Quantifying the soil organic carbon sequestration performance and carbon emissions offset potential of the City of Calgary’s *Willow Biomass and Marginal Land Reclamation Demonstration Project*

by

Chelsey Greene

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

The primary objective of this study was to measure the soil organic carbon (SOC) sequestration performance of *The City of Calgary Dewatered Biosolids Land Application Program – Willow Biomass and Marginal Land Reclamation Demonstration Project* (hereafter, "the demonstration Project") after five years of operation. The second objective was to assess the demonstration Project's potential to earn soil-based carbon offset revenue through the Alberta Emissions Offset System in the future. To accomplish the first objective, SOC stocks were measured at three sampling locations subject to different combinations of recommended management practices (RMP) for SOC sequestration by the 21st Conference of the Parties to the United Nations Framework Convention on Climate Change (COP21). The agricultural Crops + No Biosolids (Control), agricultural Crops + Biosolids (C+BS), and Willows + Biosolids (W+BS) sampling locations were subject to zero, one (organic amendments), and three (no-till, agroforestry, and organic amendments) of the COP21 RMPs respectively. When comparing SOC stocks between the Control and RMP treatment sampling locations the results were not consistent - which aligns with expectations of this study. At the 0-15 cm differences in SOC stocks between sampling locations at the 0-15 cm depth interval was not significant. In contrast, the differences in SOC stocks between the W+BS and Control sampling locations at the 15-30 cm depth interval was larger (4.5 ± 1.6 Mg C ha^{-1}) and significant. A post hoc pairwise comparison (Games-Howell test) analysis indicated that the SOC stocks at the 15-30 cm depth interval of the W+BS sampling location were significantly higher than both the C+BS and the Control sampling locations. When converting the difference in SOC stock between the W+BS and Control sampling locations at the 15-30 cm depth interval to a carbon offset equivalent, the estimated carbon offset performance of the demonstration Project within the first five years of operation was 16.5 ± 5.9 Mg CO_2e ha^{-1}. The expected (and observed) inconsistency of between the 0-15 cm sampling depth and the 15-30 cm sampling depth intervals aligned with the results of peer literature for similarly designed SOC monitoring studies at local and global scales. This is because the dynamics of SOC flux rates are influenced by numerous heterogeneous limiting (e.g. climate, and vegetation community), determining (e.g. clay content, pH, and mineral content), and reducing factors (e.g. microbial community) that shift across time, lateral distance, and depth. Therefore, differences in land management study outcomes are expected when experimental designs do not control for all the same variables. To accomplish the second objective, the knowledge gained from this study was used to develop recommendations for approaching future SOC stock monitoring studies of Willow + Biosolids systems more strategically by (1) providing guidance on conducting baseline studies to screen and compare proposed SOC sink project sites for their SOC sequestration potential, and (2) proposing more cost effective and statistically powerful study designs. This study has demonstrated that it is possible to verify measurable changes in SOC stocks at Willow + Biosolids project sites, and that SOC stocks at the demonstration Project site are likely to continue increasing and eventually plateau within the next 15 to 20 years. To move forward with developing a custom quantification protocol and gain approval within the Alberta Emissions Offset System, further research and documentation efforts are required to address the remaining key issues associated with biogenic based carbon offset protocols including; additionality, permanence, transparency, and leakage.

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Terms	
%	Percent
°C	Degrees Celsius
Al_3^+	Aluminum
C	Carbon
Ca^{2+}	Calcium ion
$CaCO_3$	Calcium carbonate
CH_4	Methane
cm	Centimeter
cm^3	Cubic centimeter
CO_2	Carbon dioxide
CO_2e	Carbon offset equivalent
Pg	Pentagram (Gigaton)
g	Gram
H^+	Hydrogen ion
ha	Hectare
K^+	Potassium ion
kg/L	Kilograms per liter
m^2	Meters squared
Mg	Metric ton
Mg^{2+}	Magnesium ion
mm	Millimeters
mS/cm	Millisiemens per centimeter
Na^+	Sodium
ppm	Parts per million

Abbreviations	
AFOLU	Agriculture, Forestry and Other Land Use
ANOVA	Analysis of variance
C+BS	Crops + Biosolids sampling location
COP21	21st Conference of the Parties to the United Nations Framework Convention on Climate Change
CEC	Cation exchange capacity
CV	Coefficient of variance
df	Degrees of freedom
F	F statistic
GHG	Greenhouse gas
IPCC	International Panel on Climate Change
ISO	International Standards Organization
MDD	Minimum detectible difference
MID	Mid-infrared spectroscopy
NA	Not available
NIR	Near-infrared spectroscopy
p	Probability
RMP	Recommended management practice
SIC	Soil inorganic carbon
SOC	Soil organic carbon
SPI	Standardized precipitation index
TC	Total carbon
SRWC	Short Rotation Woody Crop
UNEP	United Nations Environment Programme
W+BS	Willow + Biosolids sampling location
WBCSD	World Business Council for Sustainable Development
SEM	Standard error of the mean
SYLVIS	SYLVIS Environmental

Section 1—Introduction

Chapter 1

Background & Investigative Context

Over the last three decades, the rapid pace and increased geographical extent of anthropogenic climate change instigated several international climate change mitigation meetings and anthropogenic greenhouse gas (GHG) reduction commitments such as the Rio Earth Summit (1992), Kyoto Protocol (1997), the Copenhagen Accord (2009), the Cancun Pledge (2010), and the Paris Agreement (2015). The IPCC (2018 pg. 6), reports “high confidence” (high scientific community agreement and robust evidence from multiple independent studies) that anthropogenic activities are responsible for causing a 1.0°C (+/-0.2°C) increase in average global temperatures since pre-industrial times (1850–1900). If global GHG emissions continue at their current rate, scientists estimate that average global temperatures will rise to 1.5°C above pre-industrial times somewhere between 2030 and 2052. In an attempt to avoid the anticipated catastrophic environmental consequences associated with global temperatures exceeding 1.5°C above pre-industrial levels, the scientific community has developed a global carbon emission budgets to define the limits of allowable annual GHG emissions to maintain average global temperatures (UNEP, 2018 pg. XVIII). To date climate change mitigation commitments and efforts have not curtailed anthropogenic GHG emission rates (IPPC, 2018 pg. 57). In fact, annual global GHG emissions have continued to increase since 1997 (IPCC, 2018 pg. 57). Although low-carbon technology and policy strategies are readily available to achieve carbon emissions targets, lack of political will has continued to inhibit progress (IPCC, 2018 pg. 93). For example, as of 2017, the United States government announced its intent to withdraw from the Paris Agreement (UNEP, 2018 pg. 15). Similarly, in Canada, recent changes in provincial leadership has led to announcements from the Ontario Government (2018) to scale back its climate change targets (Hansard Reporting and Interpretation Services, 2018) and the Alberta Government (2019) to repeal its carbon tax.

In response to political barriers, international organizations have re-focused their recommendations, guidelines, and strategies to better incentivize sub-state global climate change mitigation action and provide more evidence supporting the synergistic benefits (e.g. biodiversity, food security, and desertification alleviation) of investment in low-carbon economies. One strategy has been to incorporate climate change mitigation discussions, research, and programs within the broader socio-ecological initiatives like the United Nations Sustainable Development Goals (Raul

Ponce-Hernandez & Antoine, 2000). By designing sustainable policies and practices aimed at generating multiple co-benefits across a broad spectrum of socio-economic and environmental goals, more opportunities can be identified to incentivize the behaviors and innovations needed to satisfy multiple societal objectives (i.e. climate change mitigation and sustaining economic growth) (IPCC, 2018 pg. 70-73).

For example, several internationally acclaimed organizations and scholars argue that the use of market-based compliance tools such as carbon pricing in the form of carbon taxes, cap-and-trade systems, or a combination of the two, are one of the most efficient and cost effective mechanisms for incentivizing climate change mitigation behavior and technological innovation (Bowen, 2011; Government of Alberta, 2018b pg. 8; WBCSD, 2017; IPCC, 2014 pg. 466, 1159). On January 17, 2019, the largest public statement of economists in history was released when 3508 United States based economists, 27 Nobel laureates, all four of the former chairs of the Federal Reserve, two former secretaries of the USA department of treasury, and 15 former chairs of the council of economic advisers signed a letter published in the Wall Street Journal titled "*Economists' Statement on Carbon Dividends*". This letter stated that "Global climate change is a serious problem" and called for immediate government action via five policy recommendations supporting carbon taxation.

By enacting carbon cap-and-trade legislation in 2007, the Government of Alberta was one of the first jurisdictions in North America to implement climate change mitigation policies (Government of Alberta, 2007). This policy signaled Alberta's commitment to climate change mitigation and established the regulatory foundation complementary to the carbon levy introduced under the *Carbon Competitiveness Incentive Regulation* in 2017 (Government of Alberta, 2018a). In between these policy developments, the Government of Alberta deployed the Alberta Carbon Offset System. The Carbon Offset System complements carbon tax policies by providing a mechanism for industry to earn revenue via carbon offset credits through application of GHG emissions reducing practices and technologies.

Most of the Alberta Carbon Offset System protocols are targeted towards agricultural management practices. Agriculture plays an extensive role in climate change mitigation because it has the capacity to reduce net GHG concentrations through improving operation efficiencies and sequestering atmospheric carbon dioxide (CO_2) into biomass and soil sinks (IPCC, 2014 pg. 24, 816). Utilizing treated domestic wastewater residuals as fertilizer for short rotation woody crop (SRWC) plantations (e.g. Willow (*Salix spp.*)) located on marginal (i.e. poor fertility) agricultural land is one emerging agricultural practice that is generating interest for its sustainable wastewater management merits and carbon offset credit potential. Since 2010, five of these integrated municipal waste residual and SRWC demonstration projects have been installed across Alberta (Keoma, Beaverlodge, Clairmont, Camrose, Ryley, Ohaton) (AROWRN, 2019). These green infrastructure projects represent the types of synergistic sustainable water management, soil restorative, and renewable energy system practises endorsed by IPCC (2018 pg. 316) which will be needed to build resilience, enhance adaptability, and mitigate future climate change conditions.

For industry and municipalities to continue investing into sustainable development and climate change mitigation projects they will require consistent signals from all levels of government indicating that climate change mitigation will remain a long-term commitment regardless of the short-term vagaries the political parties that currently hold power. Future decisions and discussions about climate change action should be evidence based rather than driven by populist political opinions.

