsoil.

When the carbon stocks presented in Table 8 are averaged across sample locations for each management zone, we find that the NW, NE, and South regions contain 60, 60 and 59 Mg

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CO₂eq/acre and the pasture contains 80 Mg CO₂eq/acre in the top one foot which may in fact represent the differences in carbon stocks due to management. However, when the carbon stock is calculated for a three-foot depth, we find that these management zone contain 153, 135, 133 and 143 Mg CO₂eq/acre which is much less consistent with regard to expected management effects. This is expected due to greater variability in the landscape position and parent material and is why we collected multiple cores from each location with multiple locations within a management zone.

At the initial five-year sampling it is expected that if a change of 1.6 Mg CO₂eq/acre occurs on average across the management zone we will be able to statistically measure that change as compared to this initial baseline sampling. As carbon is accumulated over time more detail on the impacts of management or soil type on carbon sequestration rates should appear if in fact these factors impact carbon stocks over time.

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Table 8: The organic carbon stocks as calculated as Mg CO₂eq/acre in the top one foot and three feet of soil.

Location	-----1-ft-----		-----3-ft-----	
	Mg CO ₂ /acre	CV	Mg CO ₂ /acre	CV
1	48	15	83	15
2	61	11	148	12
3	66	5	179	7
4	64	9	201	17
5	61	5	157	7
6	70	4	181	7
7	53	3	119	10
8	55	19	115	38
9	55	19	135	26
10	69	3	155	4
11	58	6	135	13
12	61	3	142	15
13	64	3	158	5
14	64	4	143	8
15	46	4	85	12
16	57	7	114	9
17	66	4	154	7
18	89	6	164	9
19	71	14	121	14

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Appendix 1 – Geographic Information System (GIS) Shapefiles of Soil Sample Locations

Sample Point	Lat	Long
1	97.3539036°W	36.5051408°N
2	97.3522760°W	36.5060068°N
3	97.3494645°W	36.5061698°N
4	97.3505098°W	36.5050499°N
5	97.3492723°W	36.5045458°N
6	97.3489082°W	36.5035144°N
7	97.3496518°W	36.5029360°N
8	97.3471680°W	36.5057192°N
9	97.3460785°W	36.5049264°N
10	97.3469565°W	36.5036906°N
11	97.3541507°W	36.5021950°N
12	97.3534731°W	36.5012155°N
13	97.3519860°W	36.5003222°N
14	97.3488651°W	36.5009887°N
15	97.3474311°W	36.5009257°N
16	97.3466950°W	36.5018673°N
17	97.3518813°W	36.5013323°N
18	97.3535934°W	36.5033721°N
19	97.3515027°W	36.5024904°N

Note: ArcGIS Spatial Data Files Provided In Records

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Appendix 2 – Table 4, 5, 6 - Soil Sampling Result Spreadsheets

Region	Depth	Depth units	Composite	Certification Program	Bulk Density (g/cm ³)	Total Carbon		Total Carbon Stock (Mg C/acre)	Carbon Stock (Mg C/hectare)	POM Soil Mass (% of Total Mass)	POM Soil Carbon (% of Total Carbon)	POM Soil Carbon (% of Total Organic Carbon)	Electrical Conductivity (mS/m)	pH	Buffer pH	Texture			
						Organic Carbon (%)	Inorganic Carbon (%)									Class	Sand (%)	Silt (%)	Clay (%)
NE	6	in	1	ROC	1.267	1.1114	0.01758791	1.09381209	85.4746846	211.2122195			234	5.7	7.1	Silt Loam	25	52.5	22.5
NE	6	in	2	ROC	1.0231	1.023	0.023	1.001	63.1641031	156.0816569			255	5.8	7.2	Silt Loam	25	52.5	22.5
NW	6	in	1	ROC	1.257	1.1145		1.1145	86.4039351	213.5084438			280	6.4		Silt Loam	22.5	52.5	25
NW	6	in	2	ROC	1.257	1.1582		1.1582	89.7918686	221.880197			259	6.2		Silt Loam	20	55	25
Pasture	6	in	1	ROC	1.15	1.8821		1.8821	133.493066	329.8680397			305	6.2		Loam	30	43.8	26.3
Pasture	6	in	2	ROC	1.15	1.557		1.557	110.434463	272.8890802			296	6.2		Loam	30	45	25
S	6	in	1	ROC	1.229	1.0519	0.02049082	1.03140918	78.1809688	193.1890829			211	5.2	6.9	Silt Loam	22.5	52.5	25
S	6	in	2	ROC	1.229	1.166	0.00639096	1.15960904	87.8985368	217.2016793			228	5.1	6.8	Loam	27.5	50	22.5
NE	10	cm	1	FAO UN	1.267	1.1192	0.01924671	1.09995329	56.4006438	139.3688108	11.3	18.8683345	19.0711847	215	5.4	7			
NE	10	cm	2	FAO UN	1.267	1.1037	0.02049082	1.08320918	55.5420816	137.2472607	12.1	20.0515539	20.2368522	211	5.6	7			
NE	10	cm	3	FAO UN	1.267	1.0417	0.00514686	1.03655314	53.1497703	131.3357398	12.1	17.9914467	18.0264912	210	5.5	6.8			
NE	10	cm	4	FAO UN	1.267	1.1166	0.02049082	1.09610918	56.2035354	138.8817462	12.4	21.5961669	21.6783018	215	5.6	6.9			
NE	10	cm	5	FAO UN	1.267	1.0479	0.0167585	1.0311415	52.8722855	130.6500611	12.5	18.7744537	18.8288191	206	5.5	7			
NE	30	cm	1	FAO UN	1.633	0.8237		0.8237	108.872564	269.0295492			302	6.6					
NE	30	cm	2	FAO UN	1.633	0.73046		0.73046	96.5485651	238.5763318			230	7.1					
NE	30	cm	3	FAO UN	1.633	0.78221		0.78221	103.388622	255.4784554			252	6.3					
NE	30	cm	4	FAO UN	1.633	0.80326		0.80326	106.170907	262.3536187			251	6.3					
NE	30	cm	5	FAO UN	1.633	0.74747		0.74747	98.7968622	244.1319863			254	6.4					
NW	10	cm	1	FAO UN	1.257	1.2149		1.2149	61.8029228	152.7181123	10.4	19.8951683	19.6328895	274	6.8				
NW	10	cm	2	FAO UN	1.257	1.1659		1.1659	59.3102541	146.5586033	10.7	19.4479544	18.2178294	364	6.9				
NW	10	cm	3	FAO UN	1.257	1.1348		1.1348	57.7281725	142.6492006	10	20.170074	19.3738479	276	6.6				
NW	10	cm	4	FAO UN	1.257	1.2971		1.2971	65.9845017	163.0510029	10.4	22.0411688	21.5339421	271	6.6				
NW	10	cm	5	FAO UN	1.257	1.1251		1.1251	57.2347258	141.4298693	12.2	19.1766954	18.7665205	249	6.2				
NW	30	cm	1	FAO UN	1.614	0.84096		0.84096	109.860626	271.4711001			367	7.3					
NW	30	cm	2	FAO UN	1.614	0.82559		0.82559	107.852733	266.5094957			308	7.2					
NW	30	cm	3	FAO UN	1.614	0.84774		0.84774	110.746346	273.6597583			322	7.2					
NW	30	cm	4	FAO UN	1.614	0.85499		0.85499	111.693465	276.0001377			338	7.6					
NW	30	cm	5	FAO UN	1.614	0.7851		0.7851	102.563234	253.4388802			305	7.2					
Pasture	10	cm	1	FAO UN	1.15	1.8646		1.8646	86.7794163	214.4362766	20.7	23.339955	23.2644013	267	6.1	7.1			
Pasture	10	cm	2	FAO UN	1.15	1.7547		1.7547	81.6646154	201.7973478	20.6	25.1010771	25.035785	299	7				
Pasture	10	cm	3	FAO UN	1.15	1.9632	0.02339373	1.93980627	90.2795538	223.0852913	21.3	31.6494652	31.7697243	272	6.1	6.9			
Pasture	10	cm	4	FAO UN	1.15	2.1036		2.1036	97.9025958	241.9222094	23.1	36.0852396	36.0105054	284	6.3				
Pasture	10	cm	5	FAO UN	1.15	2.2277	0.03251716	2.19518284	102.164907	252.4545928	21.8	24.4813305	24.7352023	271	6.1	7.1			
Pasture	30	cm	1	FAO UN	1.55	0.98625		0.98625	123.731966	305.7478752			293	6.5					
Pasture	30	cm	2	FAO UN	1.55	0.87025		0.87025	109.178954	269.7866549			291	6.3					
Pasture	30	cm	3	FAO UN	1.55	0.91676		0.91676	115.013959	284.2052442			264	6.2					
Pasture	30	cm	4	FAO UN	1.55	0.91406		0.91406	114.675225	283.3682158			258	6.2					
Pasture	30	cm	5	FAO UN	1.55	0.96599		0.96599	121.190207	299.4670621			267	6.6					
S	10	cm	1	FAO UN	1.229	1.1601	0.01883201	1.14126799	56.763965	140.2665956	13.7	18.9858547	19.2101234	201	5.2	6.7			
S	10	cm	2	FAO UN	1.229	1.1012	0.0155144	1.0856856	53.9994288	133.4352884	12.8	17.7412278	17.9594749	222	5	6.7			
S	10	cm	3	FAO UN	1.229	1.1986	0.02214962	1.17645038	58.5138535	144.5906577	13.2	15.0556649	15.1566585	187	5.1	6.4			
S	10	cm	4	FAO UN	1.229	1.1696	0.01800261	1.15159739	57.277725	141.5361223	13.2	19.7548564	19.9343151	207	5	6.3			
S	10	cm	5	FAO UN	1.229	1.156	0.02090552	1.13509448	56.4569093	139.5078457	13	15.5932526	15.6878579	201	5	6.7			
S	30	cm	1	FAO UN	1.564	0.78557		0.78557	99.445432	245.7346347			305	6.2					
S	30	cm	2	FAO UN	1.564	0.77336	0.02049082	0.75286918	95.3058302	235.5054718			246	6	7				
S	30	cm	3	FAO UN	1.564	0.83462		0.83462	105.654679	261.0779954			308	6.2					
S	30	cm	4	FAO UN	1.564	0.81044	0.01302619	0.79741381	100.944742	249.4395047			275	5.8	6.8				
S	30	cm	5	FAO UN	1.564	0.80733	0.02671134	0.78061866	98.8186408	244.1858024			276	6	6.9				

Location	Depth (in)	Bulk Density (g/cm ³)			% Total C			IC%			% Organic Carbon			% Total N			Organic C/N Ratio			Organic Carbon Stock (Mg CO ₂ eq/acre)		
		Mean	SE	CV	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV
1	4	1.3	0.0	4.1	2.3	0.1	11.4	1.4	0.2	30.3	0.9	0.3	62.7	0.1	0.0	13.2	12.2	3.7	66.9	21.5	3.0	28.0
1	8	1.7	0.0	1.4	1.7	0.3	35.7	1.2	0.2	37.7	0.5	0.1	31.9	0.1	0.0	17.8	10.4	2.1	40.2	13.6	2.1	31.2
1	12	1.7	0.0	6.4	2.1	0.6	54.3	1.4	0.4	74.4	0.5	0.1	33.5	0.0	0.0	34.5	13.7	3.0	43.9	13.0	1.9	28.8
1	18	1.7	0.0	1.8	1.2	0.1	12.6	1.1	0.3	52.6	0.3	0.1	78.8	0.0	0.0	17.1	6.0	2.6	88.6	9.5	3.7	78.7
1	24	1.8	0.0	6.3	1.1	0.2	31.2	0.9	0.2	52.3	0.1	0.1	82.1	0.0	0.0	70.1	9.9	7.5	130.4	5.7	2.5	86.6
1	36	1.7	0.0	3.2	0.9	0.2	53.8	0.7	0.2	53.4	0.3	0.1	48.3	0.0	0.0	13.8	8.6	1.8	40.8	19.9	4.8	48.3
2	4	1.3	0.0	2.2	1.1	0.0	9.6	0.0	0.0	120.4	1.1	0.0	9.1	0.1	0.0	37.9	16.1	4.2	58.0	21.4	0.7	7.2
2	8	1.7	0.0	3.9	1.0	0.1	15.7	0.1	0.0	51.1	0.9	0.1	22.7	0.1	0.0	19.4	12.1	0.7	13.4	22.7	2.5	24.7
2	12	1.7	0.0	1.0	0.9	0.0	6.4	0.2	0.1	108.2	0.7	0.1	28.8	0.1	0.0	13.6	10.6	1.0	20.5	16.8	2.2	29.0
2	18	1.7	0.0	4.8	1.0	0.1	27.7	0.2	0.2	154.6	0.7	0.1	18.7	0.0	0.0	29.5	15.7	1.3	18.7	28.5	1.9	15.2
2	24	1.7	0.0	1.9	1.0	0.1	28.8	0.3	0.1	105.4	0.7	0.1	21.6	0.0	0.0	36.1	18.8	1.8	21.4	27.8	2.5	20.4
2	36	1.8	0.0	2.4	1.0	0.1	18.9	0.6	0.1	21.7	0.4	0.1	30.5	0.0	0.0	5.8	16.9	2.5	25.9	34.3	5.6	28.4
3	4	1.3	0.0	2.1	1.2	0.0	5.5				1.2	0.0	5.5	0.1	0.0	9.8	11.8	0.7	13.6	24.1	0.5	4.9
3	8	1.7	0.0	4.5	0.9	0.0	6.2				0.9	0.0	6.2	0.1	0.0	10.9	11.4	0.5	9.2	21.6	0.6	6.5
3	12	1.5	0.0	6.7	0.9	0.1	13.0				0.9	0.1	13.0	0.1	0.0	16.4	12.3	0.4	7.0	20.4	0.9	9.9
3	18	1.4	0.1	9.9	0.9	0.0	8.8				0.9	0.0	8.8	0.1	0.0	16.9	12.9	0.8	13.6	29.2	1.1	8.1
3	24	1.5	0.1	9.5	0.9	0.0	10.4				0.9	0.0	10.4	0.1	0.0	9.1	12.9	0.9	15.2	30.7	2.4	17.1
3	36	1.5	0.0	2.3	0.8	0.0	13.2				0.8	0.0	13.2	0.1	0.0	17.0	12.7	0.7	11.6	53.3	2.9	12.0
4	4	1.2	0.0	8.0	1.2	0.1	23.9				1.2	0.1	23.9	0.1	0.0	23.4	11.3	0.3	5.9	22.9	2.5	24.2
4	8	1.6	0.0	2.8	0.9	0.0	9.4				0.9	0.0	9.4	0.1	0.0	15.9	10.9	0.4	8.3	21.3	1.1	11.3
4	12	1.6	0.0	2.8	0.8	0.1	14.4				0.8	0.1	14.4	0.1	0.0	11.3	10.8	0.7	15.1	19.6	1.3	14.4
4	18	1.5	0.0	1.7	1.0	0.1	23.7				1.0	0.1	23.7	0.1	0.0	16.1	12.1	0.8	15.5	32.6	3.2	22.3
4	24	1.4	0.0	3.3	1.0	0.1	33.9				1.0	0.1	33.9	0.1	0.0	22.1	13.2	1.1	18.7	31.6	4.6	32.6
4	36	1.5	0.0	4.6	1.1	0.2	35.0				1.1	0.2	35.0	0.1	0.0	44.0	12.3	0.5	9.5	72.7	11.6	35.8
5	4	1.2	0.1	9.8	1.3	0.0	8.4				1.3	0.0	8.4	0.1	0.0	13.9	10.6	0.5	10.3	23.9	0.7	6.6
5	8	1.5	0.0	3.4	0.9	0.0	11.5				0.9	0.0	11.5	0.1	0.0	14.1	10.7	0.6	12.5	20.0	1.0	11.0
5	12	1.6	0.0	4.2	0.7	0.0	3.2				0.7	0.0	3.2	0.1	0.0	10.2	10.0	0.4	9.2	16.9	0.5	6.3
5	18	1.5	0.0	6.4	0.7	0.1	15.6				0.7	0.1	15.6	0.1	0.0	6.9	9.8	0.8	18.7	24.5	2.0	18.6
5	24	1.4	0.0	3.8	0.8	0.1	17.9				0.8	0.1	17.9	0.1	0.0	14.6	10.6	0.7	14.9	26.4	2.2	18.3
5	36	1.5	0.0	6.1	0.7	0.1	18.4				0.7	0.1	18.4	0.1	0.0	18.9	10.0	0.5	10.7	44.8	2.5	12.2
6	4	1.2	0.0	3.7	1.5	0.1	8.5				1.5	0.1	8.5	0.1	0.0	7.3	10.9	0.1	2.4	26.2	0.8	7.2
6	8	1.5	0.0	4.7	1.0	0.0	9.5				1.0	0.0	9.5	0.1	0.0	12.7	10.1	0.3	7.4	22.8	1.0	9.5
6	12	1.6	0.0	3.2	0.8	0.0	3.8				0.8	0.0	3.8	0.1	0.0	4.8	10.3	0.2	3.6	20.8	0.4	4.3
6	18	1.6	0.0	2.6	0.8	0.0	5.6				0.8	0.0	5.6	0.1	0.0	5.9	10.3	0.4	8.3	29.5	0.7	5.5
6	24	1.5	0.0	4.7	0.9	0.1	17.9				0.9	0.1	17.9	0.1	0.0	16.5	9.9	0.2	5.0	29.4	2.3	17.2
6	36	1.5	0.0	4.6	0.8	0.1	20.9				0.8	0.1	20.9	0.1	0.0	24.0	10.0	0.3	7.8	52.4	4.1	17.5
7	4	1.3	0.0	6.0	1.1	0.0	3.5				1.1	0.0	3.5	0.1	0.0	8.7	10.8	0.4	7.4	21.7	0.5	5.3
7	8	1.6	0.0	1.7	0.7	0.0	4.6				0.7	0.0	4.6	0.1	0.0	10.3	10.1	0.4	7.9	16.5	0.3	4.4
7	12	1.6	0.0	0.8	0.6	0.0	3.0				0.6	0.0	3.0	0.1	0.0	14.5	10.2	0.7	14.5	14.9	0.3	3.6
7	18	1.6	0.0	2.0	0.6	0.0	9.3				0.6	0.0	9.3	0.1	0.0	86.5	8.3	1.6	42.6	21.1	0.8	8.9
7	24	1.6	0.0	5.7	0.5	0.0	16.9				0.5	0.0	16.9	0.1	0.0	21.4	10.0	0.9	19.2	18.1	1.0	12.2
7	36	1.6	0.1	7.2	0.4	0.1	50.3				0.4	0.1	50.3	0.0	0.0	37.7	8.7	0.8	19.4	26.6	4.9	41.0
8	4	1.3	0.0	2.6	1.0	0.0	4.4				1.0	0.0	4.4	0.1	0.0	3.3	9.4	0.2	4.8	19.3	0.4	4.4
8	8	1.7	0.0	0.6	0.6	0.0	3.0				0.6	0.0	3.0	0.1	0.0	7.1	9.1	0.3	7.5	16.1	0.2	3.0
8	12	1.6	0.0	3.9	0.7	0.2	61.5				0.7	0.2	61.5	0.1	0.0	87.7	9.5	3.2	76.0	19.2	5.6	58.2
8	18	1.6	0.0	3.0	1.6	0.5	66.6	0.9	0.3	78.7	0.7	0.2	63.5	0.1	0.0	15.2	13.2	3.9	65.5	26.0	7.6	65.7
8	24	1.7	0.0	3.4	1.6	0.2	34.5	1.4	0.3	44.4	0.1	0.1	170.8	0.0	0.0	12.1	3.7	3.0	180.4	5.9	3.9	148.9
8	36	1.7	0.0	2.3	1.5	0.3	44.2	1.2	0.2	32.2	0.3	0.2	168.4	0.0	0.0	14.8	7.8	5.9	171.2	28.1	16.7	132.7
9	4	1.3	0.0	2.9	1.0	0.0	8.4				1.0	0.0	8.4	0.1	0.0	13.5	10.4	0.5	9.9	19.1	0.7	8.4
9	8	1.7	0.0	3.4	0.7	0.2	53.7				0.7	0.2	53.7	0.1	0.0	10.2	13.0	3.1	52.5	18.5	4.5	54.8
9	12	1.6	0.0	4.5	1.4	0.4	61.7	0.7	0.4	145.5	0.8	0.1	39.8	0.1	0.0	28.5	8.9	1.2	29.7	17.9	3.1	38.6
9	18	1.5	0.0	4.9	1.7	0.5	61.3	1.2	0.5	94.7	0.6	0.0	18.1	0.1	0.0	20.2	7.8	0.2	6.3	19.0	1.9	22.3
9	24	1.5	0.1	7.6	2.7	0.3	27.9	2.0	0.3	31.3	0.6	0.1	52.6	0.1	0.0	21.6	8.8	1.5	37.1	20.7	4.3	46.0