1.1 Knowledge Gaps & Rationale

Although numerous studies have demonstrated that willow plantations have substantial carbon offset potential via biofuels production (Amichev, Kurz, Smyth, & Van Rees, 2012; McClean et al., 2015; Pacaldo, Volk, & Briggs, 2013a) the soil-based carbon offset potential of these bio-sequestration systems has been largely unaccounted for (Hu, Zeng, Ma, & Chang, 2016; Lockwell, Guidi, & Labrecque, 2012; Paustian et al., 2016). Many studies have documented the soil fertility, biomass productivity, and net GHG emission reduction benefits of substituting biosolids land application for petroleum-based fertilizers, yet limited research has been conducted to quantify the effects that biosolids have on soil carbon sequestration rates (Brown, Kurtz, Bary, & Cogger, 2011; Torri, Corrêa, & Renella, 2014). The lack of soil organic carbon (SOC) research within willow plantation systems is likely related to the complexity of SOC sequestration processes and cost of measuring and verifying carbon stock relative to biomass monitoring costs.

There do not appear to be any peer reviewed studies that have investigated the influence of combining biosolids land-spreading application and willow plantation land management treatments at marginal agricultural lands on SOC stocks and sequestration rates. To highlight the soil carbon offset potential of willow plantations and dewatered biosolids land-spreading management practices, Table 1 includes estimated SOC sequestration rates and associated carbon offset values for three land management practice scenarios; tillage, fertilizer, and crop selection practices for projects located in the dry prairie region of Alberta. These estimated net SOC sequestration rates and carbon offset values were calculated by summing the reported annual SOC emissions and sequestration rates for each land management practice combination and multiplying it by the regions SOC reserve discount factor set by the *Alberta Conservation Cropping Carbon Offset Protocol* and the carbon offset values for 2017 and 2022. One limitation of using this approach is that potential SOC stock amplification or suppression interactions between land management practices were not accounted for. This research is intended to provide more insight into the SOC sequestration performance potential of integrated Willow + Biosolids systems.

Table 1: Estimated average net emission reduction rates of carbon dioxide equivalents (CO_2e) ha^{-1} yr^{-1}) and associated carbon offset values for three different land management practice combinations

Land Management Practices	Full & Reduced-Till Chemical Fertilizer Small Grain Agricultural Crops	Full & Reduced-Till Biosolids Fertilizer Small Grain Agricultural Crops	No-Till Biosolids Fertilizer Willow
Study Treatment Name	Control	Crops + Biosolids	Willows + Biosolids
Full & Reduced Till Agriculture [a]	-0.14	-0.14	NA
Chemical Fertilizers [a]	-0.061	0	0
Biosolids Fertilizers [b]	0	6.33	6.33
Willow Plantation [c]	0	0.00	5.97
Net Rate (CO_2 e ha^{-1} yr^{-1})	-0.20	6.19	12.30
Sequestered SOC Reserve Discount Factor for Dry Prairie Region = 0.92 [a]	-0.18	5.70	11.31
Carbon Offset Credits Value at $30/Mg CO_2 e ha^{-1} yr^{-1}	$(5.55)	$170.89	$339.41
Carbon Offset Credits Value at $50/Mg CO_2 e ha^{-1} yr^{-1}	$(9.25)	$284.82	$565.69

References: [a] Government of Alberta (2012) Conservation Cropping Carbon Offset Protocols, [b] Tian et al. (2009), [c] Hansen (1993), NA = not applicable

Note: Carbon offset accounting practices consider full till (>2 till passes) and reduced till (1-2 till passes) to be equivalent (Government of Alberta, 2012). Carbon dioxide equivalents (CO2e) are a standard unit for quantifying GHG global warming impacts.

1.2 Research Goals and Objectives

The objectives of this study were to (1) determine if measurable differences in SOC stocks can be detected within the topsoil (0-15 cm and 15-30 cm depth intervals) between the Control and the two treatment sites, agricultural Crops + Biosolids and Willow + Biosolids at *The City of Calgary Dewatered Biosolids Land Application Program – Willow Biomass and Marginal Land Reclamation Demonstration Project site* (the demonstration Project) after five years of implementation, and (2) compile the background research and study results data to initiate the Government of Alberta process for developing a cost effective, technically sound, and statistically robust custom carbon offset protocol for Willow + Biosolids systems.

This study is organized into five sections and six chapters to contextualize, review, assess, and synthesis the following objectives:

1. Summarize why climate change mitigation incentives and strategies are needed (SECTION 1 INTRODUCTION, Chapter 1 Background & Investigative Context);
2. Explain how biogenic based climate change mitigation projects and protocols can contribute to developing solutions (SECTION 2 LITERATURE REVIEW, Chapter 2 Climate Change and Climate Change Mitigation, Chapter 3 Protocol Development Considerations for Willow + Biosolids Systems Carbon Offsets);
3. Demonstrate the potential for Willow + Biosolids Systems to offset carbon dioxide emissions through measuring differences in soil organic carbon stocks between control and treatment sites (SECTION 3 FIELD ASSESSMENT, Chapter 4 Project History, Chapter 5 Study Design & Overview) and;
4. Discuss the practicality of using Willow + Biosolids Systems to cost effectively generate carbon dioxide emissions offset credits (SECTION 4 SYNTHESIS, Chapter 6 Discussion, Recommendations, and Conclusions).

This study was based on *The City of Calgary Dewatered Biosolids Land Application Program – Willow Biomass and Marginal Land Reclamation Demonstration Project* which was implemented in 2013 by SYLVIS Environmental. Background data and information collected by SYLVIS is presented in SECTION 5 SUPPORTIVE INFORMATION.

Section 2—Literature Review

Chapter 2

Climate Change & Climate Change Mitigation Initiatives

It is well established that anthropogenic climate change is caused by increases in the atmospheric concentrations of greenhouse gases (GHG), including carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and fluorinated gases. These gases trap excess radiant solar energy within the atmosphere that would otherwise escape to outer space (IPCC, 2018 pg. 99). Although CO_2 has the lowest warming influence (watts/m^2) per metric ton out of the four main GHGs, it receives the most attention because it accounts for approximately 60% of the total annual GHG index (Torri et al., 2014). Since the pre-industrial times (1850–1900) atmospheric CO_2 concentrations have steadily increased from 280 parts per million (ppm) (Torri et al., 2014) to 408 ppm as of November 2018 (NOAA/ESRL, 2018). In in recent decades CO_2 concentrations have increased by about 0.5% year $(yr)^{-1}$ (Lal, 2002) which equates to approximately 2.3 ppm yr^{-1} (Lal, 2016). Without substantial advancements in global emission reduction behavior and technology continued growth in anthropogenic GHG, emissions are anticipated to increase global mean surface temperatures between 3 °C and 4 °C (compared to pre-industrial levels) by 2100 (IPCC 2018 pg. 56). To mitigate anthropogenic based climate change net GHG concentrations must decrease through either reducing GHG emission rates, sequestering and storing atmospheric carbon, or both.

2.1 Agriculture's Role in Climate Change and Climate Change Mitigation

The agricultural industry has played a major role in anthropogenic climate change. By converting approximately 40% the earth's ice-free land surface from natural ecosystems to arable lands agriculture has been the second largest generator (25%) of total GHG emissions after fossil fuel combustion (75%) (Lal, 2016, IPCC, 2014 pg. 24, 816). Agriculture generates GHG emissions by intensifying soil carbon losses through soil disturbance and utilizing fossil-fuel based inputs for machinery and fertilizers (West & Marland, 2002). It has been estimated that North American prairie soils alone have lost between 25% and 50% (~0.46 Gigatons (Pg)) of their original carbon content due to deforestation and cultivation (Lemus & Lal, 2005; Post & Kwon, 2000). The total soil carbon losses resulting from converting natural ecosystems to agriculture lands worldwide is estimated at ≥350 CO_2e Pg (Oertel, Matschullat, Zurba, Zimmermann, & Erasmi, 2016). Agricultural practices deplete soil carbon pools in three ways (1) enhancing oxidation and mineralization of SOC compounds (2) leaching and translocating dissolved organic carbon compounds, and (3) accelerating soil erosion (Lal, 2002). Additionally, annual fossil-fuel based agriculture inputs generate approximately 10-12% of total annual anthropogenic greenhouse gas emissions (IPCC, 2014 pg. 822).

By implementing SOC sequestration land management practices like the UN Framework on Climate Change (UNFCCC) 21st Conference of the Parties (COP21) recommended management practices (RMPs) (e.g. no-till agriculture, organic amendments and, agroforestry) much of these agricultural based carbon emissions can be reversed through soil re-carbonization (Paustian et al., 2016; Lal, 2016). Lal (2005, 2016) estimates that through applying SOC sequestration land management practices carbon-depleted agricultural soils have the potential to re-carbonize up to 60-70% (62 Mg C ha^{-1}, 227 Mg CO_2e ha^{-1}) of their native ecosystem SOC levels within 50-75 years. While the IPCC (2014 pg. 816) estimates that with the right combination of Agriculture, Forestry and Other Land Use (AFOLU) practices, SOC sequestration could contribute between 20% and 60% of the total cumulative GHG offsets within 15 years.

2.2 Alberta's Climate Change Mitigation Actions

In 2002, Alberta's Climate Change and Emissions Management Act was the first legislation pertaining to climate change mitigation in Canada. In 2007, Alberta became the first jurisdiction in North America to implement a multi-sectoral compliance-based GHG emissions offset program with a complementary carbon offset system (Government of Alberta, 2007). This program caps emissions for large GHG emitters (>100,000 Mg yr^{-1}) and gives them the option to buy or sell carbon offset credits when their GHG emissions are above or below this limit. Incentives to reduce provincial GHG emission reductions were broadened under the *Climate Leadership Act* (2017) by introducing carbon levies to consumer fuel purchases. As of January 2018 the price of carbon pollution in Alberta was \$30/$MgCO_2e$ and it is scheduled to rise to \$50/$MgCO_2e$ by 2022 (Environment and Climate Change Canada, 2017). By complementing the Alberta carbon offset credit system with carbon levies the provincial government has created more favorable market conditions for land owners, municipalities, and industry to invest in carbon offset projects. Revenues generated by the global carbon market reached \$22 billion USD in 2016 and \$33 billion USD in 2017 (World Bank and Ecofys, 2018). The IPCC (2014 pg. 87) indicates that the most cost effective AFOLU based carbon sequestration mitigation strategies (i.e. afforestation, sustainable forest management and agriculture management) depend on the current price of carbon offsets and the region where the AFOLU practices are applied.