9	36	1.6	0.0	4.3	1.9	0.2	22.6	1.4	0.1	17.9	0.4	0.2	85.7	0.1	0.0	19.9	8.6	1.9	43.2	39.7	11.1	55.8
10	4	1.2	0.0	4.9	1.4	0.0	4.4				1.4	0.0	4.4	0.1	0.0	8.5	10.8	0.4	8.3	24.7	0.6	5.5
10	8	1.6	0.0	0.8	0.9	0.0	3.1				0.9	0.0	3.1	0.1	0.0	8.8	11.4	0.6	11.8	21.8	0.3	3.5
10	12	1.6	0.0	3.5	0.9	0.0	3.5				0.9	0.0	3.5	0.1	0.0	11.1	11.3	0.6	11.6	22.5	0.2	1.8
10	18	1.5	0.0	3.5	0.8	0.0	5.9				0.8	0.0	5.9	0.1	0.0	7.4	11.0	0.6	11.2	26.5	0.9	7.8
10	24	1.4	0.0	6.8	0.7	0.0	7.8				0.7	0.0	7.8	0.1	0.0	6.8	10.6	0.3	7.0	22.1	1.1	11.2
10	36	1.5	0.0	2.4	0.5	0.0	7.9				0.5	0.0	7.9	0.1	0.0	8.7	10.3	0.4	8.6	37.0	1.3	8.1
11	4	1.3	0.0	5.6	1.0	0.1	15.8				1.0	0.1	15.8	0.2	0.0	61.3	7.8	1.3	37.3	20.2	1.4	15.7
11	8	1.6	0.0	5.7	0.8	0.0	7.1				0.8	0.0	7.1	0.1	0.0	18.1	8.8	0.7	16.1	20.2	0.9	9.2
11	12	1.6	0.0	3.6	0.7	0.0	7.7				0.7	0.0	7.7	0.1	0.0	13.1	9.4	0.6	15.1	17.1	0.8	10.6
11	18	1.6	0.0	2.4	0.6	0.0	13.7				0.6	0.0	13.7	0.1	0.0	12.1	8.4	0.7	17.8	20.5	1.4	15.7
11	24	1.6	0.0	3.8	0.5	0.1	29.3				0.5	0.1	29.3	0.1	0.0	29.3	8.6	0.7	18.6	16.9	2.3	30.0
11	36	1.6	0.0	5.3	0.6	0.2	60.2				0.6	0.2	60.2	0.1	0.0	48.6	8.9	0.7	16.9	40.1	10.3	57.2
12	4	1.2	0.0	7.2	1.2	0.0	3.6				1.2	0.0	3.6	0.1	0.0	6.0	10.6	0.4	9.1	22.5	0.7	7.2
12	8	1.6	0.0	2.3	0.8	0.0	4.1				0.8	0.0	4.1	0.1	0.0	6.4	10.1	0.1	2.6	19.7	0.4	4.0
12	12	1.6	0.0	5.4	0.8	0.0	11.3				0.8	0.0	11.3	0.1	0.0	10.5	10.1	0.7	14.4	18.7	0.7	8.4
12	18	1.6	0.0	6.1	0.7	0.0	15.6				0.7	0.0	15.6	0.1	0.0	17.6	10.3	0.3	7.2	23.3	1.5	14.0
12	24	1.7	0.0	5.4	0.5	0.0	9.5				0.5	0.0	9.5	0.0	0.0	30.7	12.0	1.5	27.8	20.7	1.0	10.3
12	36	1.7	0.0	3.6	0.7	0.1	44.5	0.2	0.0	43.4	0.5	0.1	50.5	0.0	0.0	16.3	12.8	2.6	45.9	37.0	7.7	46.4
13	4	1.2	0.0	1.4	1.2	0.0	4.7				1.2	0.0	4.7	0.1	0.0	5.4	11.5	0.3	6.7	22.6	0.5	5.0
13	8	1.5	0.0	4.1	0.9	0.0	5.4				0.9	0.0	5.4	0.1	0.0	7.7	10.6	0.4	9.2	20.0	0.8	8.9
13	12	1.5	0.0	6.4	0.9	0.0	6.8				0.9	0.0	6.8	0.1	0.0	12.6	11.4	0.4	7.7	21.6	0.8	8.3
13	18	1.5	0.0	3.3	0.9	0.0	1.8				0.9	0.0	1.8	0.1	0.0	4.1	10.7	0.2	3.7	28.7	0.5	4.2
13	24	1.6	0.0	1.6	0.7	0.0	3.0				0.7	0.0	3.0	0.1	0.0	7.8	10.4	0.3	6.4	24.3	0.4	3.7
13	36	1.6	0.0	1.4	0.5	0.1	21.1				0.5	0.1	21.1	0.0	0.0	13.1	11.5	1.3	25.8	40.7	3.9	21.6
14	4	1.2	0.0	4.6	1.2	0.1	11.8				1.2	0.1	11.8	0.1	0.0	8.2	10.2	0.2	5.5	22.1	0.9	8.8
14	8	1.5	0.0	2.1	0.9	0.0	5.6				0.9	0.0	5.6	0.1	0.0	10.7	10.0	0.3	6.1	21.2	0.7	7.0
14	12	1.6	0.0	4.0	0.9	0.0	7.9				0.9	0.0	7.9	0.1	0.0	4.8	10.2	0.2	4.8	20.4	0.4	4.9
14	18	1.5	0.0	1.1	0.7	0.0	11.2				0.7	0.0	11.2	0.2	0.1	143.5	8.2	1.9	50.9	23.1	1.2	11.6
14	24	1.6	0.0	4.5	0.5	0.0	8.8				0.5	0.0	8.8	0.1	0.0	30.8	8.4	1.0	27.7	19.1	0.6	7.6
14	36	1.7	0.0	2.6	0.5	0.1	27.9				0.5	0.1	27.9	0.0	0.0	13.4	11.3	1.1	22.1	36.5	4.4	26.8
15	4	1.2	0.0	6.5	1.0	0.0	5.8				1.0	0.0	5.8	0.1	0.0	3.6	9.0	0.2	6.1	18.4	0.9	11.3
15	8	1.7	0.0	5.7	0.7	0.0	8.2				0.7	0.0	8.2	0.1	0.0	13.5	8.3	0.3	7.5	17.1	0.4	4.8
15	12	1.7	0.0	5.6	0.4	0.0	13.9				0.4	0.0	13.9	0.1	0.0	10.9	6.4	0.3	11.2	10.7	0.5	11.2
15	18	1.6	0.0	3.4	0.3	0.0	22.2				0.3	0.0	22.2	0.0	0.0	17.0	6.6	0.5	18.0	11.9	1.1	20.0
15	24	1.7	0.0	2.7	0.2	0.0	23.0				0.2	0.0	23.0	0.0	0.0	3.1	5.4	0.6	24.9	9.3	1.0	23.3
15	36	1.7	0.0	1.2	0.2	0.1	49.8				0.2	0.1	49.8	0.0	0.0	26.2	7.3	2.1	63.2	17.9	3.9	49.2
16	4	1.3	0.0	7.5	1.1	0.1	14.6				1.1	0.1	14.6	0.1	0.0	6.0	10.2	0.5	10.3	21.7	1.8	18.7
16	8	1.5	0.0	3.3	0.8	0.0	7.3				0.8	0.0	7.3	0.1	0.0	6.0	9.3	0.1	3.4	18.9	0.7	8.1
16	12	1.5	0.0	4.9	0.7	0.1	18.9				0.7	0.1	18.9	0.1	0.0	23.1	9.2	0.5	11.9	16.2	1.1	14.7
16	18	1.5	0.1	8.7	0.6	0.1	25.3				0.6	0.1	25.3	0.1	0.0	26.3	8.8	0.5	12.8	19.6	1.5	16.7
16	24	1.7	0.0	5.8	0.4	0.0	21.1				0.4	0.0	21.1	0.1	0.0	13.0	7.5	0.5	13.6	16.1	1.1	15.1
16	36	1.7	0.0	4.7	0.3	0.0	15.2				0.3	0.0	15.2	0.0	0.0	5.6	5.8	0.4	13.9	21.4	1.3	12.1
17	4	1.2	0.0	4.6	1.2	0.0	5.5				1.2	0.0	5.5	0.1	0.0	16.0	11.6	0.7	13.9	21.7	0.6	5.8
17	8	1.5	0.0	1.9	0.9	0.0	2.6				0.9	0.0	2.6	0.1	0.0	15.9	11.5	0.7	14.0	20.8	0.3	2.8
17	12	1.5	0.0	2.6	1.1	0.0	6.1				1.1	0.0	6.1	0.1	0.0	17.9	11.7	0.9	17.9	23.2	0.7	7.0
17	18	1.4	0.0	4.5	0.9	0.0	11.4				0.9	0.0	11.4	0.1	0.0	13.2	12.4	0.8	14.0	30.0	1.6	12.0
17	24	1.5	0.0	4.8	0.7	0.0	13.7				0.7	0.0	13.7	0.1	0.0	6.1	11.8	0.9	17.1	24.3	1.1	10.0
17	36	1.6	0.0	3.4	0.7	0.0	10.8	0.2	0.1	69.6	0.5	0.0	14.1	0.0	0.0	7.9	12.3	0.9	16.0	34.4	1.9	12.0
18	4	1.1	0.1	10.7	2.4	0.2	15.5				2.4	0.2	15.5	0.2	0.0	12.3	11.5	0.2	3.9	41.8	2.3	11.2
18	8	1.5	0.0	4.3	1.2	0.1	17.2				1.2	0.1	17.2	0.1	0.0	20.4	11.5	0.3	5.4	26.2	1.7	14.1
18	12	1.6	0.0	4.6	0.9	0.1	17.2				0.9	0.1	17.2	0.1	0.0	18.5	11.4	0.5	10.0	21.5	1.7	17.7
18	18	1.5	0.0	1.9	0.7	0.1	18.1				0.7	0.1	18.1	0.1	0.0	12.7	11.0	0.3	6.5	24.6	1.9	17.0
18	24	1.6	0.1	9.5	0.6	0.0	12.0				0.6	0.0	12.0	0.1	0.0	17.7	10.6	0.5	10.6	21.2	1.1	11.5
18	36	1.6	0.0	1.7	0.4	0.0	13.6				0.4	0.0	13.6	0.0	0.0	24.1	9.8	0.8	18.9	29.2	1.7	13.3

19	4	1.2	0.0	7.4	1.9	0.1	14.6	1.9	0.1	14.6	0.2	0.0	7.6	10.7	0.6	12.9	34.6	3.3	21.1
19	8	1.5	0.0	4.3	0.9	0.1	16.3	0.9	0.1	16.3	0.1	0.0	5.2	9.1	0.8	18.9	21.1	1.2	12.7
19	12	1.6	0.0	6.3	0.6	0.1	30.0	0.6	0.1	30.0	0.1	0.0	8.2	7.9	0.8	22.9	15.0	1.8	26.4
19	18	1.6	0.0	1.8	0.5	0.1	30.0	0.5	0.1	30.0	0.1	0.0	13.9	8.4	1.3	35.8	18.0	2.4	29.3
19	24	1.6	0.0	5.3	0.3	0.1	49.8	0.3	0.1	49.8	0.1	0.0	11.1	6.1	1.5	56.3	11.3	2.6	51.2
19	36	1.7	0.0	2.4	0.5	0.2	94.3	0.5	0.2	94.3	0.0	0.0	19.4	11.8	6.5	123.6	20.8	4.9	47.7

Location	Depth (in)	POM Soil Mass (% of Total Soil Mass)			MAOM Soil Mass (% of Total Mass)			POM Carbon (% of Total Carbon)			MAOM Carbon (% of Total Carbon)		
		Mean	SE	CV	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV
1	4	26.7	0.6	4.9	73.3	0.6	1.8	55.1	2.3	9.4	44.9	2.3	11.5
1	8	16.9	2.8	28.7	83.1	2.8	5.9	51.7	12.7	42.5	48.3	12.7	45.4
1	12	11.9	3.4	49.3	88.1	3.4	6.7	67.2	9.7	25.1	32.8	9.7	51.4
1	18	8.1	2.8	60.2	91.9	2.8	5.3	61.0	11.5	32.6	39.0	11.5	50.9
1	24	8.7	2.8	54.6	91.3	2.8	5.2	44.4	21.1	82.3	55.6	21.1	65.8
1	36	5.8	0.3	10.3	94.2	0.3	0.6	38.5	7.4	33.4	61.5	7.4	20.9
2	4	11.2	0.1	1.6	88.8	0.1	0.2	20.5	1.3	14.0	79.5	1.3	3.6
2	8	10.5	0.3	4.5	89.5	0.3	0.5	10.4	2.7	44.4	89.6	2.7	5.2
2	12	11.4	0.1	1.9	88.6	0.1	0.3	8.1	3.7	78.7	91.9	3.7	6.9
2	18	12.2	0.9	12.6	87.8	0.9	1.8	7.5	1.8	40.9	92.5	1.8	3.3
2	24	11.9	1.2	16.8	88.1	1.2	2.3	18.7	10.5	97.5	81.3	10.5	22.4
2	36	12.7	1.3	14.6	87.3	1.3	2.1	49.6	5.8	16.6	50.4	5.8	16.3
3	4	7.5	0.3	7.8	92.5	0.3	0.6	20.7	1.3	14.4	79.3	1.3	3.8
3	8	5.0	0.4	14.7	95.0	0.4	0.8	5.0	0.9	29.8	95.0	0.9	1.6
3	12	4.4	0.2	9.7	95.6	0.2	0.5	5.3	1.9	62.8	94.7	1.9	3.5
3	18	4.5	0.1	4.6	95.5	0.1	0.2	2.2	0.2	13.2	97.8	0.2	0.3
3	24	4.1	0.5	19.5	95.9	0.5	0.8	3.9	1.5	64.7	96.1	1.5	2.6
3	36	4.8	0.6	20.1	95.2	0.6	1.0	3.2	0.9	48.3	96.8	0.9	1.6
4	4	8.3	0.3	7.5	91.7	0.3	0.7	25.1	2.9	26.1	74.9	2.9	8.7
4	8	6.1	0.4	10.7	93.9	0.4	0.7	5.0	0.5	18.5	95.0	0.5	1.0
4	12	5.7	0.8	24.7	94.3	0.8	1.5	4.5	1.3	49.4	95.5	1.3	2.3
4	18	6.8	3.0	74.9	93.2	3.0	5.5	2.6	0.2	14.5	97.4	0.2	0.4
4	24	5.4	1.2	38.4	94.6	1.2	2.2	3.7	1.3	62.0	96.3	1.3	2.4
4	36	8.7	2.3	45.2	91.3	2.3	4.3	2.4	0.3	20.7	97.6	0.3	0.5
5	4	8.1	0.1	4.0	91.9	0.1	0.4	25.5	2.2	19.2	74.5	2.2	6.6
5	8	5.3	0.3	10.2	94.7	0.3	0.6	7.5	0.1	2.6	92.5	0.1	0.2
5	12	4.3	0.5	21.1	95.7	0.5	1.0	4.4	0.7	25.8	95.6	0.7	1.2
5	18	5.6	1.1	35.1	94.4	1.1	2.1	7.2	2.0	47.9	92.8	2.0	3.7
5	24	6.1	1.2	33.4	93.9	1.2	2.2	7.1	2.1	51.7	92.9	2.1	3.9
5	36	4.4	2.5	99.0	95.6	2.5	4.6	14.6	5.5	53.6	85.4	5.5	9.1
6	4	7.9	0.1	3.9	92.1	0.1	0.3	21.9	1.0	9.9	78.1	1.0	2.8
6	8	7.1	0.1	2.9	92.9	0.1	0.2	8.9	0.3	5.6	91.1	0.3	0.5
6	12	5.9	0.2	5.1	94.1	0.2	0.3	3.9	1.1	48.0	96.1	1.1	1.9
6	18	4.5	0.5	18.5	95.5	0.5	0.9	3.4	0.2	12.5	96.6	0.2	0.4
6	24	3.9	0.6	25.5	96.1	0.6	1.0	12.5	8.8	121.0	87.5	8.8	17.3
6	36	4.8	0.7	25.6	95.2	0.7	1.3	7.6	5.3	119.8	92.4	5.3	9.9
7	4	18.2	0.6	7.0	81.8	0.6	1.6	26.6	1.9	15.6	73.4	1.9	5.6
7	8	14.6	0.3	3.5	85.4	0.3	0.6	8.6	0.4	9.1	91.4	0.4	0.9
7	12	14.3	1.0	12.3	85.7	1.0	2.1	5.0	0.4	12.3	95.0	0.4	0.6
7	18	14.0	1.9	23.2	86.0	1.9	3.8	5.3	0.8	27.6	94.7	0.8	1.6
7	24	13.6	2.0	25.4	86.4	2.0	4.0	5.4	1.2	39.6	94.6	1.2	2.3
7	36	13.4	0.3	2.7	86.6	0.3	0.4	10.7	7.8	103.0	89.3	7.8	12.4
8	4	23.5	0.9	8.8	76.5	0.9	2.7	23.6	0.7	7.0	76.4	0.7	2.2