2.3 Alberta's Carbon Offset Protocols

The Alberta carbon offset system has been designed to incentivize cost-effective reduction and sequestration of GHG emissions. Currently Alberta has 19 approved Carbon Offset Quantification Protocols. The range of carbon offset protocol project activities includes; renewable power generation (solar, water, and wind), agriculture (livestock, organic waste, biofuel and land management) and fuel efficiency. The importance of Agriculture's role in climate change mitigation opportunities is highlighted by the fact that approximately half of the current Carbon Offset Quantification Protocols have been developed specifically for this industry. However, none of these Quantification Protocols capture the full carbon offset potential of Willow + Biosolids projects; particularly in regard to SOC sequestration. Soil based bio-sequestration has been considered for carbon offset protocol status in the past. In 2006, Paragon Soil and Environmental Consulting published the *Draft Guide to Development of Customized Agricultural Soil Carbon Sink Protocol for Greenhouse Gas Emission Reductions and/or Removals under Canada's Offset System*. However, due to shifts in federal government priorities in 2006 unapproved protocols at that time were not considered further (Haak, 2007). Although substantial evidence has been generated to support the carbon sequestration potential of agricultural soils (Paustian et al., 2016) there is still considerable uncertainty around the operation of specific mechanisms driving SOC sequestration (Lehmann & Kleber, 2015). This is due to (1) the large number of environmental factors transforming SOC inputs into a diverse spectrum of carbon compounds with broad biochemical and physical properties, and (2) the limitations of SOC analysis tools to accurately detect and measure all the various SOC compounds over time. This situation creates challenges with selecting soil sampling and analysis methods that are both scientifically valid and cost effective enough to satisfy carbon offset approval and carbon offset trading market standards.

2.4 Climate Change Mitigation Potential of SOC Sequestration

Soils play a major role in the global carbon cycle. With an estimated total of 2000-2500 Gt (60% SOC and 40% soil inorganic carbon (SIC)) of soil carbon within the top 1 m, soils hold four times more carbon than the biotic pool and three times more carbon than the atmospheric pool (Sommer & Bossio, 2014). Therefore even small changes in global soil carbon pools can measurably influence atmospheric carbon pool stocks (Garten, Wullschleger, & Classen, 2011; Lal, 2016; Tian et al., 2009; Torri et al., 2014; Wijesekara et al., 2017). Within the peer literature the estimated climate change mitigation potential of increasing global SOC stocks varies as there are numerous assumptions and variables built into the GHG emissions and SOC sequestration equations that may differ between studies. Kirschbaum, (2000) estimated that a 10% increase in the global SOC pool would equate to capturing 30 years' worth of anthropogenic emissions. Similarly, Goglio et al. (2015) estimated that increasing SOC sequestration rates could offset as much as 5% to 15% (0.4 to 1.2 Pg C yr^{-1}) of global Anthropogenic GHG emissions. A three-study global meta-analysis of the GHG emission offset potential of adopting the COP21 RMP's reported total carbon emission offset potentials that ranged from 4.4% to 15% of the estimated 7.91 to 9.1 Pg yr^{-1} total global carbon emissions (Sommer & Bossio, 2014). When investigating the regional emissions offset potential of adopting COP21 RMP's, Sommer & Bossio (2014) reports that Europe, USA, Australia and Central Asia have the potential to offset 8.3% of 1.2 Pg yr^{-1}, 14% of 2 Pg yr^{-1}, 8.4% of 0.15 Pg yr^{-1}, and 16% of 0.11 Pg yr^{-1} of their total annual carbon emissions respectively.

The climate change mitigation opportunity of promoting SOC sequestration has been gaining recognition in recent years (Lal, 2016) and received global attention during the COP21 when the "*4 per Thousand*" target was proposed. The "*4 per Thousand*" proposal outlines a voluntary action plan targeted to enhance SOC content of the world's topsoil (0-40 cm depth) at the rate of 0.4% per year through adopting the COP21 Recommended Management Practices (Wijesekara et al., 2017).

Although the carbon offset potential for SOC sequestration is considerable, its potential climate change mitigation effects are finite and reversible (Lal, 2016; Goglio et al., 2015). Instead of viewing SOC sequestration as a climate change mitigation solution, it should be considered a stop-gap measure until more comprehensive and long term solutions become available (Sommer & Bossio, 2014; Grigal & Berguson, 1998).

Infobox 1. Approval Requirements for a Custom Willow + Biosolids Project Carbon Offset Protocol

To support sustainable climate change, mitigate advancements in Alberta, the current (2019) Alberta government encourages development of custom carbon offset protocols for new carbon offset activities or technologies. Approved quantification protocols require activity-specific emission reduction methodology that are (1) not already a legal requirement, (2) are tailored to Alberta-specific conditions and are (3) based on the best available science at the time. To gain approval, proposed custom protocols must satisfy the principals and requirements outlined in the International Standards Organization (ISO) ISO-14064-2 specifications for guidance at the project level for quantification, monitoring and reporting of greenhouse gas emission reductions or removal enhancements. These standardized carbon offset quantifying, monitoring and reporting methodologies provides the transparency necessary for carbon offset traders to be confident that the carbon offset credits they are exchanging are real, quantifiable and verifiable. To gain government approval for a Willow + Biosolids project based protocol, an extensive process including expert engagement and rigorous peer reviews would be required to confirm that the proposed protocol methodologies are scientifically defensible, and monitoring practices are transparent enough to satisfy carbon offset traders and auditors expectations (Government of Alberta, 2018; Goddard & Haugen-Kozyra, 2008). Apart from cost and scientific validity considerations, key policy issues associated with developing biogenic based carbon offset protocols include; demonstration that the carbon offsets are:

- **Real**: comprehensive evidence presented to support carbon offset claims
- **Additional**: demonstration that that business as usual practices would not have generated the same SOC sequestration results
- **Measurable**: standard and repeatable data collection and analysis methods applied
- **Transparent**: detailed documentation of all land management practices, data collection and analysis methods, and monitoring events
- **No-leakage**: confirmation that adoption of the carbon sequestration practices at project location would not cause an increase in GHG emissions elsewhere
- **Verifiable**: Confirmation by a third party that soil monitoring data and carbon offset calculations are correct.

Chapter 3

Protocol Development Considerations for Willow + Biosolids Systems Carbon Offsets

For policy makers and investors to support soil-based carbon offset markets, SOC monitoring plans must cost-effectively produce scientifically sound results that achieve a high degree of accuracy within a reasonable timeframe. For SOC sequestration projects to be considered cost-effective and valid, the soil monitoring and analysis costs should be less than 20% of the anticipated carbon offset value (Paragon, 2006) and produce statistically significant results ($\alpha = 0.05$, $\beta = 0.15$) (Necpálová et al., 2014). Ideally projects should also generate measurable results within five years to align with industry and political business plan cycles and maintain project support and funding (Smith, 2004). Unfortunately current standard SOC monitoring and analysis methods do not consistently satisfy all of these objectives and the scientific community has yet to reach consensus on best practices for SOC monitoring (Jandl et al., 2014). The heterogeneous and dynamic nature of SOC pools increases soil monitoring and analysis costs as a result of the large sample sizes required to maintain the high degrees of statistical power necessary to confidently differentiate natural SOC variation from SOC changes induced by new land management treatments (Conant & Paustian, 2002). Annual SOC changes are relatively small compared to background levels (Carter & Gregorich, 2006) and therefore typically take 7-10 years (Saby et al., 2008; Smith, 2004) to detect SOC rate changes above 2 Mg C ha^{-1} yr^{-1} in magnitude (Conant, Smith & Paustian, 2003; VandenBygaart, 2006). Given these constraints, the cost, time, and effort required to satisfy Quantification Protocols standards can often outweigh the value of the anticipated SOC offset credits (Singh, Murphy, & Marchant, 2012). Fortunately, substantial research efforts are being made to improve SOC monitoring plan performance and to better understand SOC sequestration dynamics. Key SOC monitoring programs steps and approaches being tested include; study design strategies (stratification), field sample collection methods (in-situ vs. ex-situ, aerial imagery), transport and processing protocols (sample integrity), laboratory analysis parameters selection, laboratory analysis methods, and data analysis procedures (Carter & Gregorich, 2006; Olander, Haugen-kozyra, & Kravchenko, 2011).

3.1 Conceptual Models of Soil Organic Carbon Dynamics

It is well-understood that the SOC sequestration processes are controlled by rates of soil carbon inputs (photosynthesis) and outputs (respiration) and is initiated by the breakdown of fresh organic matter into the soil (McClean et al., 2015; West & Marland, 2002). However the specific mechanisms driving SOC sequestration after the initial decomposition stage are still not clearly understood (Bloom, Exbrayat, van der Velde, Feng, & Williams, 2016; Rowley, Grand, & Verrecchia, 2018; Zang et al., 2018) and there are competing conceptual models explaining the composition, chemical behavior, and turnover rates of SOC compounds (Bloom et al., 2016; Lehmann & Kleber, 2015). Lehmann & Kleber (2015) indicate the three most prominent SOC sequestration conceptual models today are; classic humification, selective preservation and progressive decomposition. The classic humification model emerged in repose to early alkali-based SOC extraction methods. This model suggests that over time soil organic matter molecules undergo biochemical processes that transform them from small labile compounds into larger and more stable "humic substances" with heightened resistant to oxidation. Lehmann & Kleber (2015) argue against this conceptual model because modern soil analysis techniques have not produced evidence to support the existence of these humic substances under natural conditions. The selective preservation model (also known as preferential decomposition) assumes that organic soil inputs are composed of both labile and relatively recalcitrant compounds and that microorganisms selectively consume labile carbon compounds which leads to an accumulation of recalcitrant compounds. Although recent studies have demonstrated that the preservation model is not replicable under all conditions (Lehmann & Kleber 2015). The progressive decomposition model (also known as biopolymer degradation) describes the SOC pool as a continuum of progressively decomposing organic compounds where all organic inputs are subject to continuous degradation towards smaller molecule sizes. Lehmann & Kleber (2015) argue that from a thermodynamic standpoint the progressive decomposition model is the most robust of the three conceptual models. To build upon this model, Lehmann & Kleber (2015) developed a modified version called the soil continuum model to better explain differences in organic compound turnover as a function of variations in local biogeochemical and climactic conditions. The continued refinement and alignment of SOC dynamics conceptual models is important for future biogenic carbon offset accounting because these conceptual models can be used to increase the accuracy of SOC process-based models such as CENTURY. By improving the accuracy of SOC dynamics process model predictions for a broader spectrum of land uses and soil conditions scenarios, fewer costly SOC stock verification soil monitoring events will be required.

3.2 Biogeochemical and Climactic Factors Influencing SOC Dynamics

There are numerous complex biogeochemical processes, climatic interactions, and land management practices that influence total SOC stock capacity and flux rates to varying degrees across the landscape (Lemus & Lal, 2005; Necpálová et al., 2014; West & Six, 2007). Understanding how these factors may affect SOC dynamics and total SOC stock potential over time is important for identifying favorable SOC sink project site locations because recent studies suggest that ecosystem properties are stronger predictors of a sites SOC turn-over rates than levels of SOC recalcitrance (Rowley, Grand, & Verrecchia, 2018).