8	8	22.3	0.7	5.2	77.7	0.7	1.5	12.7	1.4	18.6	87.3	1.4	2.7
8	12	13.8	1.0	12.1	86.2	1.0	1.9	8.5	2.1	42.9	91.5	2.1	4.0
8	18	17.2	2.5	25.1	82.8	2.5	5.2	32.9	14.3	75.1	67.1	14.3	36.8
8	24	22.2	3.3	25.8	77.8	3.3	7.4	79.4	10.3	22.5	20.6	10.3	87.0
8	36	18.9	3.2	29.1	81.1	3.2	6.8	73.4	6.4	15.2	26.6	6.4	41.9
9	4	32.4	1.3	8.8	67.6	1.3	4.2	29.7	0.7	5.6	70.3	0.7	2.4
9	8	31.2	0.8	4.5	68.8	0.8	2.0	15.2	6.7	76.5	84.8	6.7	13.8
9	12	29.2	5.7	34.1	70.8	5.7	14.1	16.4	12.5	132.3	83.6	12.5	26.0
9	18	33.3	10.3	53.4	66.7	10.3	26.6	31.4	23.6	129.8	68.6	23.6	59.5
9	24	7.3	2.5	58.3	92.7	2.5	4.6	29.8	2.5	14.3	70.2	2.5	6.1
9	36	9.9	0.3	4.9	90.1	0.3	0.5	54.3	8.2	26.3	45.7	8.2	31.2
10	4	8.9	0.3	7.9	91.1	0.3	0.8	24.0	1.6	15.3	76.0	1.6	4.8
10	8	6.7	0.4	11.0	93.3	0.4	0.8	5.8	0.5	15.3	94.2	0.5	0.9
10	12	5.9	0.2	5.0	94.1	0.2	0.3	3.4	0.3	17.3	96.6	0.3	0.6
10	18	8.0	0.5	10.1	92.0	0.5	0.9	3.2	0.0	1.3	96.8	0.0	0.0
10	24	8.3	0.4	7.6	91.7	0.4	0.7	3.3	0.6	29.4	96.7	0.6	1.0
10	36	6.5	0.1	2.1	93.5	0.1	0.1	3.1	0.8	44.1	96.9	0.8	1.4
11	4	17.8	0.6	7.0	82.2	0.6	1.5	18.8	0.9	10.4	81.2	0.9	2.4
11	8	14.9	0.1	0.9	85.1	0.1	0.2	5.4	0.4	13.1	94.6	0.4	0.7
11	12	10.7	0.8	12.5	89.3	0.8	1.5	4.1	0.5	19.3	95.9	0.5	0.8
11	18	10.2	0.8	13.3	89.8	0.8	1.5	4.4	1.0	40.1	95.6	1.0	1.8
11	24	11.7	2.1	30.9	88.3	2.1	4.1	14.5	4.3	51.3	85.5	4.3	8.7
11	36	11.3	2.0	30.4	88.7	2.0	3.9	18.8	9.3	85.1	81.2	9.3	19.7
12	4	14.0	0.5	7.5	86.0	0.5	1.2	20.1	1.7	18.8	79.9	1.7	4.7
12	8	12.0	0.9	12.9	88.0	0.9	1.8	4.7	0.2	5.8	95.3	0.2	0.3
12	12	6.8	1.0	25.1	93.2	1.0	1.8	2.7	0.4	25.6	97.3	0.4	0.7
12	18	6.1	0.3	9.5	93.9	0.3	0.6	3.2	0.3	15.1	96.8	0.3	0.5
12	24	7.5	0.4	9.1	92.5	0.4	0.7	29.4	3.3	19.2	70.6	3.3	8.0
12	36	11.6	0.4	5.6	88.4	0.4	0.7	28.9	4.9	29.3	71.1	4.9	11.9
13	4	10.2	0.2	4.9	89.8	0.2	0.6	34.1	8.2	54.0	65.9	8.2	27.9
13	8	7.7	0.7	16.9	92.3	0.7	1.4	5.1	0.6	19.5	94.9	0.6	1.0
13	12	6.4	1.3	35.3	93.6	1.3	2.4	4.0	0.6	27.9	96.0	0.6	1.2
13	18	5.3	0.3	10.4	94.7	0.3	0.6	3.4	0.4	22.6	96.6	0.4	0.8
13	24	4.6	0.1	3.9	95.4	0.1	0.2	2.6	0.3	18.1	97.4	0.3	0.5
13	36	6.0	0.5	15.7	94.0	0.5	1.0	19.4	6.2	55.5	80.6	6.2	13.4
14	4	14.0	0.4	5.9	86.0	0.4	1.0	24.5	1.4	13.1	75.5	1.4	4.2
14	8	12.7	0.8	10.4	87.3	0.8	1.5	7.2	0.8	18.5	92.8	0.8	1.4
14	12	7.6	0.3	6.5	92.4	0.3	0.5	5.0	1.5	51.0	95.0	1.5	2.7
14	18	7.4	0.3	6.9	92.6	0.3	0.6	4.6	0.3	11.7	95.4	0.3	0.6
14	24	7.4	0.0	0.8	92.6	0.0	0.1	10.8	2.6	42.1	89.2	2.6	5.1
14	36	10.3	0.7	12.0	89.7	0.7	1.4	33.5	4.1	21.5	66.5	4.1	10.8
15	4	17.1	0.6	8.3	82.9	0.6	1.7	19.6	1.7	19.3	80.4	1.7	4.7
15	8	11.7	1.0	14.8	88.3	1.0	2.0	6.1	0.8	24.1	93.9	0.8	1.6
15	12	10.9	2.3	36.5	89.1	2.3	4.5	20.8	13.9	115.9	79.2	13.9	30.4
15	18	8.5	0.2	4.5	91.5	0.2	0.4	5.8	1.6	47.2	94.2	1.6	2.9
15	24	12.0	1.2	16.7	88.0	1.2	2.3	9.2	2.6	48.4	90.8	2.6	4.9
15	36	13.5	1.0	12.7	86.5	1.0	2.0	24.6	15.2	107.1	75.4	15.2	35.0

16	4	8.8	0.9	21.7	91.2	0.9	2.1	26.0	1.8	15.9	74.0	1.8	5.6
16	8	6.1	0.1	1.9	93.9	0.1	0.1	3.3	0.9	46.4	96.7	0.9	1.6
16	12	5.7	0.2	7.2	94.3	0.2	0.4	3.1	0.4	21.5	96.9	0.4	0.7
16	18	5.8	0.4	11.8	94.2	0.4	0.7	3.7	1.3	60.3	96.3	1.3	2.3
16	24	6.3	0.4	9.7	93.7	0.4	0.7	4.5	1.3	48.2	95.5	1.3	2.3
16	36	7.8	1.2	26.4	92.2	1.2	2.2	9.8	4.2	74.6	90.2	4.2	8.1
17	4	11.7	0.3	4.8	88.3	0.3	0.6	25.9	1.7	14.4	74.1	1.7	5.0
17	8	10.2	0.3	5.7	89.8	0.3	0.6	6.0	1.0	29.2	94.0	1.0	1.9
17	12	7.1	1.4	33.8	92.9	1.4	2.6	3.9	0.5	20.9	96.1	0.5	0.9
17	18	5.1	0.3	9.0	94.9	0.3	0.5	6.5	2.5	66.4	93.5	2.5	4.6
17	24	4.1	0.1	2.6	95.9	0.1	0.1	3.0	0.1	5.9	97.0	0.1	0.2
17	36	5.4	0.1	4.6	94.6	0.1	0.3	25.0	3.6	25.0	75.0	3.6	8.3
18	4	21.0	0.6	6.7	79.0	0.6	1.8	32.3	1.3	8.8	67.7	1.3	4.2
18	8	23.3	6.7	50.1	76.7	6.7	15.2	21.4	7.3	59.1	78.6	7.3	16.1
18	12	13.7	2.0	25.4	86.3	2.0	4.0	8.6	0.9	18.7	91.4	0.9	1.8
18	18	12.7	0.6	7.9	87.3	0.6	1.2	8.4	0.8	16.0	91.6	0.8	1.5
18	24	13.3	0.7	8.9	86.7	0.7	1.4	11.7	2.3	33.6	88.3	2.3	4.4
18	36	13.1	1.2	16.0	86.9	1.2	2.4	14.0	4.9	60.6	86.0	4.9	9.9
19	4	24.8	1.5	13.3	75.2	1.5	4.4	37.2	1.1	6.4	62.8	1.1	3.8
19	8	17.4	2.3	22.8	82.6	2.3	4.8	17.0	2.7	27.7	83.0	2.7	5.7
19	12	14.6	1.9	22.7	85.4	1.9	3.9	22.5	14.3	110.0	77.5	14.3	32.0
19	18	13.6	2.0	25.2	86.4	2.0	4.0	20.6	13.9	116.9	79.4	13.9	30.3
19	24	17.9	3.6	35.0	82.1	3.6	7.6	31.7	11.9	64.9	68.3	11.9	30.2
19	36	19.4	4.7	41.6	80.6	4.7	10.0	38.6	14.3	64.3	61.4	14.3	40.4

Rainmaker Farm – Baseline Soil Sampling

Final Report

Appendix 3 - Oklahoma Carbon Program Soil Sampling Protocol

Oklahoma Carbon Program

Soil Sampling to Measure
the Carbon Sequestration
Rate of Aggregated Acres

v.2013



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1.0 About the Oklahoma Carbon Program

1.1 Authority

The Oklahoma Conservation Commission has statutory authority to verify and certify carbon sequestration in Oklahoma under Oklahoma Administrative Code Title 155 to implement 27A O.S. § 3-4-101 thru 3-4-105, which authorizes the Commission to establish and administer a carbon sequestration certification program. Permanent rules for the program went into effect July 1, 2009. The rules are authorized by the Oklahoma Carbon Sequestration Enhancement Act. Persons conducting verification of agricultural carbon offsets under the Oklahoma Carbon Program (OCP) shall use protocols written or approved by the Oklahoma Conservation Commission.

1.2 Description

The Oklahoma Carbon Program (OCP) is a voluntary program for the verification, certification, and registration of voluntary carbon offsets and avoided emissions. OCP provides project verification services for aggregators and buyers of carbon offsets and also offers third-party verification of anthropogenic carbon dioxide (CO₂) stored by the oil and gas sector during enhanced oil recovery. The purpose of the OCP is to improve soil, water, and air quality by encouraging Oklahomans to voluntarily implement practices that sequester greenhouse gases (GHG). The purpose of verification is to provide an independent third party review of project sites, data, and implementation methods to determine if a project has sequestered an expected amount of GHG.

1.3 Objectives

The OCP strives to provide the following to Oklahomans:

- Oversight of carbon market transactions in Oklahoma
- Information about carbon sequestration
- Quality verification and certification of Oklahoma carbon offsets
- Financial opportunities for Oklahoma farmers, ranchers, forestland managers, oil and gas and utility operators, who take action to sequester greenhouse gases
- Funding opportunities for Oklahoma Conservation Districts

2.0 About this Document

This soil sampling methodology was developed to allow the trained non-scientist to collect soil samples from fields during field verification of agricultural practices for the purpose of expanding Oklahoma's soil carbon data set, eventually allowing for a more accurate monetary valuation of Oklahoma carbon offsets.

The soil sampling protocol described here was developed and tested to provide a consistent and cost effective way to monitor organic carbon mass (OCM) with the primary goal to determine the amount of carbon sequestered in a pool of aggregated acres under contract for carbon offset payments. The secondary goal was to use the site specific data collected to determine the impacts of soil type, land management, and geographic location on the rate of carbon sequestration. Realization of the first goal allows for accurate valuation of carbon credits generated from the aggregated acres under contract during the contract period. Realization of the second goal through long term monitoring will improve current soil carbon sequestration rate estimates and allow carbon credit payments to take into

consideration site specific variables instead of current practice-based payments based on regional default values.

Determining the amount of carbon sequestration in *each field* is not a goal of this protocol. We assume that the variability in soil carbon stocks across a typical field in the region would limit the success of efforts to determine the carbon sequestered on a field-by-field basis. During the assessment of data collected during testing of this protocol, this assumption was validated: Practical limitations to accurate determination of carbon sequestered in a four-year period on a site-by-site basis are severe. Only 4% of the sites sampled could be monitored with 10 or fewer cores being collected. It is important that sample numbers be limited so that site disturbance and costs are limited.

3.0 Applicability

This protocol was developed and tested for use in no-till cropland and planted grasslands. It is for use by persons trained in soil sampling methods. It is limited to soils that are less than 5% gravel because it has not been tested in soils with greater than 5% gravel due to the limitations of using soil probes in gravelly soil. However, if augers were used to collect the soil sample, these methods could likely be adapted to gravelly soils if accurate measures of soil mass could be achieved. When project costs allow, and with land manager consent, the OCP uses these methods to collect soil samples during verification of fields under carbon contract.

4.0 Soil Sampling Rationale

Quantification of soil organic carbon (SOC) for the purpose of monitoring carbon sequestration rates is challenging due to the dynamic nature of SOC and the high level of spatial variability relative to the total mass of SOC in most soil. Past efforts to estimate the impact of soil management on organic carbon mass (OCM) and carbon sequestration rates in Oklahoma were based on small data sets. These small data sets were insufficient to provide accurate estimates of carbon sequestration on a regional scale as is needed for the implementation of a carbon credit market based on soil carbon sequestration. This is particularly true for regions such as Western Oklahoma where there is tremendous variability in soil type, management, and climate. Accordingly, research was conducted in recent years in Oklahoma to fill gaps in current literature to ensure that the sampling protocol used by the Oklahoma Carbon Program is appropriate for soil conditions found in the Southern Plains. This supporting research is presented in the appendices at the end of this report and is intended to support the sampling methods described in this document.

4.1 Twenty-Two Sample Sites (220 Cores) are Sufficient to Measure a Statistically Significant Change of 0.8 Mg C per Hectare

The OCP includes soil sampling during field verification, when project costs allow, so we can to grow the Oklahoma soils database for future research. We assume that the variability in soil carbon stocks across a typical field in the region would limit the success of efforts to determine the carbon sequestered on a field-by-field basis. An Oklahoma State University sampling study evaluated variability in organic carbon mass measured in no-till and planted grassland soils. This study showed that soil carbon mass was affected by soil texture as determined from SURGO data but that no other SURGO data variable affected measured carbon mass or its variability within a sample site. The data analysis showed that variability between sites and within sites was strongly influenced by variability in organic carbon concentrations

and that variability in bulk density had limited impact. This suggests that an average bulk density could be used when the objective is to monitor the change in carbon mass across multiple locations but that bulk density would still be required to monitor changes at each sample site to determine the impact of site specific variables on carbon dynamics. This study was also used to determine the minimum number of samples required to measure a significant change in carbon mass if it occurs. See **Appendix A:**

Evaluation of Variation in Organic Carbon Mass.

Analysis of data from 47 sample sites located throughout Western Oklahoma was used to determine a pooled standard deviation of 2.15 for the soil carbon mass in Oklahoma soils (Appendix A). Power analysis was performed to determine the number of samples and sample sites required to be 80% confident in finding a significant real increase in carbon mass equal to the estimated sequestration rate of 0.27 Mg C ha⁻¹ yr⁻¹ currently used by the Oklahoma carbon program to calculate offset payments for conversion to no-till (OCC 2011). Power analysis allows us to determine how many samples are required to measure a given significant difference, 0.27 Mg C ha⁻¹ yr⁻¹ in this case. As shown in Table 1 below, longer intervals between sampling dates should allow for larger potential differences and, therefore, fewer required samples.

Table 1: The number of cores required to be 80% confident in finding a significant (p<0.05) increase in carbon mass in a fixed mass of soil equal to the estimated sequestration rate of 0.27 Mg C ha⁻¹ yr⁻¹ for 1 to 10 years of sequestration. The number of sites was determined assuming that 10 cores are collected from each site.

# of years	Estimated Sequestration Mg C ha ⁻¹	# of cores required	# of sites sampled
1	0.3	1997	200
2	0.5	501	50
3	0.8	224	22
4	1.1	127	13
5	1.4	82	8
6	1.6	58	6
7	1.9	43	4
8	2.2	34	3
9	2.4	27	3
10	2.7	23	2

If the sample sites are randomly selected from the aggregated acres as described in this protocol, only 22 sites are required to measure the significant increase in carbon stocks that is expected in three years based on current sequestration rate estimates (Table 1). However, given the high uncertainty about the current estimates and the potential for destruction of the sample sites, it is recommended that 60 sites be initiated to insure the appropriate level of data collection to determine the impact of soil type, landscape position, management, etc. on carbon sequestration. Experience has shown that the number of sample sites should be in excess of the actual number needed to monitor carbon because of potential for destruction of the sample sites due to tillage or oil and gas exploration activity.

Other protocols require that multiple sample sites be located within each contracted field and that the number of sites be proportional to the size of the field such that the field and its variability are properly

represented. This is optimal when funding is sufficient and when the interest in knowing the field-by-field sequestration rate is high enough to warrant the cost. Appendix A describes data collected during the testing of this protocol that suggests that an impractical number of samples (115) would be required from each site to provide site-by-site monitoring of OCM.

4.2 Use of a Soil Push Probe Provides Reliable Soil Carbon Measurement Results Versus a Hydraulic Probe

The OCP uses closed tube push probes for field sampling because they are easier to use and more cost-effective, and provide scientifically sound results. Research by Oklahoma State University concluded that carbon mass measurements were comparable to those achieved with hydraulic probes. The field sampling study evaluated the impact of soil probe type on soil carbon mass measurements. This study determined that a manually operated push probe with a cutting diameter of 2.67 cm would cause an increase in bulk density in the 0-10 cm depth increment, in turn causing an over estimation of carbon mass compared to the use of a hydraulically driven probe with a cutting diameter of 3.98 cm when the fixed depth method of calculating carbon mass was used. However, when the fixed soil mass method of calculating carbon mass in the minimum soil mass was used there were no differences among the three probe types evaluated. In fact, the push probe provided improved results because its use significantly decreased variability in carbon mass. See **Appendix B: Assessment of Three Soil Probes to Monitor Carbon mass.**

4.3 The Coefficient of Variation for Organic Carbon Concentration Increases to Above 10% when the Ratio of Inorganic C: Total Carbon is Greater Than 0.40

The OCP uses total carbon minus inorganic carbon on most samples because analytical variability in the resulting organic carbon concentration is below ten percent except when the ratio is greater than 0.40. An Oklahoma State University laboratory study evaluated the impact that inorganic carbon in a soil sample has on determination of organic carbon concentrations. This study shows that the coefficient of variation for organic carbon concentration increases to above 10% when the ratio of inorganic carbon: total carbon is greater than 0.40. See **Appendix C: Influence of Inorganic Carbon Concentrations on Variability in Organic Carbon Concentrations.**

4.4 Using the Fixed Mass Method of Calculating Soil Carbon Mass can Correct Differences in Organic Carbon Mass Caused by Shrink-Swell Soils

The OCP uses the fixed mass method of calculating soil carbon mass. An Oklahoma State University study evaluated the changes in bulk density that can occur in a shrink-swell soil due to changes in soil moisture. The data show that measured bulk density can change significantly within a 30 day period. This change appears to result from compression of macropores when soils are moist, causing a higher bulk density compared to when soils are dry. The resulting difference in organic carbon mass can be corrected by using the fixed mass method of calculating soil carbon mass. See **Appendix D: Changes in Bulk Density and Carbon Stock Estimates in Shrink-Swell Soils.**

5.0 Sampling

5.1 Timing

Sampling is aligned with field verification timing for each agricultural practice type. Typically this would be during the months of October through January for winter wheat fields and 30-60 days after planting spring wheat.

5.2 Frequency

The OCP will sample as the project budget allows. When funding allows, the goal for each project is to collect at 50 different fields to sufficiently represent the variation of soil types and get a robust sampling of the management, climate and soil within the aggregated acres. Based on past experiences we expect that the integrity of sample sites will be lost for various reasons such as oil and gas pipeline installation, fire, or tillage. Fifty sites is the number based on the expectation of returning to 22 intact sites at a three year frequency. Research conducted by Oklahoma State University shows that sampling 22 sites will allow for statistically significant measurement of expected organic carbon sequestration.

5.3 Sample Site Selection

Sample sites are selected randomly in each field under contract. This ensures that the samples collected provide a best estimate of carbon sequestration in the aggregated acres under contract. Sample sites in each field are selected using the random point generator in ArcGIS. This provides latitude and longitude coordinates that are used to locate the center of the sampling site. The sample site is a three meter radius circle surrounding the center of the sample site.

5.4 Number of Sample Sites

Ideally, a sample site would be placed in each field under contract for carbon credit payment. However, since time and money are typically limited, a minimum number of sample sites are utilized to monitor the aggregate sequestration. The number of samples required is determined by the variability in carbon mass.

5.5 Soil Sample Collection Field Form

The soil sample collection field form records the latitude and longitude of the sample site, and other information needed to track the sample back to its origin. It is labeled with a 10 digit identifier that is comprised of the producer identification number, field number, location number (county code), core number (there are 10 cores taken per site), and depth (the core is cut at three depths). The form contains a key to explain the 10 digit identifier.

6.0 Methods

The protocol has been evaluated using three different types of probe. The first probe used is a hydraulic probe (HP) (Giddings #25-TS Model HDGSRTS, cutting diameter 3.99 cm). The second probe is a push/hand probe (PP) (cutting diameter 2.67 cm). This probe is commercially available from AMS Inc. and is described as a 1 1/4" x 24" plated replaceable tip soil recovery probe. This push probe can be fitted with a hammer head cross handle to allow for sample collection during dry conditions. The third sampler evaluated is the slide hammer probe (SH) (cutting diameter 4.8 cm). This sampler is also commercially available from AMS Inc. as the 2" x 12" soil core sampler.

Appendix B provides data demonstrating that any of these probes can be used interchangeably to monitor soil carbon stocks, given that the fixed mass method is used to calculate carbon stocks. It must also be noted that the probe tube must be a solid tube such that contamination does not occur as it is extracted from the soil.

Equipment Needed

- GPS
- Solid tube soil probe with tip and probe cap
- Soil probe hammerhead attachment and hammer
- Crescent wrench for probe cap
- Wooden dowel
- Tape measure
- 11 surveyor flags
- Spatula
- Clipboard
- Sharpie markers and ink pen
- Field sheets
- Ice chest / Cooler
- Plastic ziplock bags (1-quart size, 3 per core sample, 30 per site)
- Cradle for cutting core

Equipment Preparation

Cradle for cutting core. Cut in half a 4 inch diameter PVC pipe to use as a cradle for the soil core. Mark the cradle at 4, 8, and 12 inches.

Assembling the probe. Attach the probe tip to the bottom of the probe by screwing it in. Attach the hammer head top by screwing it on until it won't turn anymore. Make sure it is on straight, with the threads aligned, before beginning to hammer or push.

Labeling sample bags. 30 baggies are labeled for each sample location. That is 10 bags for each depth increment as follows: 0-4 in., 4-8 in., 8-12 in.

6.1 Soil Core Collection with Push Probe

Ten cores are collected from each site to a depth of 30 cm and segmented into 0-10, 10-20, and 20-30 cm segments. Probes can be pushed or hammered into the soil to a depth no less than 30 cm. It is suggested that the probe be forced to a depth of approximately 35 cm because the bottom of the core will often fall from the tip of the probe or be damaged while trying to push the core from the probe and it is necessary to collect the top 30 cm of soil intact.

6.1.1 Identifying the Sample Location

1. Use a GPS with WAAS capability to pinpoint the sample location coordinates (provided).
2. Place a flag at the GPS coordinate to mark the center of the sample location. Place flags in a circle within a radius of 10 ft (3m) around the center of the sample locations.

3. Prepare to collect 10 cores at random points within a 10 ft radius of the sample location by locating the core sample sites: Walk back and forth across the sample location in a zigzag pattern and place the tip of the probe down at random sample points.

6.1.2 Obtaining the Soil Core

1. Remove crop residue from the surface at each sample site **before** forcing the probe into the soil, but **do not** disturb or scrape away the soil surface. If the probe tip comes down on the crown of an intact plant, simply move the probe tip to one side just off of the crown. If a probe is pushed through residue or the crown of a living plant the resulting core can become distorted, which can cause errors in the bulk density value.
2. Collect 10 cores at random points within a 10 ft radius of the sample location: Push without twisting, or hammer, the probe into the soil vertically to a depth of 14 inches (**Fig.1**). The depth greater than 12 inches is used to ensure that a 12 inch core is extracted: Sometimes a portion of the core can be lost from the tip due to the suction created when pulling it from the soil. Do **not** twist the probe into or out of the ground.



Figure 1: Probe hammered 14 inches into the ground

3. Remove the probe from the soil by bending your legs, pushing and pulling the probe back and forth, while using your leg strength to pull straight up on the hammer head handles. Use a lever if necessary to avoid straining your back.
4. Take the probe to the cradle on a flat work surface.

6.1.3 Removing the Core

1. Place the opened plastic ziplock bag labeled "0-4" at the end of the cradle (**Fig. 2**).
2. Place a spatula on the outside of the bag pressed firmly against the cradle (**Fig. 2**). If you are working on the ground, use the hammerhead and clipboard to brace the spatula against the cradle.



Figure 2: The spatula held firmly against the cradle assures that the core can be pushed flush to the end of the cradle before cutting. If working on the ground instead of a truck, place the hammer head and clipboard behind the spatula.

3. Unscrew the hammer head from the probe tube and place it at the end of the cradle, if working on the ground.
4. Lay the probe into the cradle with the top end of the probe touching the spatula through the bag.
5. Place the wooden dowel into the bottom end of the probe (end that went into soil) and use it to push the core **gently out the top of the probe** onto the cradle while pulling the probe toward you (**Fig. 3**). For best results, pull the probe up the push rod (broom stick works well as a push rod) instead of pushing the rod into the probe. This will allow the core to lay down on the PVC cradle instead of being pushed up the PVC, this is particularly important for dry sandy soils or dry soils with small aggregates near the surface that easily fall apart.

Note: When clay type soils are wet, they may stick to the internal walls of the probe. To prevent this, lubricate as necessary. A silicon-based lubricant is recommended to prevent potential contamination from petroleum-based lubricants, particularly when using probes with a cutting diameter less than 3.2 cm. The smaller cores contain less soil mass, which increases sensitivity to contamination.



Figure 3: A broom stick handle with one end cut flat can be used to push the core from the probe tube.

6. Position the core so that the top of the core is at the zero mark of the cradle. Keep the end of the soil core flush with the end of the cradle and touching the spatula through the inside of the ziplock bag (**Fig. 4**). Try to keep the cylindrical shape of the core intact as much as possible to prevent mixing of the different soil depths. **Note:** If the entire soil core is removed and then pushed into place, looser soil will fall apart and result in mixing of soils from different depths, which will cause inaccurate laboratory results when the soil is analyzed.



Figure 4: Core must be pushed flush to the end of the PVC cradle. This can be achieved by bracing the spatula or clipboard at the end of the cradle and pushing the dowel forward while pulling the probe back. If necessary, push the core gently forward with the spatula.

6.1.4 Cutting the Core

1. Cut the core with the spatula at the 4 inch mark and push the cut portion of the core into the “0-4” bag (Fig. 5-6) that has been labeled with the sample identifier. Remove and close the bag.



Figure 5: Rounded putty knife fits into the PVC cradle for cutting the core

2. Realign the core at the 4 inch mark because the core remaining in the cradle may have moved during the cutting process.
3. Place the bag labeled “4-8” at the end of the cradle, and cut the core with the spatula at the 8 inch mark. Remove and close the bag.
4. Realign the core at the 8 inch mark. Place the bag labeled “8-12” at the end of the cradle, and cut the core with the spatula at the 12 inch mark. Remove and close the bag.
5. Discard onto the ground the remaining 2 inches of soil left in the cradle.



Figure 6: Core is cut at marks on the PVC cradle at 4, 8, and 12 inches from the end and pushed into labeled ziplock bags

6.1.5 Sample Labeling, Storage and Shipment

1. Mark each soil sample bag with the sample identification number (provided on soil sample information sheet).

2. Place the soil samples in an ice chest (if during summertime) to minimize fluctuations in temperature and **transport to a refrigerator as soon as possible** (particularly important if samples are collected when air temperatures high).
3. Label the soil sample information sheet with the date and time the samples were collected.
4. Include on the form a precise measure ($\pm 1/16^{\text{th}}$ of an inch) of the cutting diameter of the probe tip used.
5. Place the information sheets in a box with the soil samples and ship with 2-3 day delivery (in summertime ship with overnight delivery) as directed.

Appendix A: Evaluation of Variation in Organic Carbon Mass

Objectives

The purpose of this study is to 1) determine if SSURGO data can be related to measured carbon stocks and variability in those measurements, 2) determine the minimum number of samples required to measure expected significant change in carbon stocks, 3) determine if the fixed mass method for calculating carbon stocks can reduce variability resulting from variability in bulk density.

Materials and Methods

Sampling and Sample Preparation

The study was conducted on 47 fields in Alfalfa, Major, Caddo, Garfield, Greer, and Washita counties of Oklahoma. Out of 47 fields, 32 fields are under no-till cropland systems and 15 fields are under grasslands management. Winter wheat dominates the no-till cropping systems in the region and is generally planted two or more years in a row. If crop rotation is practiced, the producers generally utilize sorghum, canola, or cotton in a one crop per year system. However, most fields had been in continuous wheat since initiation of no-till management. The grass fields represented Bermuda grass as well as native mixed grass.

A list of producers participating in the Oklahoma Carbon Program was obtained from the Oklahoma Conservation Commission. Participation means that the producers agree to maintain the fields in permanent no-till or planted grass during the contract period and will receive a carbon credit payment annually. Producers were contacted and asked if they would allow the collection of soil samples from the contracted fields. When producers agreed to sample collection, the legal descriptions and management information were obtained from the Oklahoma Conservation Commission. Field boundaries were drawn in ArcMap 10 (ESRI) and the random point generator in the ArcToolbox was used to generate sample points within each field.

At each sample point a 3 m radius circle was marked around the random point and 10 cores were taken in that circle with a tractor-operated hydraulic probe. The tractor-operated hydraulic probe (cutting edge diameter of 3.98 cm) used in this study was a Giddings #25-TS Model HDGSRTS (Giddings Machine Company, Windsor, CO). The GPS location at which each core was extracted was recorded using a Trimble GeoXH GPS receiver.

Cores were extracted to a depth of 30 cm and cut into 0-10, 10-20, and 20-30 cm segments. The segments were packed in ziplock plastic bags and placed in an ice chest until transported and stored in a refrigerator at 4°C. Ziplock bags with wet soil were weighed and a subsample (~20 gm) was weighed into an aluminum weigh boat. This subsample soil was dried at 110°C for 24 hours and then weighed to determine moisture content. The soil remaining in the ziplock bag was transferred to a paper bag and allowed to dry at 65°C for one week and then ground and sieved through a 2 mm sieve. Moisture content was used to determine dry soil mass in the ziplock bag and further to determine bulk density. The sieved soil was analyzed for total carbon after weighing 0.2400-0.2500 g into a tin foil cup using the dry combustion method (Kalembasa and Jenkinson, 1973) in a Leco analyzer. Inorganic carbon was determined using Pressure Calcimeter (Sherrod et al., 2002). Soil pH was determined on a 1:1, soil:deionized H₂O mixture after a 30 minute equilibration period.

Measured values of carbon stocks were pooled by various mapping unit variables from the SSURGO data to determine if SSURGO data could be used to estimate carbon stocks and the variability expected.

Lastly, power analysis was used to determine the minimum number of samples required to observe significant change expected to occur if sequestration is occurring at the current estimated rate.

Calculation of Carbon Stock

Previous research has shown that variation in the measurement of bulk density can cause significant errors in the quantification of soil carbon density (Wilson, 2011; Ellert and Bettany 1995; Ellert et al. 2002). Gifford and Roderick (2003) proposed the cumulative mass method as an alternative to the fixed depth method. The cumulative mass method calculates carbon density found in a constant mass of soil instead of a constant depth and therefore may reduce errors associated with changes in bulk density. In this method, depth varies so that each sample contains the same dry mass per unit area.

The fixed mass approach to calculating OCM was adopted from Gifford and Roderick (2003). Specifically, the total length of the core was represented by Z_b and the surface subsection is represented by Z_a with the cumulative dry soil masses to the respective depths denoted by $m_s(z_b)$ and $m_s(z_a)$ and the cumulative mass of soil C, $c_s(z_b)$ and $c_s(z_a)$. The target or "Fixed" cumulative mass of dry soil is denoted by $m_s(t)$ and the corresponding cumulative mass of soil C that we are looking for is denoted as $c_s(t)$. Through linear interpolation, the resulting equation is

$$c_s(t) = c_s(z_a) + \frac{c_s(z_b) - c_s(z_a)}{m_s(z_b) - m_s(z_a)} (m_s(t) - m_s(z_a)).$$

The soil mass up to 30 cm depth for each core was calculated. The minimum soil mass among the cores collected was selected as the fixed mass which was 3690 Mg ha⁻¹ this is equal to a bulk density of 1.23 g cm⁻³ in the surface 30 cm of soil. The soil mass of 0-20 cm depth of every core is subtracted from this fixed mass. The resulting mass of soils is multiplied by the concentration of carbon found in the 20-30 cm segment of the core. This value is then added to the mass of carbon in the 0-10 and 10-20 cm depths, which is simply calculated by multiplying the mass in each depth by the OCC found in each depth.

It is expected that use of the cumulative mass method will reduce variability in the carbon density resulting from variability in bulk density measurements, thereby decreasing the standard deviation and minimizing the number of samples required to measure carbon sequestration over time.

The soil texture data was obtained from SSURGO. SSURGO data for each county involved in this study was downloaded from USDA Soil Data Mart (Web Soil Survey). Each soil mapping unit in SSURGO data has multiple soil series; however, most of the mapping units involved in this study were consociations except one which was complex. Therefore, soil texture for the major soil series in the mapping unit was used. Since there is variation in the depth of different horizons in a single soil series, the depth of horizons were averaged. Major soil series in most of the mapping units had average A horizon depth of more than 30 cm. For those which had average A horizon depth less than 30 cm, the texture of subsequent horizons was evaluated. In those soils having an A horizon less than 30 cm, the texture of the horizon immediately below the A horizon was not different from the A horizon.

Statistical analysis

The PROC MIX procedure in SAS was used to calculate the difference of least squares means for mean separation presented. The PROC POWER procedure in SAS was used to determine the fractional N total (cores required) based on the standard deviation of means calculated for each sample location and the pooled standard deviations. A nominal power of 0.80 was used to set the type II error at 0.20, and the

type I error was set at 0.05 for all power analyses. The standard deviation measured at each site, as well as the pooled standard deviation, was used in these analyses. The pooled standard deviation for the data set was calculated as the square root of the mean squared error term in ANOVA generated by the PROC GLM procedure in SAS. These power analyses were conducted for soil organic carbon mass as calculated on a fixed mass (3478 Mg soil) basis.

Results and Discussion

Samples were collected from a wide range of soil types containing broad range of organic carbon mass (OCM) in the surface 30 cm (Table A-1). The average OCM as calculated using the fixed mass method found in two Grandfield loamy sand soils was 3.8 Mg C ha⁻¹. In contrast, an Aspermont silt loam contained 28.0 Mg C ha⁻¹. This difference demonstrates the diversity in

Table a-1: Average organic carbon stock per hectare (OCM) in different soil series and their corresponding average coefficient of variation (CV) as calculated using fixed mass method

Series ¹	N	OCM -----Mg ha ⁻¹ -----	SD	CV %
Abilene Silt Loam	1	22.5	0.8	3.4
Aspermont Silt Loam	1	28.0	1.0	3.5
Burford-Tillman complex	1	20.6	5.0	24.4
Carey Loam	2	21.9	1.0	4.7
Grandfield Loamy Sand	2	3.8	1.1	29.8
Grant Silt Loam	1	25.7	3.2	12.6
Lovedale Fine Sandy Loam	1	14.8	1.6	10.5
Meno Loamy Fine Sand	4	10.1	1.6	17.1
Minco Sandy Loam	3	19.5	2.4	12.4
Nobscot Fine Sand	3	11.0	1.8	17.8
Obaro Silty Clay Loam	2	20.6	2.7	14.9
Pond Creek Silt Loam	1	23.3	1.6	7.0
Port Silt Loam	1	15.8	0.6	3.6
Reinach Very Fine Sandy Loam	1	11.3	2.5	22.2
Renfrow Silty Clay Loam	2	27.1	4.0	14.9
St. Paul Silt Loam	8	24.5	1.7	6.2
Tillman Clay Loam	2	26.4	1.7	12.1
Westview Silt Loam	1	27.3	1.7	6.3

¹ Soil series used is the major series in the mapping unit. Most of the mapping units are consociation with major series occupying more than 75% of the area, except Burford-Tillman complex system

OCM represented within this study. A wide range in the amount of variability in OCM is also presented in Table A-1. The coefficients of variation (CV) presented were as little as 3.4% for one Abilene silt loam and as large 29.8%, which was the average CV for the two Grandfield loamy sand soils. When OCM was calculated on a fixed depth basis (Table A-2), the relative difference among the soil series did not differ; however, the OCM were higher for the fixed depth method. The standard deviation for OCM as calculated with the fixed mass method was lower than the standard deviation of OCM calculated with the fixed depth method. On the other hand, CVs for the fixed mass method are higher (with few exceptions) than those calculated with the fixed depth method. It should be noted that the mass selected for the fixed mass calculation was the minimum among the cores and all other cores were truncated to achieve it, such that the lower OCM values in fixed mass calculation were expected. The

higher CVs resulting from using the fixed mass method are a result of the decrease in average OCM and a slight change in standard deviation.

Table A-2: Average organic carbon stock per hectare (OCM) in different soil series¹ and their corresponding percent coefficient of variation (CV) as calculated using fixed depth method

Series ¹	N	OCM -----Mg ha ⁻¹ -----	SD	CV %
Abilene Silt Loam	1	27.8	1.2	4.4
Aspermont Silt Loam	1	30.5	1.3	4.3
Burford-Tillman Complex	1	25.0	7.3	29.3
Carey Loam	2	26.6	1.4	4.7
Grandfield Loamy Sand	2	4.2	1.1	27.1
Grant Silt Loam	1	30.0	4.6	15.4
Lovedale Fine Sandy Loam	1	17.5	1.9	11.0
Meno Loamy Fine Sand	4	10.9	1.7	16.7
Minco Sandy Loam	3	25.8	2.4	9.5
Nobscot Fine Sand	3	13.2	2.4	19.2
Obaro Silty Clay Loam	2	25.1	3.2	14.0
Pond Creek Silt Loam	10	27.2	1.9	7.1
Port Silt Loam	1	18.1	0.5	3.0
Reinach Very Fine Sandy Loam	1	13.2	2.6	19.4
Renfrow Silty Clay Loam	2	32.1	4.1	12.8
St. Paul Silt Loam	8	31.0	2.7	8.7
Tillman Clay Loam	2	32.8	2.2	6.5
Westview Silt Loam	1	32.1	1.5	4.8

¹ Soil series used is the major series in the mapping unit. Most of the mapping units are consociation with major series occupying more than 75% of the area, except Burford-Tillman complex system

The variation observed between soil types as well as within sample locations presents challenges to efforts to monitor organic carbon stocks and provide useful estimates of carbon sequestration or loss. Therefore, an effort was made to determine if SSURGO data and land management information could be used to categorize the soil types into those that may allow for easier monitoring of carbon stocks and those that present challenges.