The most prominent factors governing mechanisms of SOC stabilization and turnover includes; climate (temperature and precipitation), parent material properties (soil texture i.e. % clay and available mineral content i.e. calcium (Ca^{2+}), magnesium (Mg^{2+}), iron ($Fe^{2+/3+}$), and aluminum (Al^{3+})), biota (vegetation and microbial community composition) and land management practices (crop selection, nutrient amendments, and crop selection) (Corsi, Friedrich, Pisante, & Sà, 2012; Grigal & Berguson, 1998). Mcconkey et al., (2000) separates environmental factors governing SOC dynamics into three categories; limiting factors, determining factors, and reducing factors. Limiting factors control the quantity of carbon inputs (biomass) entering a system. Biomass production rates are generally controlled by regional factors such as climate and vegetation community composition. Determining factors set the upper limits of a sites total SOC stock capacity potential through SOC stabilization and primarily include the physical (soil depth, density, etc.) and chemical (organo-mineral complex potential) constraints of a site (Lemus & Lal, 2005).

Physical SOC stabilization occurs by protecting SOC compounds from decomposing organisms and factors of aerobic decomposition (i.e. oxygen and moisture) through physical separation. The physical protection of SOC compounds from decomposers may occur over large (via freezing or waterlogged soil conditions) and small scales (via soil aggregate formation) (Rabbi, Lockwood, & Daniel, 2010; Rowley et al., 2018). Chemical SOC stabilization occurs when SOC compounds form organo-mineral complexes with minerals that prevent microbes from accessing the carbon molecules. The role of calcium in chemical SOC stabilization is especially strong (Rowley et al., 2018). Reducing factors influence the rate of SOC stock turnover. Reducing factors include any land management practice or biogeochemical or climatic condition that accelerates microbial community activity or increases soil erosion. Regions with the highest total carbon stock capacity are those with the most favorable combinations of biogeochemical characteristics (productive vegetation communities on deep fine-textured soil with high cation concentrations), climatic conditions (warm and humid climates), and land management practices (minimal soil disturbance, high rates of carbon inputs) for amplifying biomass inputs, enhancing SOC stabilization and suppressing microbial decomposition (protection from soil disturbance) (Lemus & Lal 2005).

3.3 SOC Sequestration Rate Trends

Understanding how SOC stocks and flux rates and stocks are likely to change over time is an important consideration for SOC sink project planning (Corsi et al., 2012; West & Six, 2007). When the total SOC stock of a SOC sink project is graphed over time, it typically follows a sigmoid curve pattern (Sommer & Bossio, 2014) where the rates of SOC sequestration are relatively slow at both the beginning and end and relatively fast in the middle of a SOC sink project's lifespan as shown in Figure 1. Active SOC sequestration typically lasts up to 30 years at SOC sink project sites (Sommer & Bossio 2014). Because the project was five years old at the time of the study, its position along the SOC sequestration curve is most likely within the initial slow to medium SOC sequestration rate ranges. Depending on how the soil microbial community responded to the demonstration project implementation land management practices, the site may also have undergone temporary loss in SOC stocks during the first few years due to soil priming (discussed in more detail below). The width of the sigmoid curve represents the duration of the SOC sink project's productive SOC sequestration life-span and the height represents the difference in total SOC stock between project establishment and maximum total carbon sink capacity (steady-state) for given set of conditions. Depending on a sites land management practices and biogeochemical and climatic conditions, the time period between SOC sink project establishment and steady-state typically takes between 20-40 years (Sommer & Bossio 2014; Corsi et al., 2012; Government of Alberta, 2012; West & Post, 2002). The SOC sequestration sigmoid curve begins with a ramp-up period represented by slow to medium SOC sequestration rates while SOC sequestration processes recalibrate to new land management practices. This ramp-up period generally ranges between year 0 and 10 (West & Six, 2007).

A common explanation for the initially slow SOC sequestration rates (lag phase) is due to the time it takes to shift the soils microbial communities from a bacterial dominated community that persist in cultivated agricultural systems to fungal dominated microbial communities that persists in no-till agroforestry systems (Six et al 2006). As microbial communities' transition towards fungal dominated systems, the SOC stocks increase because soil fungi organisms have higher carbon to nitrogen ratios and they support micro-aggregate formation which physically protects SOC compounds from decomposition (Corsi et al., 2012; Six et al., 2006). The conceptual models and explanations of microbial community responses to land use changes are still under debate within the literature (Hydbom, 2017) and further research is needed to reveal the full spectrum of conditions and factors influencing their dynamic responses to environmental changes.

In some instances, the SOC stocks may also drop during the first few years of a SOC sink project due to a phenomenon called “priming” which is caused by a temporary acceleration of soil bacteria activity due to a surge in labile carbon and increased soil disturbances from SOC sink project installation activities (Fontaine, Bardoux, Abbadie, & Mariotti, 2004). In these situations, little to no increase in SOC stocks may be observed within the first 5 years of a SOC sink project (West & Marland, 2002). The ramp-up period is followed by an acceleration period represented by fast SOC sequestration rates resulting from an increase in SOC sequestration processes and low SOC stocks relative to the steady-state SOC stock. This period generally occurs between year 5 and 20 (West & Six, 2007; Corsi et al., 2012). The acceleration period (exponential phase) is followed by a deceleration period (stationary phase) where SOC sequestration rates begin to decrease due to total SOC stocks approaching their SOC steady-state levels. This period generally begins after year 15 (West & Six, 2007). The steady-state period occurs when the net SOC sequestration rate is approximately zero because the rates of soil carbon input (leaf and root litter) and output (respiration) are relatively equal (West & Six, 2007). Steady-state is generally reached before year 30 (Sommer & Bossio 2014). The steady-state level (height of sigmoid curve) of a SOC sink project site may be adjusted by altering the land management practices at the site which can influence soil carbon input and mineralization rates (Sommer & Bossio 2014, Lal 2016).

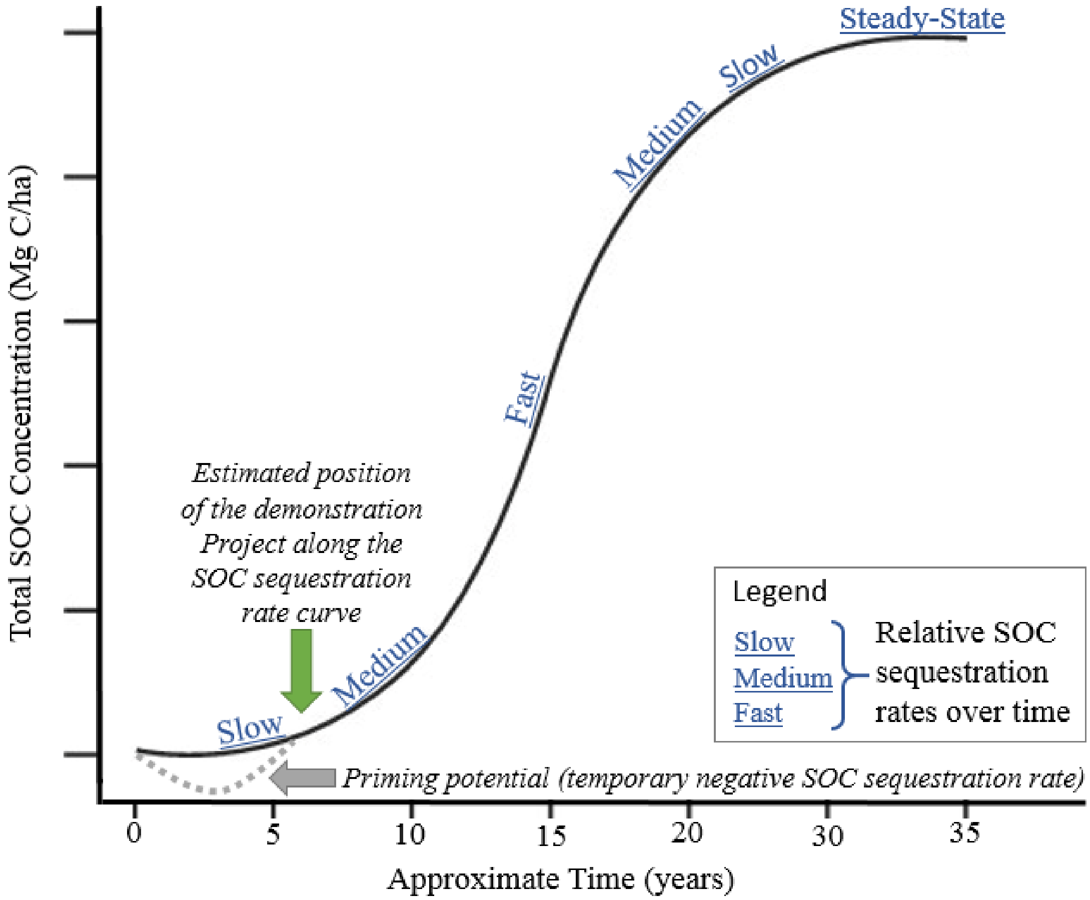

Figure 1: Typical SOC sequestration rates expected for a SOC sink project over time (adapted from Sommer & Bossio 2014).

3.4 Study Design Considerations for Monitoring SOC Stocks

SOC stock monitoring studies requires strategic planning as there are numerous trade-off decisions between sampling costs, sample size, sample timing, statistical power, minimum detectable difference, and study duration expectations when making budget and scheduling decisions (Smith, 2004).

Sample Size and Statistical Power Considerations

The most cost-effective SOC monitoring plans are those which prescribe the smallest sample size necessary to attain minimum measurable detection levels of SOC stock changes within accepted accuracy standards and specified time periods (Conant & Paustian, 2002). However without detailed information of SOC stock variability trends across space and time, the risk of falsely accepting the null hypothesis (Type II error) due to the low statistical power of a small sample size can far outweigh the cost of collecting additional soil samples (Necpálová et al., 2014; Carter & Gregorich, 2006 pg. 52; Kravchenko & Robertson, 2011). Due to the high cost of soil sample collection and analysis, small sample sizes are a common problem in SOC research (Necpálová et al., 2014) and decisions based on these low statistical power studies can negatively impact future SOC research and related policy decisions (Kravchenko & Robertson, 2011; Singh et al., 2012, Carter & Gregorich, 2006). Therefore, it is important to be mindful that the absence of a significant difference found in low statistical power studies does not necessarily indicate an absence of the ecological processes being investigated (Kravchenko & Robertson, 2011).