The effect of land management can be observed in Table A-3. In this study there were 15 grass fields and 32 no-till cropland fields. The OCM, standard deviation, and CV were not affected by management system (Table A-3). It should be pointed out again that the grass fields were previously cultivated. Some fields were planted to improved grasses for hay production and others were planted as a result of enrollment in the conservation reserve program. The common practice in the area is to plant grass to those cropland fields that are low in grain crop productivity or highly erodible. Therefore, the results of this analysis are likely confounded by crop history and soil type. However, the diversity in soil types did not allow for analysis of management within each soil type.

Table A-3: Average organic carbon mass (OCM), average standard deviation (SD) and average coefficient of variation (CV) under grassland and no-till system as calculated through fixed depth and fixed mass method.

Method	Management System	OCM -----Mg ha ⁻¹ -----	SD	CV %
<u>Fixed Depth</u>				
	Grass (N = 15)	22.1a	2.4a	13.9a
	No-till (N = 32)	25.1a	2.3a	10.2a
<u>Fixed Mass</u>				
	Grass	19.1a	2.1a	14.8a
	No-till	20.8a	1.7a	9.5b

Table A-4 shows the effect of texture category on OCM and CV. When using both the fixed depth and fixed mass method, the soils containing > 60% sand had significantly lower OCM than the remaining texture categories. The remaining textural classes were not significantly different from each other. Our results are in agreement with findings of Gosling and Parson (2013) and Meersmans et al. (2008) who reported that soils with large amounts of sand have lower OCM when compared to those containing higher clay contents. Meersmans et al. (2008) used the Belgian textural triangle, where clay soils have >30% clay. Goslings and Parsons (2013) (for Great Plains of US) and Meersmans et al. (2008) (for soils in Belgium) in their separate studies reported a significant effect of texture on total soil organic matter. Clay and silt appear to retain the heavy fraction of soil organic matter by protecting it in micropores out of the reach of microbes or by forming organo-mineral complexes with clay minerals (Christensen 1996; Hassink 1992; Schimel 1985; Sorensen 1981).

Table A-4: Average of organic carbon mass (OCM) and coefficient of variation (CV) in soils with different proportion of sand, silt and clay

Method	Sand -----%-----	Silt	Clay	Soil type	Number of Sites	OCM Mg ha ⁻¹	CV %
<u>Fixed Depth</u>							
	<15	>60	<25	1	26	28.7b	7.7a
	<10	>60	>30	2	2	25.1b	14.0ab
	<30	<40	>30	3	5	31.0b	13.6b
	<35	<45	<20	4	2	24.0b	4.4a
	>60	<25	<15	5	12	11.3a	19.0b
<u>Fixed Mass</u>							
	<15	>60	<25	1	26	23.8b	7.3ab
	<10	>60	>30	2	2	20.6b	14.9bcd
	<30	<40	>30	3	5	26.7b	13.2c
	<35	<45	<20	4	2	20.0b	4.3a
	>60	<25	<15	5	12	9.9a	19.3d

Soils having more clay fraction have a higher proportion of micropores of diameter <0.2 micro meter (Hassink, 1992; Hassink, 1993), which does not allow microbes to decompose the organic matter/biomass trapped in these pores. Similarly, soils with higher clay content due to their higher aggregation capacity trap the organic matter in aggregates or encapsulate it, thus protecting it from decaying by microbes (Kölbl and Knabner, 2004; Krull et al 2003). In contrast, Hassink (1994) did not find any relation of carbon decomposition with soil texture. In addition, Epinet et al. (2013) reported that texture along with precipitation influences decomposition rates, where sands have higher rates of

decomposition than clay for a given amount of precipitation. The available water capacity of soil influences net primary productivity, where the available water capacity increases with silt content of the soil (Burke et al. 1989). However, there is a feedback mechanism with respect to the impact of precipitation which supports productivity as well as enhances decomposition of soil organic matter (Burke et al. 1989).

In addition to containing the lowest OCM, the soils with > 60% sand (Table A-4) had the highest average CV, regardless of calculation method. In fact, it was significantly higher than all other textural categories except for the soil containing < 10 sand, > 60 silt, and > 30 clay. Those texture categories with < 25% clay and < 35% sand had the lowest CVs. This indicates that loam soils have lower levels of variability within a sample site compared to sandy and clayey soils.

Table A-5: Organic carbon concentration (OCC) and percent coefficient of variation (CV) in soil with different proportion of sand, silt, and clay.

Sand	Silt	Clay	Soil type	Number of Sites	OCC	CV
-----%-----					g kg ⁻¹	%
<15	>60	<25	1	26	6.7a	7.5a
<10	>60	>30	2	2	5.9a	14.5ab
<30	<40	>30	3	5	7.5a	14.4b
<35	<45	<20	4	2	5.8a	4.5a
>60	<25	<15	5	12	2.7b	19.6b

The organic carbon concentration (OCC) follows the same trend as OCM with respect to the impact of texture (Table C- 5). The OCC in soils containing >60% sand was significantly lower than in the remaining textural classes.

The variation in OCC was highest in sandy soils followed by soils with >30% clay. This is in agreement with findings of Merry and Spouncer (1988), who reported an increase in CV of carbon concentration with decrease in carbon concentration. Merry and Spouncer (1988) analyzed the interaction of sample weight, furnace temperature and carbon concentration for four different soils. They observed a maximum CV of 7.1% in soils having a minimum OCC of 4.8 g kg⁻¹. The high CV is a result of being near the detection limit of the analysis (dry combustion method). In the current study, the second highest CV was observed in soils with > 30% clay content, and most of these clayey contained carbonates. The average total carbon concentration in soils with >30% clay was 13.3 g kg⁻¹, which is approximately twice the OCC. Thus, the higher total carbon concentration (organic carbon + inorganic carbon) in soils containing carbonates might have elevated CV (refer to Appendix C). The suitable weight for a majority of the soils is 0.2-0.3 g (Carr, 1973), which has been used in this study. The current study shows high variation in sandy soils with the lowest organic carbon concentration and in soil with the highest total carbon concentration.

The bulk density was not significantly affected by soil texture (Table A-6); however, soil texture had a significant effect on the CV for bulk density (Table A-4). The bulk density CVs were lowest for the soils containing >60% sand but only significantly lower than the CV for the soils containing >60% silt.

Table A-6: Bulk density (BD), its standard deviation (SD) and percent coefficient of variation (CV) in soil with different proportion of sand, silt and clay.

Sand	Silt	Clay	Soil type	Number of Sites	Avg. BD Mg m ⁻³	CV %
-----%-----						
<15	>60	<25	1	26	1.44a	3.6b
<10	>60	>30	2	2	1.42a	5.7c
<30	<40	>30	3	5	1.41a	3.5ab
<35	<45	<20	4	2	1.39a	3.0ab
>60	<25	<15	5	12	1.48a	2.8a

Table A-7 simplifies the data by presenting OCM found in 3 texture classes and the average SD and CV for both calculation methods. This presentation shows that the absolute error as indicated by the SD is similar for soils containing > 60 % silt or sand which have lower SD than compared to the soils containing > 30% clay. In contrast, because the soils containing >60% silt have OCM values equivalent to the soils containing > 30 % clay, the CV for the silt soils is approximately half of that found for the sand and clay soils.

Assessment of Tables A-4, 5, and 6 suggests that the relative variability in OCM as evaluated using CV is dominated by variability in the OCC as indicated by the fact that the effects of texture class on the CV values for OCM and OCC were similar and that the CV in bulk density did not follow a similar trend. In order to fully understand how measured OCC and bulk density values influenced absolute error in the OCM estimate, regression analysis was used to determine if the average OCC or average bulk density in the 0-30 cm sample depth were related to the average SD values for the OCM values calculated using fixed mass and fixed depth. In addition, the SD values for OCC and bulk density for each depth and the average across depths were also regressed against the average SD and average CV values for OCM values calculated

Table A-7: Average organic carbon (OCM) mass as calculated in soils with different dominating textural components and their average standard deviation (SD) and percent coefficient of variation (CV)

Method	Textural component	% in Soil	OCM -----Mg ha ⁻¹ -----	SD	CV %
Fixed Depth	Clay	>30	29.3b	3.8b	13.7b
	Silt	>60	28.7b	2.2a	7.7a
	Sand	>60	11.3a	1.9a	19.0c
Fixed Mass	Clay	>30	24.9b	3.1b	13.7b
	Silt	>60	23.5b	1.7a	7.8a
	Sand	>60	9.9a	1.6a	19.3c

using fixed mass and fixed depth. The coefficients of determination (r^2) presented in Table A- 8 show that OCM was highly correlated with the OCC. The strong relationships between OCC and OCM for fixed mass and depth methods suggest that OCC is the dominant factor resulting in differences among sample sites. Table A-8 also shows that the standard deviation of OCM (OCM SD) is not related to the average OCC, indicating that the variability in OCM is not consistently affected by the OCC.

Table A-8: Coefficients of determination for the linear relationship between average OCM and the corresponding average standard deviation for the OCM and the average organic carbon concentrations found in each sampled depth and the average concentration in the 0-30 cm.

Method		Avg. OCC (g kg ⁻¹)			
		0-30cm	0-10cm	10-20cm	20-30cm
Fixed Mass	OCM (Mg ha ⁻¹)	0.9923	0.8681	0.9359	0.8410
	OCM SD (Mg ha ⁻¹)	0.0378	0.0221	0.0412	0.0406
Fixed Depth	Fixed depth				
	OCM (Mg ha ⁻¹)	0.9673	0.7956	0.9166	0.8712
	OCM SD (Mg ha ⁻¹)	0.0758	0.0210	0.1057	0.1024

Table A-9 shows that the SD for OCC values was not related to the OCM. However, the SD for OCC was strongly related to the SD for OCM. This indicates that the within sample site variability in OCM measurements is dependent on variability in OCC.

Tables A-10 and 11 show that neither the average bulk density nor the SD for bulk density is related to the OCM or the SD for OCM. This analysis suggests that variation in bulk density has no impact on variability in OCM within a sample site or among sample sites. In fact, differences in OCM between sample sites and within sample sites were more dependent on OCC than on bulk density. The fact that variation in bulk density does not influence variation in OCM can be explained in part by the fact that bulk density did not vary as a function of soil type. Specifically, recall that Table C-6 shows no difference in bulk density among the different texture classes evaluated. In addition, the average bulk density measured to a depth of 30 cm across all sample sites was 1.45 g cm⁻³. Figure A-1 shows that 20 of the sample sites had average bulk densities within the range of 1.49-1.41 g cm⁻³ which is +/- 2.5% of the 1.45 g cm⁻³ average. Furthermore, all bulk density values were within 15% of average bulk density. In contrast the range of OCC values was 0.6 to 10.0 g kg⁻¹ or +/- 90% of the mean of 5.7 g kg⁻¹. This wide range and more even distribution (Figure A-2) of OCC values explains its dominant effect on OCM.

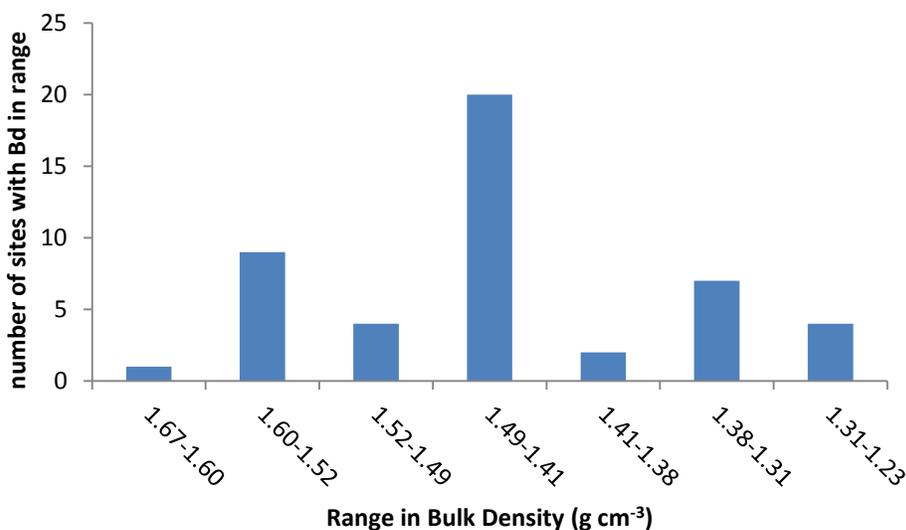


Figure A-1: Histogram showing the distribution of bulk density values measured at each of the 47 sample sites.

Table A-9: Coefficients of determination for the relationship between average OCM and the corresponding average standard deviation for the OCM and the average standard deviation of the organic carbon concentration found in each sampled depth and the average standard deviation in the 0-30cm.

Method		Ave. Stdev of OCC (g kg ⁻¹)			
		0-30cm	0-10cm	10-20cm	20-30cm
Fixed Mass	OCM (Mg ha ⁻¹)	0.0601	0.0605	0.0056	0.0314
	OCM SD (Mg ha ⁻¹)	0.7669	0.499	0.5463	0.3479
Fixed Depth	OCM (Mg ha ⁻¹)	0.0695	0.0553	0.0126	0.0395
	OCM SD (Mg ha ⁻¹)	0.8308	0.1736	0.3973	0.7452

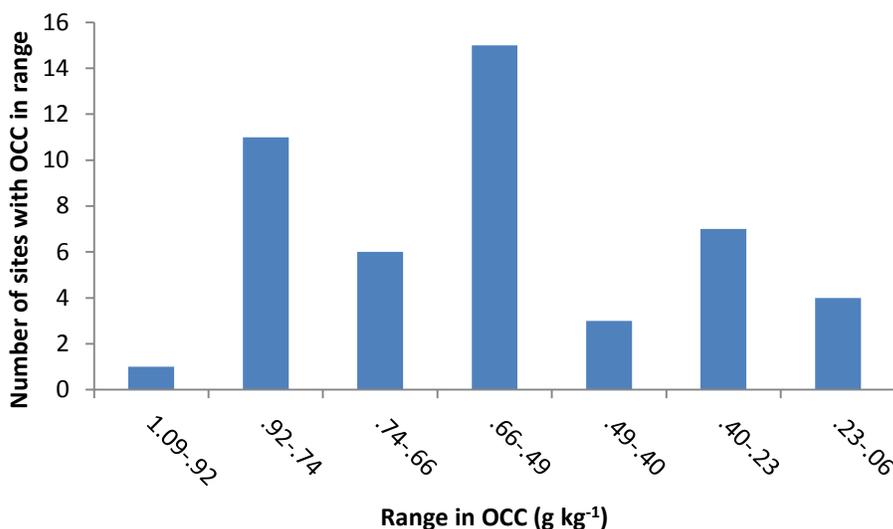


Figure A-2: Histogram showing the distribution of organic carbon concentration (OCC) values measured at each of the 47 sample sites.

Table A-10: Coefficients of determination for the relationship between average OCM and the corresponding average standard deviation for the OCM and the average bulk density found in each sampled depth and the average bulk density in the 0-30cm.

Method		Ave. Bd (g cm ⁻³)			
		0-30cm	0-10cm	10-20cm	20-30cm
Fixed Mass	OCM (Mg ha ⁻¹)	0.1593	0.1200	0.1054	0.1461
	OCM SD (Mg ha ⁻¹)	0.0004	0.0036	0.0096	0.0107
Fixed Depth	OCM (Mg ha ⁻¹)	0.0770	0.0479	0.0461	0.0887
	OCM SD (Mg ha ⁻¹)	0.0043	0.0024	0.0141	0.0000

Power Analysis

Power analysis was performed to determine the number of cores and sample sites required to be 80% confident in finding a significant ($p < 0.05$) increase in OCM equal to the estimated sequestration rate of $0.27 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$. The first analysis assumed a four-year sample rotation to monitor the change in OCM on each sample site. Specifically, the analysis was performed to determine the number of samples required to measure an increase in OCM of 1.1 Mg C ha^{-1} at each site based on the standard deviation in OCM in each site. Figure A-3 shows that the standard deviations observed in the data range from 0.5 to 5.0 Mg C ha^{-1} , resulting in a sample requirement of 10 to 659 with an average of 115 samples from each site to measure a site specific change in OCM equal to 1.1 Mg C ha^{-1} if in fact it occurred.

Table A-11: Coefficients of determination for the relationship between average OCM and the corresponding average standard deviation for the OCM and the average standard deviation of the bulk density found in each sampled depth and the average standard deviation in the 0-30cm.

Method		Ave. Stdev. of Bd (g cm^{-3})			
		0-30cm	0-10cm	10-20cm	20-30cm
Fixed Mass	OCM (Mg ha^{-1})	0.1468	0.0227	0.1638	0.1010
	OCM SD (Mg ha^{-1})	0.0524	0.0204	0.0266	0.0563
Fixed Depth	OCM (Mg ha^{-1})	0.1897	0.0425	0.1963	0.1892
	OCM SD (Mg ha^{-1})	0.1349	0.1025	0.0632	0.1076

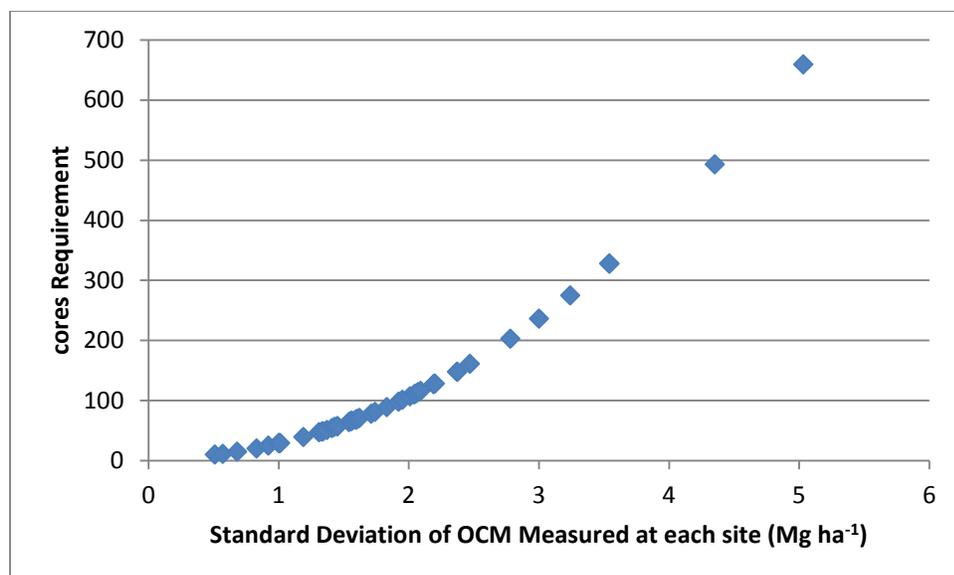


Figure A-3: The number of cores required to be 80% confident in finding a significant ($p < 0.05$) increase in carbon mass equal to 1.1 Mg C ha^{-1} at the standard deviation of OCM calculated for each site using the fixed mass method at each site.

This analysis of the core requirement to monitor carbon at each site demonstrates the futility in monitoring carbon on a site-by-site basis. In addition to the analysis costs, collection of the average number of required cores (115) from each sample site would seriously damage the integrity by simply forming an excessive number of holes in the soil surface.

Because of the high number of cores required to monitor carbon at each site, it may be more useful to assess the average carbon sequestration across all sample sites. In order to determine the number of sample sites required, analysis of data from the 47 sample sites was used to determine a pooled standard deviation of 2.15 Mg C ha⁻¹ for the OCM. Power analysis was again performed to determine the number of cores and sample sites required to be 80% confident in finding a significant (p<0.05) increase in OCM equal to the estimated sequestration rate of 0.27 Mg C ha⁻¹ yr⁻¹. This analysis found that 1997 cores would be required to measure this annual change in OCM as calculated using the fixed mass method. If 10 cores were collected from each sample site, 200 sample sites would be required. Further analysis demonstrates the potential impact of sample frequency on sample requirement. For example, if fields are sampled every four years and we expect that the sequestration rate is constant at 0.27 Mg C ha⁻¹ yr⁻¹, then in 3 years the soil would accumulate 0.8 Mg C ha⁻¹. Two hundred and twenty-four samples would be required to be 80% confident in finding a significant increase of this magnitude. Given that 10 cores are collected from each site, this would require 23 sample sites. Table A-12 provides further assessment of the impact of sample frequency on the number of cores required to measure a significant change in OCM.

Table A-12: The number of cores required to be 80% confident in finding a significant (p<0.05) increase in carbon mass in a fixed mass of soil equal to the estimated sequestration rate of 0.27 Mg C ha⁻¹ yr⁻¹ for 1 to 10 years of sequestration. The number of sites was determined assuming that 10 cores are collected from each site.

# of years	Estimated Sequestration Mg C ha ⁻¹	# of cores required	# of sites sampled
1	0.3	1997	200
2	0.5	501	50
3	0.8	224	22
4	1.1	127	13
5	1.4	82	8
6	1.6	58	6
7	1.9	43	4
8	2.2	34	3
9	2.4	27	3
10	2.7	23	2

Summary

The analysis shows that OCM is influenced by soil texture and that the variability in OCM increases in sandy soils due to low concentrations of OCC which decrease the reproducibility of the laboratory method. The increased variability in OCM found in the clayey soils appears to be the result of the presence of inorganic carbon in these soils. The dominant factor influencing within-site variability at a six-meter scale, and ultimately the number of samples required to successfully monitor carbon stocks, is the variability in OCC. Although OCC has a strong influence on OCM, using the fixed mass method of calculating OCM further improved the relationship between OCC and OCM by decreasing variability in OCM due to variation in bulk density.

The fact that bulk density was not significantly influenced by texture and is not a major contributor to variability suggests that perhaps a single/constant bulk density value may be used along with OCC

analysis to monitor carbon stocks. This may be particularly true if the goal of monitoring is to determine the rate of change in a large land base such as the aggregated acres in a carbon program. However, the procedure is likely insufficient if monitoring is meant to determine the rate of change in carbon stocks at individual locations because site specific bulk density will be needed. Power analysis found that, based on the standard deviation in OCM values at each site, the average number of cores required to monitor OCM would be 115. This would be costly as well as damaging to the integrity of the sample site for long term monitoring due to excessive number of holes left in the soil surface. Therefore, it is likely that multiple samples sites will be required to monitor the aggregate change in OCM.

Utilizing multiple sites to monitor the aggregate change in OCM is prudent for a variety of other practical reasons as well. First, it improves the representation of the contracted acres. Secondly, it prevents loss of site integrity due to tillage or other soil disturbance. Lastly, it provides potential opportunity to gain knowledge about the impact of soil type, management, and location on changes in OCM. Therefore, collection of 10 cores per sample location will be sufficient to monitor carbon on a three-year sample frequency given that no fewer than 23 samples sites are used. However, experience suggests that at least 1.5 times this many sites will be required to insure that site integrity is not compromised by tillage or other activity such as pipeline excavation over time.

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Appendix B: Assessment of Three Soil Probes to Monitor Carbon Stock

Objectives

The objective of this study was to determine the impact of probe type on measured bulk density, organic carbon concentrations, and organic carbon mass in cropland soils of the U.S. Southern Plains. A secondary objective was to evaluate the utility of the fixed mass method in reducing variability in measured organic carbon mass.

Materials and Methods

Three probe types were used in this study. The first was a tractor mounted hydraulic probe (HP), Giddings #25-TS Model HDGSRTS, with a diameter of 3.98 cm. The second probe is referred to as the push/hand probe (PP) and has a diameter of 2.67 cm. This probe is commercially available from AMS Inc. as the 1 1/4" x 24" plated replaceable tip soil recovery probe. This push probe can be fitted with a hammer head cross handle allowing for sample collection during dry conditions. The third sampler evaluated is referred to as the slide hammer probe (SH), with a diameter of 4.8 cm. This sampler is also commercially available from AMS Inc. as the 2" x 12" soil core sampler. These samplers were selected because they include solid recovery tubes that will prevent contamination as the core is extracted. They also allow sampling to 30 cm or greater.

Nineteen fields participating in Oklahoma Carbon Program were sampled. These fields were located in four Oklahoma counties (Major, Garfield, Washita, and Caddo). The fields were cropland, with wheat as the primary crop. A wide range of soil types were included in this study. Legal descriptions of each field and management information were obtained from the Oklahoma Conservation Commission. Each field was assigned an alphabetic character for unique identification. Field boundaries were drawn in ArcMap 10, and the random point generator in the ArcToolbox was used to generate sample points within each field.

A 3 m radius circle was marked around each random point, and samples were taken in that circle. In these fields, 10 cores were collected using the hydraulic probe, and 5 cores each were collected with the push probe and slide hammer probe.

Cores were extracted to a depth of 30 cm and cut into 0-10, 10-20, 20-30 cm segments. The segments were packed in ziplock plastic bags and placed in an ice chest until transported and stored in a refrigerator at 4°C. Ziplock bags with wet soil were weighed and a subsample (~20 g) was weighed into an aluminum weigh boat. This subsample of soil was dried at 110°C for 24 hours and then weighed to determine moisture content. The soil remaining in the ziplock bag was transferred to a paper bag and allowed to dry at 65°C for one week and then ground and sieved through a 2 mm sieve. Moisture content was used to determine dry soil mass in the ziplock bag and further to determine bulk density. The sieved soil was analyzed for total carbon using the dry combustion method (Kalembasa and Jenkinson, 1973) in a Leco CN analyzer after weighing 0.2400-0.2500 g into a tin foil cup. Inorganic carbon was determined using a Pressure Calcimeter (Sherrod et al., 2002) for samples with pH greater than 7.2. Soil pH was determined on a 1:1, soil:deionized H₂O mixture after a 30 minute equilibration period.

The organic carbon concentration of samples was calculated as the difference between the total carbon and inorganic carbon. The organic carbon mass (OCM) in each depth was determined by multiplying the concentration by the bulk density.

The fixed mass approach to calculating OCM was adopted from Gifford and Roderick (2003). Specifically, the total length of the core was represented by Z_b and the surface subsection is represented by Z_a with the cumulative dry soil masses to the respective depths denoted by $m_s(z_b)$ and $m_s(z_a)$ and the cumulative mass of soil C, $c_s(z_b)$ and $c_s(z_a)$. The target or “fixed” cumulative mass of dry soil is denoted by $m_s(t)$ and the corresponding cumulative mass of soil C that we are looking for is denoted as $c_s(t)$. Through linear interpolation, the resulting equation is this:

$$c_s(t) = c_s(z_a) + \frac{c_s(z_b) - c_s(z_a)}{m_s(z_b) - m_s(z_a)} (m_s(t) - m_s(z_a)).$$

The soil mass up to 30 cm depth for each core was calculated. The minimum soil mass among the cores collected was selected as the fixed mass which was 3690 Mg ha^{-1} this is equal to a bulk density of 1.23 g cm^{-3} in the surface 30 cm of soil. The soil mass of 0-20 cm depth of every core is subtracted from this fixed mass. The resulting mass of soils is multiplied by the concentration of carbon found in the 20-30 cm segment of the core. This value is then added to the mass of carbon in the 0-10 and 10-20 cm depths, which is simply calculated by multiplying the mass in each depth by the OCC found in each depth.

Sample points were considered replicates and the probe type was the treatment. The mean bulk density, organic carbon concentration, and organic carbon mass was calculated for each sample point and probe at each depth. The mean organic carbon mass for the cumulative depth of 30 cm and the cumulative mass of 3690 Mg ha^{-1} was also calculated for each sample point and probe. The coefficients of variation around these means was then determined.

All of the three probes were treated as treatments and the fields as replications. Randomized complete block analyses of variance were performed using the SAS PROC GLM procedure (SAS Institute, 2001) to determine significant treatment effects on the analyzed variables and the average coefficient of variation for those variables. Fisher’s protected LSD was used to separate significant differences.

Results and Discussion

Table B-1 shows the impact of probe type on the bulk density measurements. The average bulk density as measured with PP was significantly higher than HP and SH in the surface 10 cm. The probable reason for higher bulk density at surface 10 cm for PP would be the compaction caused by its small cutting edge diameter (2.26 cm), such that the cross sectional area of the PP’s sample is less than half of the SH (cutting diameter of 4.8 cm) and HP (cutting diameter of 3.98cm). The granular structure in the surface layer with low bulk density tends to be compressed easily. There was no significant difference among the average bulk density readings of all three probes at 10-20 cm and 20-30 cm depth.

Table B-1: Average bulk density and coefficient of variation (CV) from 19 fields at various depths (0-10, 10-20, and 20-30cm) collected using each of the three probe types.

Depth cm	Probe Type†	Bulk Density g cm ⁻³	CV %
<u>0-10</u>	PP	1.46a‡	7.9a‡
	SH	1.32b	7.7a
	HP	1.33b	7.8a
<u>10-20</u>	PP	1.51a	7.0a
	SH	1.55a	4.8b
	HP	1.53a	6.1ab
<u>20-30</u>	PP	1.56a	6.5a
	SH	1.56a	5.3a
	HP	1.53b	5.2a

†PP, Push probe with cutting edge diameter of 2.26cm; SH, Slide Hammer probe with cutting edge diameter of 4.8cm; HP, Hydraulic probe with cutting edge diameter of 3.98cm.

‡Values within each depth with different lower case letters are significantly (p<0.05) different

Further, the average coefficient of variation (CV) in bulk density for each probe was not significantly different for the 0-10 or 20-30 cm soil layers. However, the average CV for PP was significantly higher than the SH at 10-20 cm depth, but not different from the HP.

Table B-2 shows that the average organic carbon concentration (OC) was not significantly different among the three probes at any depth. The average CV for OC at 0-10 cm depth for HP was significantly higher than average CV for PP. However, the CVs were not significantly different among the probes at the remaining depths.

Table B-2: Average organic carbon (OC) concentration and coefficient of variation (%) and organic carbon mass (OCM) and coefficient of variation (CV) from 19 fields at 0-10, 10-20, and 20-30 cm depths, collected with all three probe types using fixed depth method.

Depth cm	Probe†	OC g kg ⁻¹	Avg. CV of OC %	OCM Mg ha ⁻¹	Avg. CV of OCM %
<u>0-10</u>	PP	7.0a	13.1a	10.0a	13.2a
	SH	7.1a	15.1ab	9.2a	16.4a
	HP	7.0a	17.3b	9.2a	16.7a
<u>10-20</u>	PP	5.3a	14.9a	7.9a	16.8a
	SH	5.2a	12.3a	8.0a	13.5a
	HP	5.2a	14.9a	7.8a	16.8a
<u>20-30</u>	PP	5.2a	20.2a	7.8a	21.4a
	SH	5.1a	15.2a	7.9a	16.1a

HP	5.0a	17.0a	7.5a	17.7a
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†PP, Push probe with cutting edge diameter of 2.26cm; SH, Slide Hammer probe with cutting edge diameter of 4.8cm; HP, Hydraulic probe with cutting edge diameter of 3.98cm.

‡Values within each depth with different lower case letters are significantly ($p < 0.05$) different In parenthesis is the lsd value for corresponding variable and depth

Despite the differences in bulk density in the 0-10 cm depth, there was no significant difference in the OCM among probe types at this depth or the lower depths. Also, there was no difference in the average CV of organic carbon mass among probe types. This indicates that the probes could be used interchangeably to determine carbon mass on a fixed depth basis. However, inspection of the data shows that the PP estimated 10.0 Mg OC ha⁻¹ compared to 9.2 and 9.2 Mg ha⁻¹ for the SH and HP in the 0-10 cm depth (averaged across all fields). The difference between the PP and remaining probes is approximately 0.8 Mg OC ha⁻¹, respectively. Although not statistically significant, this difference is larger than the current estimated carbon sequestration rates (0.14-0.4 Mg OC ha⁻¹ year⁻¹) used to calculate carbon offsets for management of cropland in the area (OCC 2011). Therefore, this difference is important at a practical level, and effort should be made to minimize it. This error might be reduced if the initial carbon value was also determined using PP or if the error due to compression of the PP core can be removed from the analysis.

Table B-3 shows that the cumulative OCM measured in the 0-30 cm depth with the PP is significantly higher than the OCM calculated with HP to a fixed depth. In fact, the PP resulted in an OCM that is 1.2 Mg C ha⁻¹ (4.9%) larger than that measured with the HP. This shows that the elevated bulk densities (Table B-1) of the PP at surface 10 cm resulted in significantly higher average total OCM. No significant difference was observed in the average %CV for each probe.

Table B-3: Average organic carbon mass (OCM) as measured in 19 fields with three different probe types using fixed depth and fixed mass methods.

	Probe†	Average OCM Mg ha ⁻¹	CV %
<u>Fixed Depth</u>			
	PP	25.7a	8.9a
	SH	25.0ab	10.2a
	HP	24.5b	10.7a
<u>Fixed Mass</u>			
	PP	21.8a	8.4a
	SH	21.6a	9.8ab
	HP	21.3a	11.0b

†PP, Push probe with cutting edge diameter of 2.26cm; SH, Slide Hammer probe with cutting edge diameter of 4.8cm; HP, Hydraulic probe with cutting edge diameter of 3.98cm.

‡Values within each depth with different lower case letters are significantly ($p < 0.05$) different

Table B-3 also shows the average OCM measured to a fixed mass of 3690 Mg ha⁻¹ using three different probes in all 19 fields. There was no significant difference in average OCM calculated from different probes. In fact, the fixed mass method decreased the difference between the PP and HP to 0.5 Mg C ha⁻¹ or 2.2%. However, the coefficient of variation for the PP is significantly lower (Table B-3) than the coefficient of variation of the SH and HP. This result is in agreement with the study of Wuest (2009).

Wuest (2009) used square sampling probes of different cutting edge cross-sectional area for taking samples in tilled and compacted soils for water content measurement. Using the fixed depth method, different equipment gave different results, but the use of equivalent mass eliminated the difference, although equipment effect on bulk density was not reported.

Wilson et al. (2011) studied the variation in bulk density of a silt loam soil at different moisture levels (Appendix D in this protocol). They took samples three times in 18 days up to a depth of 90 cm. During the first sampling event, soil was in the driest condition, while it was wettest on second event and had intermediate moisture during the third sampling episode. No significant difference in mean bulk density was observed in the surface 30 cm, but the bulk density was lower in the driest condition as compared to wet conditions. This change in the bulk density gave a significant difference in the measurement of total carbon mass, when there should be a negligible change in the carbon mass during such a short time. However, the equivalent mass method gave no significant change in the total mass of carbon. This is consistent with the findings of the current study in that differences in measured organic carbon density resulting from differences in bulk density can be corrected regardless of the cause of the changes in bulk density. There are different ways of using fixed mass method. For example, Ellert et al. (2002) compared the fixed depth and the equivalent mass methods of measuring carbon on non-amended and coal-amended (serving as carbon sequestered) plots. The equivalent mass method recovered 91-106% of applied coal as compared to 62-84% when the fixed depth method was used. They chose the value of equivalent mass for each layer arbitrarily, and the authors reported that whatever value of equivalent mass is chosen does not have any effect on recovery of organic carbon.

On the other hand, Lee et al. (2009) adjusted the bulk density based on an assumption that tillage would consistently decrease the bulk density compared to a no-till system. They compared changes in soil carbon mass using adjusted bulk density under these systems with the equivalent mass of each layer adjusted to minimum, maximum, and original soil mass. The authors reported greater accuracy when using minimum soil mass, where the bulk density increases such as in no-till, and where the direction of change in bulk density is not known. The maximum mass was suitable for sites where bulk density was decreasing and the original mass accurately known.

As described in the materials and method section in our study, we have adjusted the soil mass of all cores to the minimum cumulative mass found among the cores/samples. Thus, there can be different ways of using the equivalent/fixed soil mass method. However, both the studies presented above and our study resulted in a reduction of error or uncertainties in calculation of OCM due to uncertainty in bulk density measurements.

Summary

This study shows that small diameter soil sampling probes can compress the surface layers of the soil, thus giving higher bulk density measurements. Differences in bulk density can impact soil organic carbon stocks when calculated with fixed depth methods. The fixed mass method eliminates the significant differences in carbon stocks due to difference in sampling probes. This is crucial where bulk density is involved in the calculation of soil properties, especially for monitoring temporal changes in soil organic carbon stocks, since data collection may be conducted by different people and with different tools at different times. Removal of the difference in carbon stocks due to bulk density error by fixed mass enables the use of inexpensive push probes in places where tractor mounted hydraulic probes cannot be used or where funding restricts its use.

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Appendix C: Influence of Inorganic Carbon Concentrations on Variability in Organic Carbon Concentrations

Objectives

The objective of this study was to evaluate the impact of inorganic carbon (carbonates) on the determination of organic carbon concentrations in soils.

Materials and Methods

Three soils (Woodward, Konawa, and Osage) containing a range of inorganic carbon and organic carbon concentrations were collected, dried at 65°C, and ground to pass a 2 mm sieve. These soils were spiked with Agricultural lime, charcoal, and peat (Table C-1). The lime was sieved to create three particle sizes of <0.05 mm, 0.05-0.5 mm and 0.5-2.0 mm to determine if the size of inorganic carbon particles influenced organic carbon analysis. Each amendment was added to each soil at 5% and 25% by weight. This resulted in 30 treatments which were replicated three times. Each replicate of the soil:amendment combination was mixed and sieved to homogenize the sample. Each treatment was then analyzed for total carbon using the dry combustion method (Kalembasa and Jenkinson, 1973) in a Leco CN analyzer and inorganic carbon using a Pressure Calcimeter (Sherrrod et al., 2002). The difference between these two analytical results was used as the organic carbon concentration.

Table C-1: The initial total C, inorganic C, and organic C concentrations found in soils and additives used in this study.