Predefining expectations the SOC stock minimum detectible difference and monitoring duration of a SOC sink project is an important study design planning consideration as these factors can have major influences on SOC monitoring costs. For example, Olander et al. (2011) estimates that a 10% decrease in the SOC coefficient of variation would reduce the soil sampling and analysis costs by approximately 30%. To further illustrate the integrated relationship between SOC coefficient of variation, minimum detectible difference, and sample size requirements, Necpálová et al. (2014) estimates that a 15% coefficient of variance for SOC stocks would require a sample size of 400 to detect a 1% change in SOC stock from the mean (e.g. a 0.5 Mg C ha^{-1} change in a 50 Mg C ha^{-1} pool). Conversely much smaller samples sizes are required when minimum detection limits are higher. For comparison Conant & Paustian (2002) estimated that a sample size between 14 and 28 is sufficient to detect a SOC stock change of 2.3 Mg C ha^{-1}. However, SOC stock changes of this magnitude can take years to develop. Smith (2004) reported that it is difficult to detect changes in SOC stocks in projects that are less than 7-10 years old without very large sample sizes (>100).

Use of P Values to Define Effect Outcomes — Pros and Cons

Within the recent peer reviewed literature (Nature, 2019; Wasserstein, Schirm, & Lazar, 2019), debates have been building around the appropriateness of using arbitrary p values for determining ecological study effects. Some researchers argue that the use of a single p value to determine experimental effects is a too narrow of an approach for effective scientific inquiry and discovery because it creates conditions that promotes bias, false positives, and overlooked effects (Wasserstein et al., 2019). Instead Wasserstein et al., (2019) recommends taking a more thoughtful approach to data analysis which includes a broader set of study variables and data analysis approaches to more holistically explore and evaluate research development and results. Although adopting more holistic methods for scientific inquiry exploration would be more challenging than using arbitrary p values, many researchers argue that this approach would lead to a better understanding of the scientific phenomenon being explored and more clarity on why similar studies generate different results (Wasserstein et al., 2019). With regards to SOC stock and dynamics monitoring, adopting a holistic approach to data analysis for determining effects would likely generate higher quality insights than single arbitrary p values because (1) SOC stocks represent the net balance between an infinite number of interactions between multiple variables across broad scales of space and time, and (2) due to the nature of ecological systems measured soil parameters (e.g. pH, bulk density, %SOC, clay content, etc.) often do not satisfy parametric statistical test assumptions (Halvorsen Okland, 2007) – which were also observed in this study.

Managing SOC Stock Variability

Accurately measuring and monitoring global SOC stocks and fluxes is challenging due to naturally high variability of SOC stocks. Approximately 44% of SOC variability has been linked to spatial scale and soil profile depth (Maillard, McConkey, & Angers, 2017). To increase SOC stock change detection sensitivity without dramatically increasing sample size, SOC monitoring study designs need to separate natural SOC variation from SOC changes induced by new land management practices (Kravchenko & Robertson, 2011; Smith et al., 2012). This separation of natural SOC variation from land management induced SOC changes is generally accomplished through paired repeat measures sampling designs and stratified sampling plans (Heim, Wehrli, Eugster, & Schmidt, 2009; Olander &

Haugen-Kozyra, 2012). Stratified sampling plans partition sampling categories by biophysical, chemical, climatic, and land management variables that influence SOC dynamics; including topographic position, depth interval, soil texture, vegetation communities, and land management practices (Necpálová et al., 2014; Olander, Haugen-kozyra, & Kravchenko, 2011).

3.5 Soil Organic Carbon Analysis Parameters

Measuring Total Soil Organic Carbon

Traditionally, SOC analysis was performed to rate soil productivity and fertility; quantifying the relative differences of SOC stock between samples yielded an acceptable level of accuracy (Carter & Gregorich, 2006). However, because carbon offset credits are legally binding, the standards for measuring total SOC are substantially higher now (Chatterjee, Lal, Wielopolski, Martin, & Ebinger, 2009). Contemporary research efforts now focus on improving the speed, accuracy, precision and cost of total SOC monitoring.

Currently, ex-situ SOC analysis methods (e.g. chemical oxidation and thermal oxidation) are the standard analytical approaches for measuring the organic carbon content of soil samples (Stockmann et al., 2013). Both methods decompose carbon compounds within soil samples to expel carbon dioxide which is measured to calculate the soil samples total SOC content (Schumacher, 2002) – though each method has some drawbacks. Chemical oxidation tends to underestimate SOC content by not completely oxidizing a samples organic carbon and the thermal oxidation tends to overestimate organic content by degrading some of the sample's inorganic carbon (Chatterjee et al, 2009). Studies show that thermal oxidation methods, specifically the dry combustion with infrared reflectance spectroscopy, tend to be the most precise (Carter & Gregorich, 2006) with reported standard deviations ranging from 5-8% (Australia Government, 2014). However, when the soil analysis error is combined with the sampling error introduced during the soil collection, handing, and processing stages, ex-situ methods are generally not sufficient enough to detect small SOC stock changes short time spans to the standards needed for sustaining a robust carbon offset market (Chatterjee et al., 2009; Stockmann et al., 2013).

Alternative SOC monitoring approaches such as in-situ SOC analysis are being explored to improve the speed, accuracy, and cost-effectiveness of measuring SOC stocks (Chatterjee et al., 2009). Two complementary in-situ SOC measuring methods being evaluated are near-infrared spectroscopy (NIR) and mid-infrared spectroscopy (MIR) (Stockmann et al., 2013). These methods work by utilizing the near-infrared and mid-infrared regions of the electromagnetic spectrum to identify and quantify organic compounds known to vibrate at specific wavelengths (Chatterjee et al., 2009). These methods provide a faster and more efficient means of measuring total carbon content and its fractions than ex-situ methods. Although, the calibration methods of these technologies are not yet reliable (Chatterjee et al., 2009; Soriano-Disla, Janik, Rossel, MacDonald, & McLaughlin, 2014).

Measuring Soil Organic Carbon Fractions

The heterogeneous mix of SOC compounds ranging from stable to labile are primarily differentiated by mean turn over time and are analytically segregated by physical (size, density, aggregation) and chemical (solubility, mineralogy) fractionation processes (Lockwell et al., 2012; Stockmann et al., 2013). In the early stages of a SOC sequestration project, the labile carbon pools (particulate organic carbon, dissolved organic carbon and, microbial biomass carbon) are particularly valuable as early indicators of SOC responses to land treatments because they react most strongly to changes affecting carbon mineralization and accumulation rates (Ghani, Dexter, & Perrott, 2003; Lockwell et al., 2012). Labile SOC pool flux rates are controlled by microbial (bacteria and fungi) activity, community composition, and soil enzyme activity which can be analysed to represent SOC stock dynamics (Torri, Corrêa, & Renella, 2014). Examples of two soil products strongly tied to these labile carbon fractions attributes are hot water extractable carbon and amino sugars (Lockwell et al., 2012). Hot water extractable carbon is a highly labile carbon fraction and is well correlated with microbial biomass size and root exudates. Amino sugars are more stable of the two labile carbon fractions and are generated during microbial mineralization of organic matter (Lockwell et al., 2012). Together, these products can help evaluate the influence of microbial activity in SOC dynamics and its contribution to SOC sequestration.

3.6 SOC Dynamics Research on Agricultural Land Management Practices

Some of the most prominent climate change mitigation land management practices include; conversion from till to no-till agriculture, adoption of perennial crop systems, and application of organic amendments (Stockmann et al., 2013). Research on quantifying the biogenic carbon offset potential of these land management practices has focused on characterizing the biomass and biofuel production potential of these land management practices. Comparatively little research has explored the SOC sequestration benefits of these practices, especially when multiple land-use management practices are combined. The following sections summarize the best available research on the SOC sequestration potential of these three land management practices.

Conversion from Till to No-till Agriculture

Adopting no-till agriculture reduces net CO_2 emissions by decreasing demand for fossil-fuel based agricultural inputs, and increasing SOC sequestration rates (West & Marland, 2002). When accounting for net emission reductions of these two outcomes together, West & Marland (2002) calculated the relative net flux of no-till compared to reduced till to be -1.35 $CO_2e\ ha^{-1}\ yr^{-1}$ while the Government of Alberta (2012) calculates it to be -0.2 $CO_2e\ ha^{-1}\ yr^{-1}$ for the dry prairie region of Alberta.

No-till agriculture usually decreases the rate of GHG emissions by reducing the number of machinery field passes and the size and power of the machinery required to manage the agricultural crops at a site (West & Marland, 2002; Corsi et al., 2012). On average conversion from a till to a no-till agricultural system reduces fuel consumption unit input per unit area by 35-80%, decrease the number of land passes by 50-54%, and reduce the size of the machinery required to manage the land by 50% (Corsi et al., 2012 pg.31).

By minimizing soil disturbances and increasing soil organic residues, no-till agriculture supports SOC sequestration by increasing supply of carbon inputs and reducing exposure of SOC compounds to oxidative elements (Garcia-Franco, Albaladejo, Almagro, & Martínez-Mena, 2015). As presented in Table 2, global meta-analysis research studies have shown that adopting no-till agriculture generates a wide range of SOC sequestration results depending on the biogeochemical and climatic conditions of the region. For example, VandenBygaart, Gregorich, & Angers (2003) found that the Chernozemic soils of the western Canada prairies sequestered more carbon than the Gleysolic soils of the eastern Canadian provinces after no-till agriculture is adopted.

Table 2: A summary of studies using global meta-analyses of average and range of CO_2e offsets generated by SOC sequestration resulting from converting from till to no-till agriculture

Reported Mean Mass and range of Sequestered SOC (Mg CO_2e ha^{-1} yr^{-1})	**Sampling Depth (cm)**	**Experiment Details**	**Mean Land Use Duration (years)**	**Reference**
1.27 -0.43 - 3.62	NA	Meta-analysis of 250 studies located across the United States	NA	Olander et al., (2011)
1.54 -0.14 - 3.22	24 ± 6	Meta-analysis 35 literature studies consisting of 96 paired treatments located across south eastern United States	10 ± 5	Franzluebbers (2005)
10.61 5.85 - 15.37	10-45	Meta-analysis of 12 studies across consisting of 35 paired treatments located across western Canada	11.4 ± 1.5	VandenBygaart, et al., (2003)
0.21 0.16 - 0.26	23 ± 0.5	Meta-analysis of 67 studies consisting of 276 paired treatments located world wide	15	West & Post, (2002)
1.23 NA	30	Meta-analysis of 76 long-term studies located across the United States	NA	West & Marland (2002)
0.08 NA	18 ± 2	Meta-analysis of 7 studies consisting of 39 comparisons located in temperate climates world wide	13	Paustian et al., (1997)

Notes: NA= not available

Short Rotation Woody Biomass

Afforestation, the process of replanting woody biomass in areas previously deforested by agricultural, is one of the simplest and most efficient climate change mitigation practices available (Hu et al., 2016). Willow are ideal afforestation species, especially on marginal lands, because they are fast-growing, adapted to grow on marginal soils, and require few land management inputs (Agostini, Gregory, & Richter, 2015). Most of the climate change mitigation attention that willow plantations have received is because of their biofuel production potential. A second climate change mitigation contributing factor of willow systems that is often overlooked is their SOC sequestration potential. Grogan & Matthews, (2002) and Hu et al. (2016) estimate that as much as 20% of the carbon offset potential of willow systems can be generated by SOC sequestration. Willow plantations support SOC sequestration processes through leaf and root litter soil inputs (Lemus & Lal, 2005; Hu et al., 2016) and once established receive few soil disturbances.

To-date willow plantation SOC sequestration studies have produced inconsistent results (Paul, Polglase, Nyakuengama, & Khanna, 2002) which has led to uncertainty around the SOC sequestration benefits of converting conventional agricultural fields to willow plantations (Zang et al., 2018). Some of the initial loss or low initial sequestration rates of SOC stocks at willow plantations has been attributed to soil priming due to the soil disturbances caused by site preparation and planting activities (Lemus & Lal, 2005; Grigal & Berguson, 1998), which can take up to 10 years to recover (Bashkin & Binkley, 1998). Others (Olson & Al-Kaisi, 2015) suggest that inconsistencies in total soil sampling depths and sampling depth interval lengths between studies are responsible for some of the inconsistent and inconclusive willow SOC study results because SOC variance increases with both the depth and length of a sampling interval and much of a sites SOC stock data is lost when soil sampling depths are too shallow. Therefore more standardized long-term studies (>25 years) of willow plantations with sampling depths that correspond to root system depths are needed to better characterize their SOC sequestration rates and stability (Agostini et al., 2015). Table 3 summarizes the SOC sequestration results from short-rotation woody biomass plantation studies conducted globally.

Table 3: A summary of global meta-analyses of average and range of CO_2e offsets generated by SOC sequestration resulting from converting conventional agriculture to short rotation woody crop (willow and poplar) plantation systems

Reported Mean Mass and Range of Sequestered Carbon ($Mg\ CO_2e\ ha^{-1}\ yr^{-1}$)	Reported Sampling Depth	Experiment Details	Mean Treatment Duration (years)	Reference
1.5 NA	NA	Sweden willow a plantation	7	Rytter (2012)
0.15 NA	60	Study of 2 willow plantation sites located in Southwestern Quebec	3	Zan, Fyles, Girouard, & Samson (2001)
4.25 3.66-4.83	100	Meta-analysis of 11 studies with poplar trees located North Dakota, Minnesota, Iowa, and Wisconsin	12-18	Hansen (1993)
No significant difference NA	25	Paired study of 5 poplar tree planation compared to adjacent crop lands located across Minnesota	6-15	Grigal & Berguson (1998)
No significant difference NA	80	Paired study of 21 willow/crop land sites located on former crop land in central Europe	8	Walter, Don, & Flessa (2015)
No significant difference NA	30	Replicated plantations with willows (*Salix* spp.) on former arable land at 5 sites across Sweden.	5	Rytter, Rytter, & Högbom (2015)
2.05 NA	15-70	Meta-analysis of 7 long-term field trials for willow plantations located in Sweden, Ohio, Germany, and New York.	13	Agostini et al., (2015)

Notes: NA= not available

Biosolids as Organic Based Fertilizer

Because of their high nutrient content, treated biosolids can be provide a high quality substitute for synthetic fertilizers (Xue et al., 2015). Studies have shown that application of biosolids on willow plantations can improve soil fertility and increase biomass productivity - especially on nutrient and carbon depleted soils (Adegbidi, Briggs, Volk, White, & Abrahamson, 2003; Athamenh, Salem, Et-Zuraiqi, Suleiman, & Rusan, 2015; S. Brown et al., 2011; Quaye & Volk, 2013; Torri et al., 2014; Wijesekara et al., 2017). The rapid growth rates of willow species make them particularly well suited to transform nutrient rich biosolids into biomass (Quaye & Volk, 2013). By substituting synthetic fertilizers with biosolids, the net energy ratio of the biofuels produced has been reported to increase by 34% (Quaye & Volk, 2013) to 40% (Heller, Keoleian, & Volk, 2003). The high carbon content of biosolids, which can range from 40-70%, also provides the soil with an excellent source of labile carbon (Torri, Corrêa, & Renella, 2014). A recent study conducted by Wijesekara et al., (2017) reported that biosolids application increased surface soil (0-15 cm) carbon concentrations by 45% on both sandy and clay soils. Another study conducted by Tian et al., (2009) on a 32-year (1974-2004) reclamation project located in Fulton County Illinois that was vegetated with herbaceous crops and fertilized with biosolids reported average SOC sequestration rates of 6.33 Mg CO_2e $ha^{-1}yr^{-1}$.

Although biosolids have demonstrated potential to support SOC sequestration directly through soil carbon inputs and indirectly through willow biomass production support (Wijesekara et al., 2017), few studies have investigated the SOC sequestration potential of adding biosolids into willow plantations. In an extensive biosolid management life cycle meta-analysis review, Yoshida, Christensen, & Scheutz (2013) reported that only 3 out of 35 studies considered SOC sequestration and only one of these three studies acknowledged Willow + Biosolids systems. This study reported that biosolids can offset approximately 0.68 g CO_2e for every MJ of energy produced by biomass when the following variables are accounted for; direct and indirect fuel use; N_2O emissions from applied fertilizer and leaf litter; and carbon sequestration (soil and below ground biomass) (Heller, Keoleian, & Volk, 2003). No studies to date have been found to have specifically investigated the SOC sequestration CO_2e offset contribution of biosolids within willow plantations.

Section 3—Field Assessment

Chapter 4

Project History

In 2010 the City of Calgary determined that their biosolids management program was approaching maximum capacity. To address this issue, the City teamed with SYLVIS Environmental (SYLVIS) to explore biosolids management diversification options. Together they developed The *City of Calgary Dewatered Biosolids Land Application Program – Willow Biomass and Marginal Land Reclamation Demonstration Project* (the demonstration Project). Implementation of the demonstration Project involved applying municipal biosolids (treated wastewater sludge) to marginal (low fertility) agricultural land and planting willows (*Salix* spp.) to an area 350 hectares (ha) in size. The purpose of the demonstration Project was to assess the economic and environmental merits of land-spreading the City's excess dewatered biosolids onto marginal agricultural land planted with willow. The purpose of this study was to determine if measurable differences in SOC stocks could be detected between land management treatments within the Study Area to evaluate the carbon emissions offset potential of the demonstration Project. The Study Area for this SOC monitoring study of the demonstration Project is located within a small portion of the entire demonstration Project footprint.

4.1 Study Area Overview

Study Area Location

The Study Area was located at the southwest quarter of 07-026-25 W4M within Wheatland County which is approximately 70 km east of the City of Calgary, Alberta and 13 km east of the Hamlet of Keoma. Maps of the Study Area location relative to regional communities and local secondary roads are presented in Appendix A (Figure A 1 and Figure A 2 respectively).

Biophysical and Climatic Conditions

The Study Area is located within the foothills fescue natural subregion (Natural Regions Commitee, 2006). This subregion experiences semiarid climate conditions. The Canadian prairies often experience drought conditions (Watson, 2016). The Alberta Agriculture and Forestry (2019) 180 day (April through October) standardized precipitation index (SPI) maps indicate that the soil moisture conditions at the demonstration project region were near normal between 2012 and 2015 (SPI = -0.44

to 0.44), less than 1-in-6 year wet (SPI= 0.97 to 1.39) in 2016 and less than 1-in-3 year dry (SPI= -0.97 to 0.44) in 2017. The nearest weather station with historical climate data records available during the demonstration Project operation period is Beiseker AGCM Alberta (Climate ID 3020610). This weather station is located approximately 20 km north of the Study Area (12 U 335766 m E, 5694727 m N). A summary of the annual key climate parameters averages is presented in Appendix A (Table A 1).

Topography, Soil Classification and Soil Characteristics

The Study Area's soils are classified as well-drained Orthic Black Chernozems which are generally composed of moderate to medium textured soils (Alberta Forestry and Agriculture, 2016). The Alberta Forestry and Agriculture (2016) Soil Information Viewer, indicates that the entire Study Area is located within Soil Polygon 11501 which is classified as high gradient undulating landscape (slope gradient 4%, slope length 250 m, and 5 m relief) represented by the Midnapore, Rockyview and Delacour soil series in order of predominance. The elevation within the Study Area ranges from 955 m to 960 m (Alberta Forestry and Agriculture, 2016). Collectively these soil series top soils are typically sandy, with fine texture, non to very weakly saline (<4 mS/cm) saline), non-calcareous (<1 calcium carbonate ($CaCO_3$) equivalent percent (%)), and medium SOC concentrations (2.2-3.4%) (Alberta Soil Information Centre, 2016; Government of Canada, 2018; Alberta Agriculture Food and Rural Development, 2005). Summaries of the typical physical and chemical properties of these soil series top soils under agricultural land management according to the Government of Canada, (2018) soil classification system are presented in Appendix A (Table A 2 and Table A 3).

The topsoil of undisturbed native Chernozem soils have relatively high SOC concentrations, well developed soil structure, and high mineral content (i.e. Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) cations) (Watson, 2016). Although the SOC content of cultivated Chernozem top soils is often low due to mixing with carbon poor subsurface layers (Watson, 2016).

Land Management History

Agriculture, in the form of grazing and till cropping of short season crops, is the principal land use in the Foothills Fescue Natural Subregion and occupies approximately 80% of the landscape (Natural Regions Committee, 2006). According to historic Google Earth imagery and landowner management reports (Lavery, J. (SYLVIS Environmental), personal communication, March 15, 2019a), the non-willow plantation Study Area was used for conventional agricultural purposes (e.g. small grain and oil seed rotations) prior to and during demonstration project operations. The historic air photos from July and November, 2011 show relatively uniform landscape patterns (vegetation, coloration, and agriculture machinery lines) between each sampling location within the Study Area are presented in Appendix A (Figure A 3 and Figure A 4 respectively). This visual consistency suggests that the biophysical conditions and land management practices were relatively uniform at each sampling location for at least two years prior to the demonstration Project implementation.

From 2012-2017 the Study Area (except for the willow plantation) was planted with spring wheat, canola, barley, and oats and received numerous fertilizer, herbicide, and fungicide treatments. A summary of the 2012-2017 land management practices is presented in Appendix B (Table B 7 through Table B 6). Between harvests, the landowner left the fields as stubble and occasionally used a heavy harrow to manage crop residue as part of reduced-till agriculture practices (Lavery, J. (SYLVIS Environmental), personal communication, March 15, 2019a).