Material	Total C	Inorganic C	Organic C
	-----g kg ⁻¹ -----		
Woodward	49.3	38.2	11.2
Konawa	17.1	0.0	17.1
Osage	19.5	0.1	19.4
Lime (0.5-2.0 mm)	120.6	116.7	3.9
Lime (0.05-0.5 mm)	114.7	114.7	1.8
Lime (<0.05 mm)	118.6	108.7	9.9
Charcoal	471.2	12.6	458.6
Peat	441.8	0.0	441.9

Results Summary

Additions of lime, charcoal, and peat did not consistently influence the recovery of organic carbon in samples analyzed (Data not shown). However, the presence of inorganic carbon did influence the variability in organic carbon concentrations. Figure C-1 shows the change in the coefficient of variation in organic carbon concentrations as a function of the ratio of inorganic carbon to total carbon increases. Notice that when this ratio is in excess of 0.40, meaning that 40% of the total carbon is inorganic carbon, the coefficient of variation (CV) increase to above 10%.

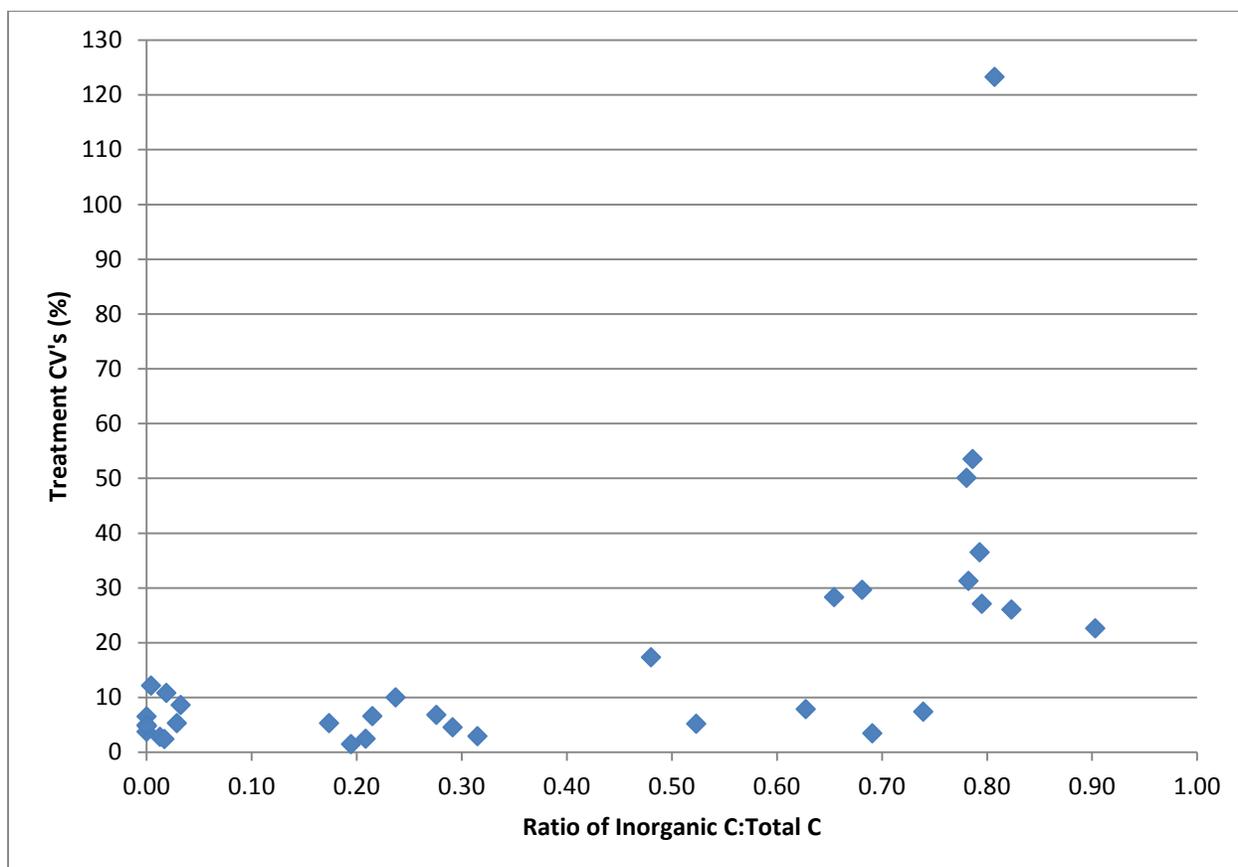


Figure C-1: The response of the coefficients of variation for the mean organic C concentrations in each treatment to the ratio of inorganic C to total C measured in those treatments.

This data show that when inorganic carbon represents 40% or more of the total carbon in a soil sample the variability will dramatically increase. This will require more analytical replicates to overcome this variability or the use of an alternative method for determining organic carbon should be used.

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Appendix D: Changes in Bulk Density and Carbon Stock Estimates in Shrink-Swell Soils

Objective

This study was conducted to determine if using the fixed mass method would improve the precision of soil C stock measurements in a high shrink/swell soil under variable soil moisture conditions. This research will be useful in determining if changing from the current fixed depth method to the fixed mass method is needed in order to monitor soil C stocks for the purpose of determining soil C sequestration rates in shrink/swell soils.

Materials & Methods

This experiment was located in Stillwater, OK, on a Kirkland silt loam (Fine, Mixed, Superactive, Thermic Udertic Paleustoll). This soil was chosen for its high clay content and corresponding shrink/swell that causes cracks in the soil for some time during most years. This soil was selected using the NRCS Soil Characterization database that provided bulk density data demonstrating that the bulk density, as determined using the clod method (Brasher et al., 1966; Blake and Hartge, 1986; Grossman and Reinsch, 2002), of this soil can change by as much as 30% between field capacity and permanent wilting point. This experimental location was planted to soybeans under conventional tillage. An area measuring five meters by seven meters was sectioned into 32 individual sample areas. The experimental area was sampled three times to provide three different soil moisture conditions. The sample areas were randomly assigned to a sample time (treatment) such that 10 cores would be collected during each treatment time. The remaining two sample areas would not be sampled unless an error was made in collecting from the other areas. Due to the short experimental period (two weeks), it is assumed that changes (decomposition/deposition) in organic C stocks would be minimal.

Soil samples were collected August 13th, 25th, and 30th of 2010 to capture various soil moisture conditions. Sampling dates were chosen to represent a range from very dry to moist soil conditions. August 13th was quite dry, between August 13th and 25th the location received approximately 5.7 cm of rainfall, and on the 24th the plots were irrigated with approximately 2.5 cm of water and sampled on the 25th. On the 28th, the plots were again irrigated with approximately 2.5 cm of water and sampled two days later. These sampling dates are referred to as T1 (13th), T2 (25th) and T3 (30th).

Soil samples were collected using a tractor-mounted hydraulic probe with a cutting diameter of 7.45 cm. The probe was pushed to a depth of approximately 125 cm. Soil from the bottom of some of the cores fell out as the core was extracted from the soil. Therefore, only 90 cm of soil was used for this analysis because this depth was consistently extracted throughout each sampling time. Each hole created after sampling was measured for depth. The depth of the hole created was then compared to the length of the core to gauge compression if any. The cores were placed in a cradle made from PVC pipe with a diameter of 10 cm and cut into 10 cm sections using a curved knife, such that soil was not lost from each section. Soil samples were then placed in a plastic bag and stored in an ice chest until they were delivered to a refrigerator for storage at 4°C. Each soil core section was initially weighed to determine bulk density. After the initial weight was determined, the sample was mixed and a subsample (20 g) was dried at 110°C to determine the moisture content. The bulk density was then adjusted to a dry weight basis. The remaining sample was transferred to a paper bag and placed in a greenhouse to air-dry. Each sample was then ground to pass through a 2 mm sieve. Each sample was analyzed for total C and N using a TrueSpec CN analyzer (LECO, Inc. St. Joseph, MI). Soil pH was determined on a 1:1, soil:

deionized H₂O mixture after a 30 minute equilibration period. Soil inorganic C was determined on soil samples with a pH > 7.0 using a pressure calcimeter method (Sherrod et al., 2002). Soil organic C was determined by the difference between total C and inorganic C.

In addition, the coefficient of linear extensibility (COLE) was determined on three randomly selected soil samples from each depth using the method of Schafer and Singer (1976).

Two methods were used to calculate C stocks for each sampling period. The first method is the commonly used spatial coordinate method. In this method, the sampling depth, z , is specified and therefore constant. The soil volume, V (m³), contains a dry mass, m_s (kg), and total mass, m_t (kg), including water. The C mass within the volume is

$$c_s = \frac{c_s}{m_s} \frac{m_s}{V} V = f_c \rho_b V, \quad (1)$$

where $f_c = c_s/m_s$ is the mass fraction of C within the total dry mass, and $\rho_b = \frac{m_s}{V}$ (kg m⁻³) is the mass concentration of the dry material (“dry bulk density” or “bulk density”). Since V equals area (A) times depth (z), soil C per unit area is

$$\frac{c_s}{A} = f_c \rho_b \frac{V}{A} = f_c \rho_b \frac{Az}{A} = f_c \rho_b z. \quad (2)$$

The second method used is the cumulative mass method proposed by Gifford and Roderick (2003) as an alternative to the spatial coordinate method. The cumulative mass method calculated C stocks found in a constant mass of soil instead of a constant depth and therefore may reduce errors associated with changes in bulk density resulting from shrink swell. In this method, depth varies so that each sample contains the same dry mass per unit area (m_s/A). In Eq. 2, $\rho_b z$ is equivalent to the dry soil mass per unit area. Therefore, in the cumulative mass method, as ρ_b increases, the sampling depth (z) is reduced, thereby maintaining the product of the two terms as a constant.

In order to find the cumulative mass of soil C, Gifford and Roderick (2003) use linear interpolation to allow for variation with depth in both the mass fraction of C and bulk density. This is accomplished by dividing the core into two sections; for example, a core taken to 40 cm would be divided into one section of 0-20 cm and another of 20-40 cm. The total length of the core is represented by z_b and the surface subsection is represented by z_a with the cumulative dry soil masses to the respective depths denoted by $m_s(z_b)$ and $m_s(z_a)$ and the cumulative mass of soil C, $c_s(z_b)$ and $c_s(z_a)$. The target or “fixed” cumulative mass of dry soil is denoted by $m_s(t)$ and the corresponding cumulative mass of soil C that we are looking for is denoted as $c_s(t)$. Through linear interpolation, the resulting equation is

$$c_s(t) = c_s(z_a) + \frac{c_s(z_b) - c_s(z_a)}{m_s(z_b) - m_s(z_a)} (m_s(t) - m_s(z_a)). \quad (3)$$

Analysis of variance and contrast analysis were performed using the SAS PROC GLM procedure (SAS Institute, 2001), to determine significant treatment affects on measured response variables.

Results and Discussion

COLE values increased with depth (Figure D-1). The COLE values of 0.10 or greater found below 20 cm in this profile indicate very high potential for shrink-swell in a soil. The values obtained for this sample site meet the criteria for a Vertic suborder classification as defined by the Soil Survey Staff (2010).

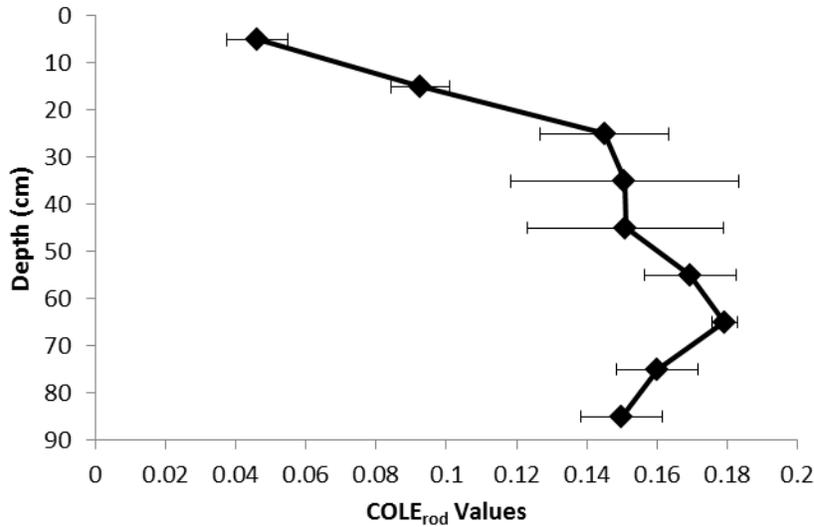


Figure D-1: The average COLE_{rod} values for 3 randomly selected samples from each depth.

Soil Moisture. Analysis of variance found that all sample dates had significantly different soil moisture at the 0-10 cm increment, with T1 being the driest at 0.08 g g⁻¹ moisture, T3 being the intermediate moisture level at 0.15 g g⁻¹ and T2 being the wettest with 0.19 g g⁻¹ soil moisture. Analysis of the 10-20 cm, 30-40 cm, and 60-70 cm increments all revealed T1 to be significantly drier than T2. The T3 soil was not significantly different from either T1 or T2 at any of these increments. No significant differences were found at depth increments below 70 cm. (Figure D-2).

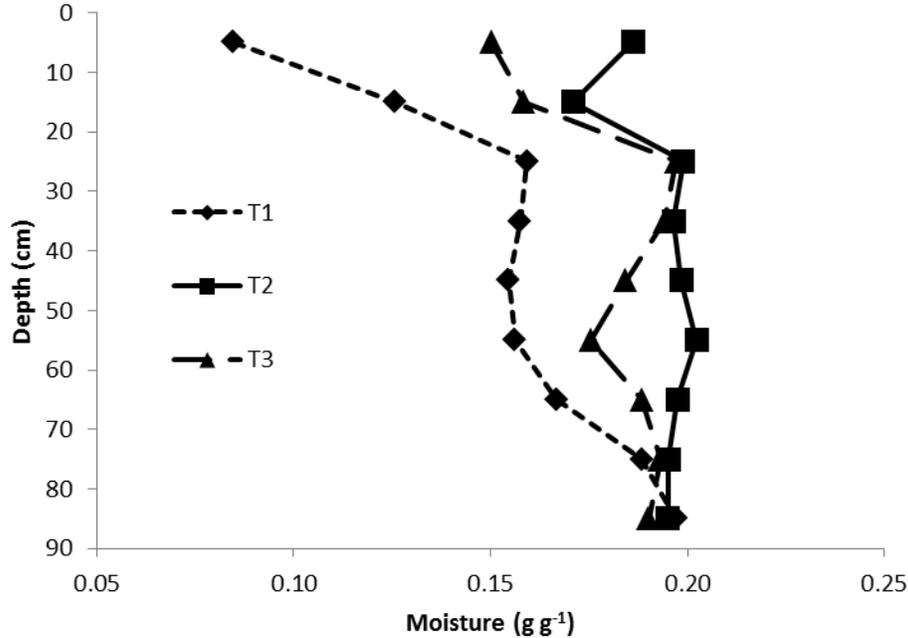


Figure D-2: Soil moisture as measured at sample date T1, T2 and T3. Values right of data points are least significant differences (LSD) at the 0.05 probability level. Data points without LSD values were not significantly different.

Soil Bulk Density. Analysis of variance showed no significant differences in mean bulk density for the surface 30 cm. At 30-40 cm, T1 was found to have a significantly lower bulk density of 1.35 g cm^{-3} than either T2 or T3 with bulk densities of 1.49 and 1.50 g cm^{-3} , respectively. From 40-60 cm, no significant differences were found. At 60-70 cm, the bulk density of T1 was again significantly lower at 1.46 g cm^{-3} compared to the T2 and T3 dates which both had bulk densities of 1.62 g cm^{-3} . The bulk density of T1 was significantly higher than the remaining sampling dates in the 70-80 cm increment. No significant differences were found at the 80-90 cm depth. (Figure D-3)

The differences in soil moisture help to explain the differences found in the bulk densities. The T1 soil profile was generally drier than the T2 and T3 profiles. At depths where significantly different bulk densities were observed, the T1 soils had lower bulk densities except for at 70-80 cm where it had greater bulk density. This is contrary to the hypothesis that shrinkage of soils upon drying would result in an increase in bulk density in these soils with apparent shrink/swell capacity as indicated by measured COLE values. Recall that the bulk density as measured by the clod method does increase with decreasing soil moisture because the individual clods shrink upon drying. However, it appears that the differences in bulk density found at 30-40 and 60-70 cm resulted from compression of the T2 and T3 cores.

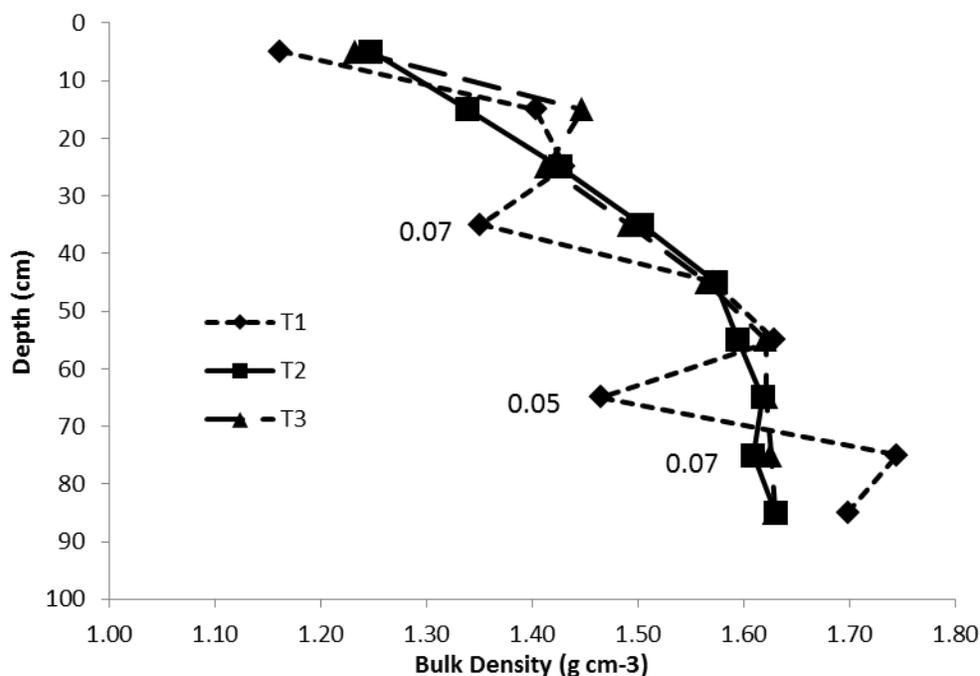


Figure D-3: Soil bulk density as measured at sample date T1, T2 and T3. Values left of data points are least significant differences (LSD) at the 0.05 probability level. Data points without LSD values were not significantly different.

In order to assess compression, core lengths and hole depths were measured. Table D-1 shows the average measured whole core lengths and the average depth of holes created during sampling. Notice that whole core lengths were approximately 1 cm longer than the depth of holes for T1 and T2 and that the core length was approximately equal to hole depth for T3.

Table D-1: The date of sample collection, the average measure whole length of soil cores extracted and the average depth of holes after core extraction.

Sample Date	Sample Date ID	Core Length	Hole Depth
8/13/2010	T1	104.8	103.7
8/25/2010	T2	105.1	104.1
8/30/2010	T3	122.0	122.2

Recall that during the sampling process the probe was pushed to an approximate depth of 125 cm. At T1 and T2 portions of the core fell out of the probe tube before the tube could be lifted from the hole. This did not apparently occur at T3. Therefore, it appears that the section of soil falling back down the hole did not set firmly back from where it came, which explains the fact that, on average, the hole depth is shallower than the length of core for T1 and T2. This illustrates the difficulty in estimating small amounts of compression by measuring core length and hole depth. In fact, when the compression of the T2 and T3 cores is calculated from the average bulk density values measured to 90 cm, it is found that equivalent mass of soil in T2 and T3 would be 0.57 and 1.27 cm shorter than T1, respectively. Wuest (2009) states that the same soil sampled when bulk density is higher will remove more soil than when

the soil has a lower bulk density. In other words if the soil is measured to specific depth more soil will be removed. However, if a soil could be measured to a specific mass, a shorter core would be extracted. The realities of core extraction prevent full explanation of the mechanisms by which bulk densities varied between sampling times; however, the data does allow for an assessment of the impact of this variable bulk density on carbon concentration and carbon mass measurements.