4.2 Summary of Study Area land Management Practises (2013-2017)

Demonstration Project Land Preparation

To prepare the Study Area for the demonstration Project land management practices in 2013, the Willow + Biosolids site was deep tilled to a minimum depth of 15 cm, burying remaining crop stubble / residue remaining after harvest, using a disc Horsch Anderson Joker disc during the fall of 2013 (J. Lavery (SYLVIS Environmental), personal communication, March 15, 2019a).

Dewatered Biosolids Land-spreading Application

In the fall of 2013, biosolids were land-spread evenly across the Study Area at a rate of 23.8 Mg ha^{-1} using a Bunning vertical auger manure spreader and incorporated into the top 15 cm of the surface soil with two passes of a Horsch Anderson Joker disc (J. Lavery (SYLVIS Environmental), personal communication, March 15, 2019b). In 2016, biosolids were applied to the Study Area rate of 21.1 Mg ha^{-1}. Within the recently harvested (coppiced) portion of the Study Area, a cultivator was used to incorporate the biosolids with the leaf litter and organic debris on the soil surface. The small grain crop portions of the Study Area received biosolids via a Bunning® vertical auger manure spreader which were incorporated into the top 1-3 cm of soil using a Case TurboTill 330 vertical tillage disc (J. Lavery (SYLVIS Environmental), personal communication, March 15, 2019b)

Dewatered Biosolids Characteristics

The treatment and application of biosolids at the demonstration Project site was conducted according to the Guidelines for the *Application of Municipal Wastewater Sludges to Agriculture Lands* (2001) criteria to optimize the environmental benefits and mitigate potential risks of biosolids land-spreading application. The biosolids were treated at the City's wastewater treatment plant via aerobic and anaerobic digestion involving biochemical breakdown and stabilization of municipal wastewater sludge using microorganisms in the presence and then absence of oxygen. This process was followed by dewatering via filtration to separate the biosolids from the wastewater liquids. A summary of the Biosolids chemical analysis for both the 2013 and the 2016 land-spreading application events is presented in Appendix B (Table B 7). The mean chemical analysis results of 2013 (n=12), 2016 (n=11), indicated that key chemical parameters to this study such as % total organic carbon and pH were the same (TOC = 27% in 2013 and 2016) or similar, (pH = 7.7 (2016), 7.4 (2013)) while other SOC sequestration co-factors such as cation exchange capacity and available nutrients were generally 35% lower in 2016 than in 2013.

4.3 Willow Plantation Site Preparation, Planting and Harvesting

During the fall of 2013, willows were planted at the demonstration Project in various sized blocks according to the number of available willow stakes for each cultivar variety. Each block included one of 12 different willow cultivar varieties. 'Milbrook' is the willow cultivar sampled within the Study Area (J. Lavery (SYLVIS Environmental), personal communication, March 15, 2019d). 'Milbrook' is a hybrid of *Salix purpurea* '95026' crossed with *Salix miyabeana* 'SX64' and was published under the US Plant Patent No.17, 646 in 2007. 'Milbrook' was bred for rapid growth rate, disease resistance, and adaptability to a wide range of soil and moisture conditions; characteristics ideal for biomass production (Cornell University, 2010). After biosolids land-spreading of the Study Area in 2013, the willow portion of the Study Area was sprayed with glyphosate for weed control prior to planting and SureGuard® weed control was used 1-3 days after willow planting for pre-emergent weed control (J. Lavery (SYLVIS Environmental), personal communication, March 15, 2019d).

Willow Biomass Production

In 2016, the mean biomass collected from the Milbrook planting plot during was 19.4 ±3.3 Mg dry tonnes ha^{-1} (n=4) (J. Lavery (SYLVIS Environmental), personal communication, March 15, 2019d). Belowground biomass was not sampled.

4.4 Environmental Monitoring

For environmental regulatory purposes, SYLVIS conducted environmental soil monitoring prior to, and on an annual basis after the demonstration Project was established to monitor the site's soil nutrient and mineral concentrations over time. To collect soil samples, SYLVIS used methods outlined in the Alberta Guidelines for the Application of Municipal Wastewater Sludges to Agricultural Lands (2001). Between April 2013 and July 2017 SYLVIS conducted five sampling events where composite soil samples composed of 6 sub-samples collected at the 0-15 cm and 15-30 cm depth intervals were collected from four sampling ellipse located within the north, east, south, and west portions of the Study Area. A schematic of the sampling protocol used by SYLVIS is presented in Appendix B (Figure B 1). The SYLVIS environmental monitoring sampling results for the sampling events that occurred prior to biosolids land-spreading application (2013) and after two biosolids land-spreading application events (2017) are presented in Appendix B (Table B8)

4.5 Study Sampling Locations

Three sampling locations representing three different land management practices; agricultural Crops + no biosolids (Control), agricultural Crops + Biosolids (C+BS), and Willow + Biosolids (W+BS), were included in this study. Each sampling location was chosen using a random number generator to select a northing and easting UTM coordinate pair within the range of possible coordinates available for each land management practice area already in place at the time of the Study. The Study Area, sampling areas (zones within Study Area representing the Control, C+BS, and the W+BS land management practices), and sampling locations (sampling plot representing each of the three land management practices) are presented in Figure 2 and the UTM coordinates for each sampling location are presented in Table 4. Photos of each sampling location are presented in Figure 3 through Figure 5.

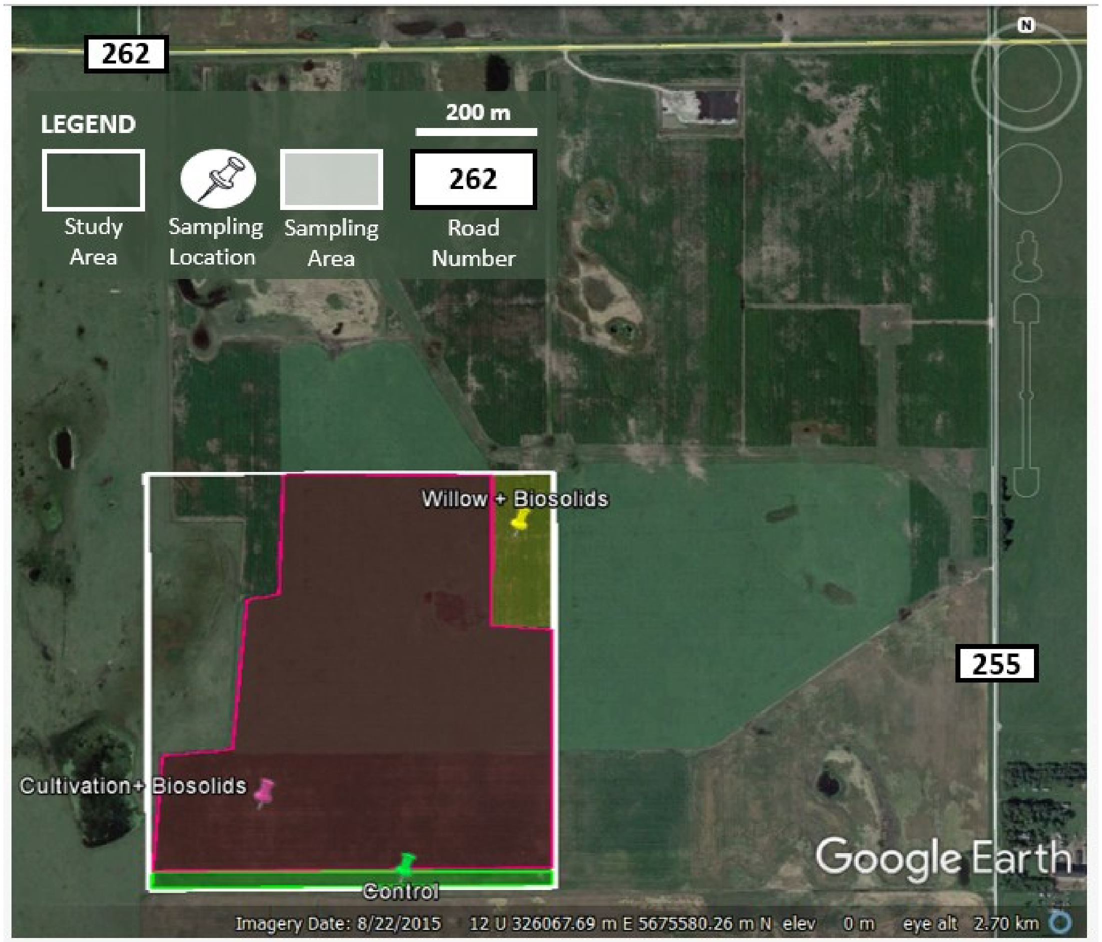

Figure 2: View of Study Area location on August 22, 2015 with an overlay of the treatment sampling areas and the treatment sampling locations. Image date (Google Earth, 2018a).

Table 4: UTM coordinates and land management practice treatments of study soil sampling locations

Sampling Locations	Zone	Easting	Northing	Crop Type	Total Tones of Dried Biosolids
Willow + Biosolids (W+BS)	12 U	326531m E	5675443 m N	Willow	45 Mg ha^{-1}
Agricultural Crops + Biosolids (C+BS)	12 U	326024 m E	5674940 m N	Small Grains	45 Mg ha^{-1}
Agricultural Crops + No Biosolids (Control)	12 U	326289 m E	5674789 m N	Small Grains	0 Mg ha^{-1}

Figure 3: View of the Control sampling location looking south

Figure 4: View of the agricultural Crops+ Biosolids (C+BS) sampling location looking west

Figure 5: View of the Willows + Biosolids (W+BS) sampling location looking east

Chapter 5
Study Overview & Design

Because the demonstration Project was in operation for five years prior to the study, a space-for-time substitution experiment design was the best of few options available to investigate changes in soil organic carbon (SOC) stocks across the Study Area. The space-for-time substitution experimental design uses a paired sampling approach by linking treatment sites with control sites. To avoid sampling bias in SOC space-for-time substitution studies all spatial and temporal variation of SOC and its sequestration cofactors must be relatively equal (Blois, Williams, Fitzpatrick, Jackson, & Ferrier, 2013; Lal, 2005). To support this requirement, the locations of treatment and control sampling plots should be relatively close to minimize natural spatial variation (Maillard et al., 2017) and exhibit the same biological, chemical, and physical properties prior to land management treatments (Johnson & Miyanishi, 2008). Therefore, under these uniform soil conditions it can be assumed that any differences detected in SOC stocks between control and treatment sites are a result of changes in land management practices (Smith et al., 2012). Based on apparent consistencies in land management history prior to demonstration Project implementation (Figure A 3 and Figure A 4); and that the entire Study Area is located within the same soil classification polygon (Alberta Forestry and Agriculture, 2016), this study was based on the assumption that each sampling location exhibited the same biological, chemical and physical properties prior to demonstration Project implementation. However, results of this study revealed that differences in soil physical and chemical properties between sampling locations did exist.