Carbon Concentration. Analysis of variance found no significant differences in total C, soil inorganic C, or soil organic C between the treatments. Figure D-4 shows that C concentrations generally decreased with depth and given the short experimental period, one would not expect to see significant differences between the sampling dates.

Carbon Stocks. Analysis of variance of the mean C stocks in each depth increment showed that at 30-40 cm the C stocks in the T1 samples were 11.9 Mg C ha⁻¹, which was significantly lower than the 12.8 and 13.3 Mg ha⁻¹ found at this depth in T2 and T3 respectively. At the 60-70 cm increment the T1 samples contained 12.6 Mg C ha⁻¹, which was significantly lower than 13.9 Mg C ha⁻¹ found in T3 but not different from the 13.3 Mg C ha⁻¹ found in T2 (Figure D-5). Despite these significant differences found at each depth increment, no significant differences were found in the cumulative C stocks when calculated on a fixed depth basis (Table D-2).

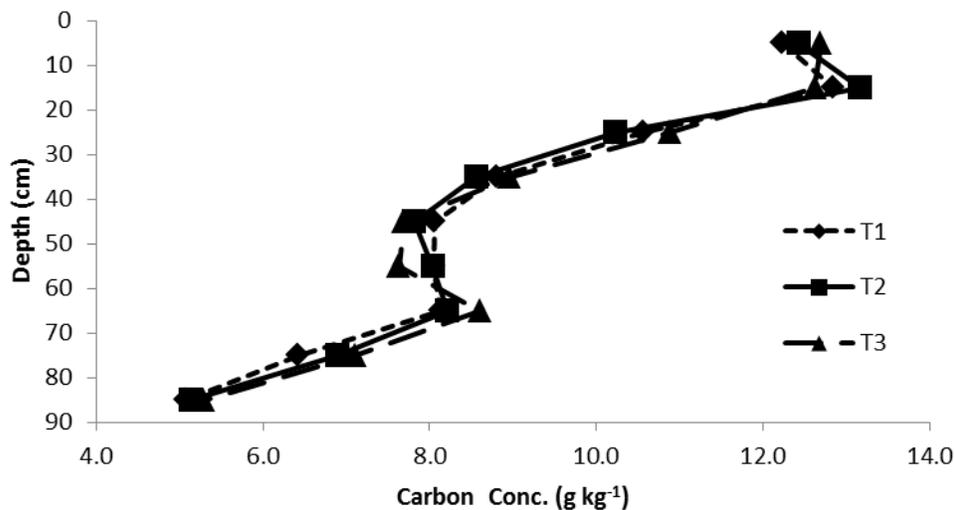


Figure D-4: Organic carbon concentrations as measured at sample date T1, T2 and T3. Values left of data points are least significant differences (LSD) at the 0.05 probability level. Data points without LSD values were not significantly different.

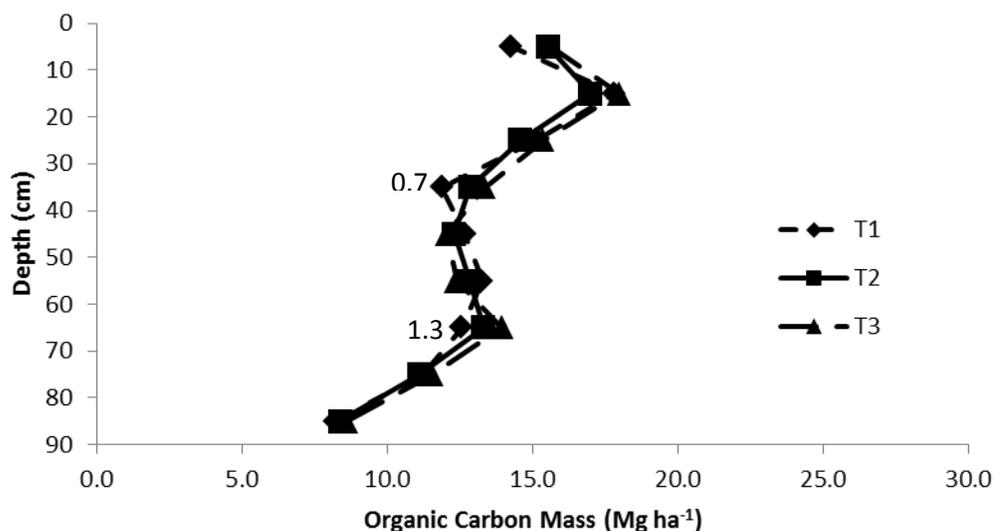


Figure D-5: Organic carbon mass in each depth increment as measured at sample dates T1, T2 and T3. Values left of data points are least significant differences (LSD) at the 0.05 probability level. Data points without LSD values were not significantly different.

Calculating the soil C stocks on a fixed mass basis, Table 15 shows the C stocks found in a range of soil masses corresponding to depth increments from 16-90 cm. Here again there were no differences among the three sampling dates. However, the absolute differences in C stocks when calculated using the fixed depth method (Table D-2) are greater than the absolute differences when C stocks are calculated using the fixed mass method (Table D-3).

Table D-2: The cumulative carbon stocks as measured to each depth on a fixed depth basis for each sample date (T1, T2, and T3) and the maximum difference among sampling dates.

Depth Cm	T1	T2	T3	LSD [†]	Max Difference
	-----Mg ha ⁻¹ -----				
10	14.2	15.5	15.6	ns	1.4
20	32.0	32.5	33.6	ns	1.5
30	47.1	47.0	48.9	ns	1.9
40	58.9	59.9	62.2	ns	3.3
50	71.6	72.7	74.3	ns	2.7
60	85.7	85.5	86.7	ns	1.1
70	98.7	98.8	100.6	ns	1.9
80	109.5	109.9	112.1	ns	2.6
90	117.7	118.3	121.2	ns	3.6

[†]LSD, Least significant difference at the 0.05 probability level.

Table D-3: The cumulative carbon stocks as measured in each increment of soil mass on a fixed mass basis for each sample date (T1, T2, and T3) and the maximum difference among sampling dates.

Fixed Mass Mg	Estimated Depth T1†	Estimated Depth T2	Estimated Depth T3	T 1	T 2	T 3	LSD‡	Max. Difference
	-----cm-----			-----Mg ha ⁻¹ -----				
2000	16	16	16	25.0	25.5	25.2	ns	0.5
3000	23	23	23	36.9	36.8	37.1	ns	0.3
4000	29	29	29	47.5	46.8	47.9	ns	1.1
5000	36	36	36	56.2	55.6	57.1	ns	1.5
6000	43	42	42	64.5	64.4	65.5	ns	1.1
7000	50	49	49	73.4	72.1	73.2	ns	1.3
8000	56	55	55	81.3	80.1	80.8	ns	1.2
9000	63	61	61	89.5	88.0	88.7	ns	1.5
10000	70	68	68	97.6	96.2	97.3	ns	1.4
11000	76	74	74	104.4	103.7	105.0	ns	1.3
12000	83	81	81	110.3	110.2	111.9	ns	1.7
13000	90	87	87	115.5	115.6	117.9	ns	2.4

†The depth was estimated from the relationship between cumulative mass and depth (Figure D-6).

‡ LSD, Least significant difference at the 0.05 probability level.

The fixed mass method removed error associated with the significantly different bulk densities found at 30-40 and 60-80 cm. The remaining variability could be due to spatial variability or analytical variability in the C analysis. In fact, when the fixed mass method was used to calculate C stocks, the largest difference between sample dates was 2.6% found in the surface 5000 Mg of soil (Table D-3). In contrast, when the fixed depth method was used, the maximum difference observed in the 0-10cm depth was 9.3 % of the average C stock found in this depth. The difference observed at 0-40 cm was 5.5 % of the average C stock (Table D-2). This analysis is consistent with the findings of Ellert and Bettany (1995) who stated that use of the fixed mass method eliminates sensitivity to bulk density.

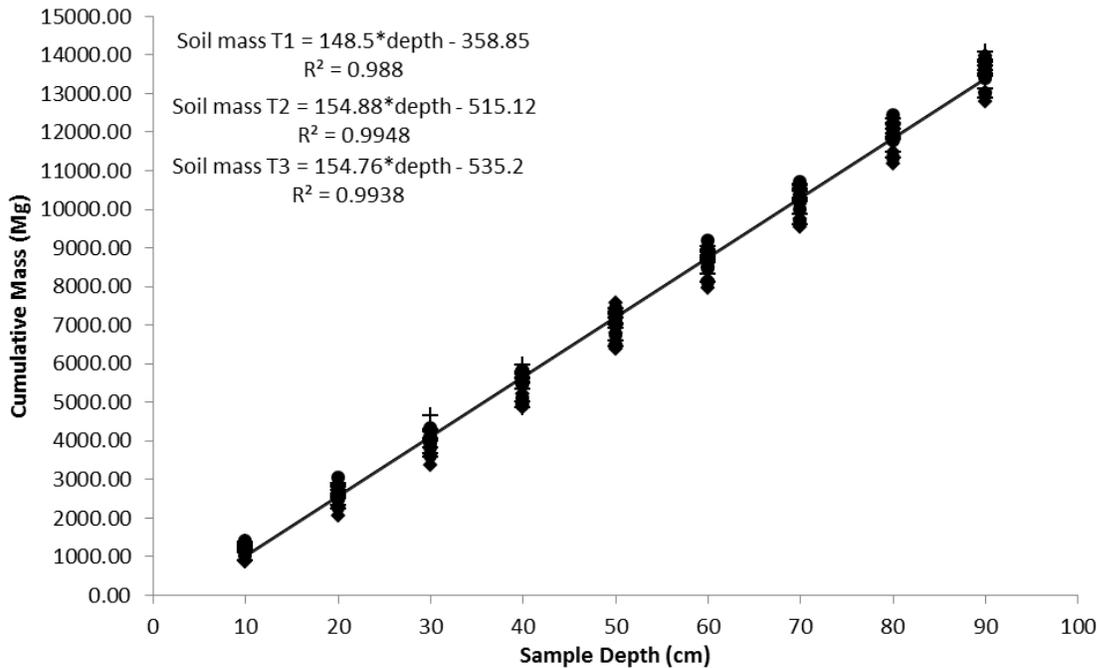


Figure D-6: Relationship between cumulative mass of soil and sampling depth, data includes all sample dates.

Figure D-7 shows the C mass in each depth increment normalized based on equivalent mass of soil. Notice that significant differences found in Figure 9 at 30-40 and 60-70 cm are eliminated when equivalent mass is used to calculate C stocks within each soil layer. This supports the findings of previous research (Gifford and Roderick, 2003; VandenBygaart and Angers, 2005) that small differences in bulk density can change how much C mass is reported. If scientists are to understand global climate change, then accurate and standardized reporting of soil C stocks is essential. Currently, the Oklahoma Carbon Program estimates C sequestration to be $0.3 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ after conversion to no-till in Western OK. This data demonstrates that the magnitude of error that can occur when monitoring C is quite large compared to the potential average annual changes. Using the fixed mass method to calculate C can at least reduce variability associated with changes in measured bulk densities.

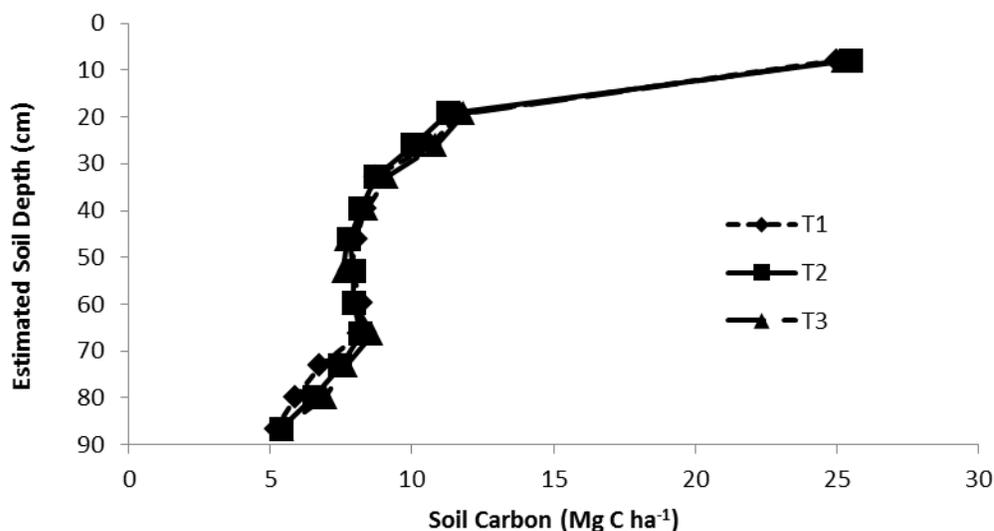


Figure D-7: Organic carbon mass in each estimated depth increment (increments were created from relationship between cumulative mass and soil depth in Figure D-6) as measured at sample date T1, T2 and T3. Data points without LSD values were not significantly different at the 0.05 probability level.

Therefore, it may be a more appropriate method than the current fixed depth method suggested by the IPCC, particularly for monitoring C changes over time or when data from different sources or methods is to be compared.

Conclusions

Soil bulk density did not increase with decreasing soil moisture as was expected in this high shrink/swell soil. Alternatively, under moist soil conditions, discrete depth increments were susceptible to compaction during sampling, presumably because internal structure was compressed in these depth increments. This compaction did result in significant difference in soil C stocks at these depth increments. Because there were only two weeks between sampling dates in this experiment, these changes in C stocks must be attributed to error imposed by the compression of these soil layers while moist. The fixed mass method removed these errors and provided a more precise estimate of soil C stocks.

Methods of analysis, while generally standardized, can still have a huge impact on soil C measurements. The method tested here, the fixed mass method, as proposed by Gifford and Roderick (2003), allows for correction of biases imposed by differences in sampling equipment and sampling conditions that result in different measured bulk densities. This may allow for a broader basis for comparisons of soil C measurements between sites, conditions, times, and researchers.

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Appendix E: Soil Sample Analysis

E.1 Soil Analysis

Ziplock bags with wet soil are weighed, and a subsample (~20 g) is weighed into a weighing tin. This subsample is dried at 110°C for 24 hours and then weighed to determine moisture content. The soil remaining in the ziplock bag is transferred to a paper bag and allowed to dry at 65°C for 24 hours and then ground and sieved through a 2 mm sieve. Moisture content is used to determine dry soil mass in the ziplock bag and further to determine bulk density. The sieved soil is analyzed for total carbon using the dry combustion method (Kalembasa and Jenkinson, 1973) in a Leco CN analyzer after weighing 0.2400-0.2500 g into a tin foil cup. Inorganic carbon is determined using a Pressure Calcimeter (Sherrod et al., 2002) for samples with pH greater than 7.2. Soil pH was determined on a 1:1, soil:deionized H₂O mixture after a 30-minute equilibration period. The organic carbon concentration (OCC) of samples is calculated as the difference between the total carbon and inorganic carbon.

Note: The presence of inorganic carbon can cause variability in organic carbon concentration to increase. In fact, laboratory analysis of samples with 0 to 90% of the total carbon as inorganic carbon showed that the coefficient of variation increases above 20% when the inorganic carbon is greater than 40% of the total carbon (Appendix C). Therefore, laboratory replicates will be required for soils in which the inorganic carbon is greater than 40% of the total carbon in order to reduce the variability in organic carbon analysis.

E.2 Calculating Organic Carbon Mass

Traditionally the OCM in a unit area of soils is calculated using a fixed depth approach. Using this approach, the OCC is multiplied by the bulk density (Bd) and divided by the sample depth:

$$\text{OCM} = \text{OCC} * \text{Bd} / \text{depth}$$

However, measurements of bulk density are highly variable and can be influenced by probe diameter (Appendix B) and soil moisture (Appendix D). Therefore, the fixed mass approach to calculating OCM will be used in this protocol. This approach decreases error associated with differences in bulk density measurements, thereby reducing variability in OCM measurements (Appendices B and D).

The fixed mass approach to calculating OCM was adopted from Gifford and Roderick (2003). Specifically, the total length of the core was represented by Z_b and the surface subsection is represented by Z_a with the cumulative dry soil masses to the respective depths denoted by $m_s(z_b)$ and $m_s(z_a)$ and the cumulative mass of soil C, $c_s(z_b)$ and $c_s(z_a)$. The target or “fixed” cumulative mass of dry soil is denoted by $m_s(t)$ and the corresponding cumulative mass of soil C that we are looking for is denoted as $c_s(t)$. Through linear interpolation, the resulting equation is

$$c_s(t) = c_s(z_a) + \frac{c_s(z_b) - c_s(z_a)}{m_s(z_b) - m_s(z_a)} (m_s(t) - m_s(z_a)).$$

The soil mass up to 12 inch depth for each core is calculated. The minimum soil mass among the cores collected during the method development was selected as the fixed mass which was ~9118 Mg acre⁻¹ this is equal to a bulk density of 1.23 g cm⁻³ in the surface 12 inches of soil. The soil mass of 0-8 inch depth of every core is subtracted from this fixed mass. The resulting mass of soils is multiplied by the concentration of carbon found in the 8-12 segment of the core. This value is then added to the mass of carbon in the 0-4 and 4-8 inch depths, which is simply calculated by multiplying the mass in each depth by the OCC found in each depth.

Note: The fixed mass of soil was set equal to the minimum soil mass among the cores collected during the method development because this allows all cores to be included in the analysis while maximizing the fixed mass value. This mass may change as an increasing number of cores are added to the database. For example, if a core with a bulk density less than the lightest soil currently in the database is collected, its mass per unit area will then be used as the fixed mass.

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Abbreviations

OCM, the organic carbon mass in a unit area of soil to a specific fixed depth or fixed mass of soil

OCC, the concentration or organic carbon in a soil

Appendix F: Soil Sample Collection Form

Soil Sample Collection

010101

Producer Name: _____

Producer #: 01

Date: ___/___/_____

Field Identification #: 0101

Sample location #: 01

Sampling Location: Longitude, Latitude: _____

Sample ID	Depth (inches)	Time Collected	Notes
0101010104	0-4		
0101010108	4-8		
0101010112	8-12		
0101010204	0-4		
0101010208	4-8		
0101010212	8-12		
0101010304	0-4		
0101010308	4-8		
0101010312	8-12		
0101010404	0-4		
0101010408	4-8		
0101010412	8-12		
0101010504	0-4		
0101010508	4-8		
0101010512	8-12		
0101010604	0-4		
0101010608	4-8		
0101010612	8-12		
0101010704	0-4		
0101010708	4-8		
0101010712	8-12		
0101010804	0-4		
0101010808	4-8		
0101010812	8-12		
0101010904	0-4		
0101010908	4-8		
0101010912	8-12		
0101011004	0-4		
0101011008	4-8		
0101011012	8-12		

0101010104