5.1 Pre-hoc Power Analysis for Sample Size Selection

To estimate the minimum sample size necessary to measure the Study Area SOC stocks with within 10% of the true mean 90% of the time, an estimate of the Study Areas SOC stock (Mg C ha^{-1}) variation was required. The Study Areas SOC stock variation was estimated using the coefficient of variation equation (Equation 1) and five years of previous soil sampling data collected by SYLVIS during their environmental monitoring events. The coefficient of variation was calculated for the SOC stock (Mg C ha^{-1}*)* results (n=4) for each of the five sampling events that occurred from 2013 through to 2017 using Equation 1.

Equation 1: Coefficient of variation = sample data standard deviation / sample data mean

The coefficient of variation data was then used to estimate the minimum sample size necessary to generate statistically significant results using Equation 2 from Aynekulu, Vagen, Shephard, & Winowiecki, (2011).

Equation 2: $n = (N * S)^2/[n^2 \times (E^2/t^2) + (N \times S^2)]$

Where: n = sample size

E = allowable error calculated by multiplying the mean carbon by the desired precision i.e. mean carbon stock x 0.1 (for 10% precision)

t = the sample statistic from the t-distribution for the 95% confidence interval

N = the number of sampling units in the population

S = standard deviation of the stratum

The sample size was selected based on the largest recommended sample size calculated from Equation 2 across all five of SYLVIS' historic sampling events within the Study Area at both sampling depth intervals (0-15 cm and 15-30 cm). The largest recommended sample size calculated using Equation 2 was 23. To improve the study's statistical power and to implement a symmetrically balanced sampling plot, a sample size of 25 was chosen for the soil parameters directly associated with measuring SOC stocks i.e. %SOC and soil bulk density. For the remaining SOC sequestration cofounding factors, where high statistical power was less critical to the study research questions, a sample size of 15 was chosen.

5.2 Soil Sampling Methods

The sampling plot size and spacing pattern was modeled after the Australian National Carbon Accounting system (McKenzie, 2000). Each sample site was a 20 m x 20 m grid divided into 25 sampling sites spaced 5 m apart as shown in Figure 6. A 30 m Komelon 6611IM Fiber Reel tape measure was used to mark out the sampling site boundaries and sampling locations. Soil sampling locations were recorded with a Samsung Galaxy Tab S2 9.7 tablet, using the Locus Maps-Pro mapping software (version 3.34.1) and a Garmin GLO^{TM} GPS Bluetooth antenna to improve GPS accuracy. GPS accuracy ranged between 2 – 4 m at each sampling location.

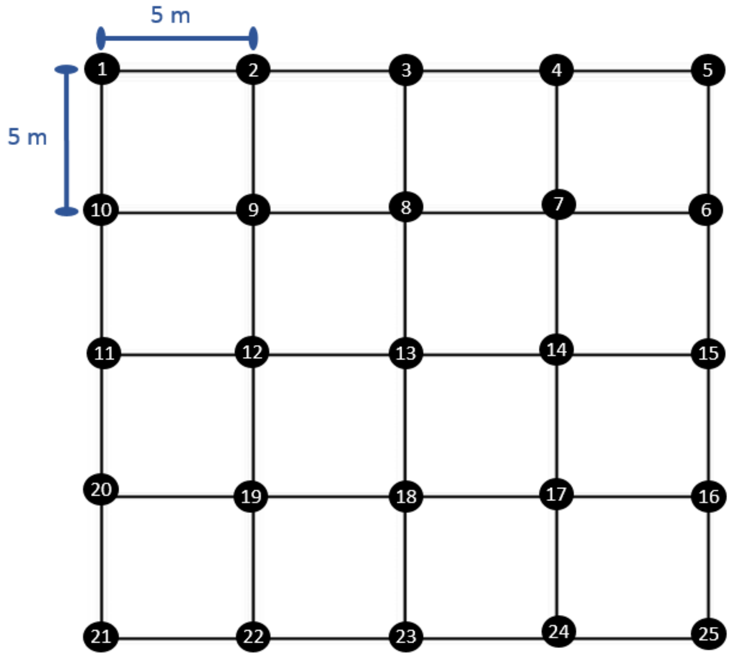

Figure 6: Study soil sampling grid pattern

At each of the 25 soil sampling sites, one soil sample was taken from the entire length of each depth interval, 0-15 cm and 15-30 cm, for laboratory analysis. Soil samples taken for laboratory analysis were retrieved using a hand operated AMS 2 3/4" Signature Regular Auger and placed into a labelled sealable plastic bag provided by the laboratory including sampling location code, sample replicate number, and laboratory analysis codes information. Sample depths were measured using a 1 m foldable fiberglass ruler. The soil analysis parameter data used for calculating SOC stock i.e. %SOC and bulk density was collected from each of the 25 sampling locations. SOC sequestration co-factor parameters i.e. soil pH and texture were analyzed for 15 of the 25 soil samples submitted to the lab. The soil samples selected for analyzing SOC sequestration cofactors were selected using a random number generator.

A second soil sample for was taken approximately 5 cm east of each laboratory soil analysis sampling location for soil bulk density analysis. The soil bulk density collection methods presented in the United States Department of Agriculture (1998) and Brown & Wherrett, (2014) were followed. Soil samples taken for bulk density analysis were retrieved using AMS Bulk Density 5 cm X 5 cm Liners. A soil bulk density liner >4 cm was chosen to avoid compaction of the soil core and compromise the integrity of the soil bulk density sample (Australia Government, 2014). Three soil core liner lengths (5 cm each) totaling 15 cm were taken for each sampling depth interval (0-15 cm and 15-30 cm). The volume of soil taken for each sampling depth interval totaled 295 cm^3; the soil sample was placed in labelled sealable plastic bags with information on sampling location code, sample replicate number, and depth interval.

All soil samples collected for chemical analysis were submitted to Exova Laboratories (Exova) located at No. 5, 2712 - 37 Avenue N.E., Calgary, Alberta on the same day that they were retrieved. Exova (www.exova.com) is an independent laboratory accredited through the Canadian Association for Laboratory Accreditation Inc. (CALA). Soil samples were analyzed for total carbon, pH, and texture. Soil samples were taken during the spring and late summer of 2018.

5.3 Physical Soil Parameter Analysis Methods

Soil Bulk Density

The methods used to measure bulk density were adapted from Brown and Wherrett (2018). The contents of each soil sampling bag were transferred to a tarred 20 cm x 20 cm x 3 cm tin baking sheet, weighed and baked in a kitchen oven at 170°C for 3 hours. After removal from the oven, the dried soil samples were re-weighed. All the soil samples were weighed using a Smart Weight Pro Pocket Scale with an accuracy to 0.1 g which was calibrated using two, 200 g Newer® weight kits.

Soil Texture

Soil texture analysis was performed by Exova using a modified version of the soil hydrometer method No. 55.3 prescribed in Carter (2008). A detailed description of Exova’s soil texture procedures are presented in Appendix C. Soil texture was classified based on the proportion of sand (50 µm - 2 mm), silt (2 µm - 50 µm), and clay (<2 µm) particles in each sample. The soil texture classes were based on the Canadian system of soil classification.

5.4 Chemical Soil Parameter Analysis Methods

Prior to chemical analysis soil samples were thoroughly mixed, subsampled, air dried to each parameter analysis specifications, and ground until all the soil sample contents could pass through a 2 mm sieve. A detailed description of Exova’s drying and grinding procedures are presented in Appendix C.

Total Organic Carbon

Percent total soil organic carbon (SOC) was measured via dry combustion with a LECO Truspec Analyzer using modified procedures from Nelson and Sommers (1996) and British Columbia Ministry of Environment (2014) to accommodate instrument set up and calibration procedures. A detailed description of Exova's total organic carbon soil analysis procedures are presented in Appendix C.

Soil pH

Soil pH was determined using a modified version of the 1:2 extraction method No. 4.12 from McKeague (1978). The soil pH was determined by mixing soil in water in a 1:2 ratio and recording the pH meter values. A detailed description of Exova's pH analysis procedures are presented in Appendix C.

5.5 Data Analysis Approach

Soil bulk density, SOC stock, texture, and pH calculations were conducted, tabulated, and graphically presented using the Windows Excel 2013 software program.

Soil Bulk Density Calculation

Soil bulk density was calculated by dividing the oven dried soil weight by total core volume as shown in Equation 3. The total core volume was calculated using the volume of a cylinder equation as shown in Equation 4.

Equation 3: Soil Bulk Density = weight of dry soil (g)/volume of soil from the cylinder (cm^3)

Equation 4: Volume of a cylinder: $= \pi * \text{cylinder radius}^2 * \text{cylinder height}$

$$= \pi * 2.5\ cm^2 * 15.0\ cm$$

$$= 294.52\ cm^3$$

Soil Organic Carbon Stock Calculation

To calculate the stock of SOC at each soil sampling site in terms of the mass of carbon in metric tons per hectare (Mg C ha^{-1}), the percent total carbon %TC results were multiplied by their corresponding soil bulk density results at each sampling location, the soil depth interval, and a unit area scale correction factor as presented in Equation 5. The %TC results represented %SOC because the soil conditions of the Study Area were too acidic (<7.2) for soil inorganic carbon (SIC) compounds to form (Brown et al., 2011).

Equation 5: %TC * bulk density (kg/L) * 0.15 m (thickness of sampling interval) * 100 (unit area scale correction factor) = Mg C ha^{-1}

Statistical Analysis Approach

Statistical analysis of the data was conducted using the Windows Excel 2013 and the R Studio version (1.1.456) software programs. Excel was used to generate the descriptive statistics tables and graphics presenting the mean and standard error of the mean (SEM) of each parameter, soil sampling location and depth interval. The statistical tests selected to detect differences in parameters where chosen based on the outcomes of the statistical test decision tree presented in Figure 7. Normality of the data was assessed by reviewing the skewness and kurtosis coefficients generated by the descriptive statistics data analysis feature in Excel and the Shapiro-Wilk test for normal distribution. The data set passed the normality assumption if the skewness and kurtosis coefficients were between ±1.96 (Rose et al., 2014)) and if the results of the Shapiro-Wilk test were $p \geq 0.5$. Homogeneity of the residuals variance were assessed using the Leven's test. When the data were not normally distributed, non-parametric tests were used and the data medians were used for pairwise comparisons rather than the data means. When parameters were compared across soil depth interval pairs, the Wilcoxon signed-rank test was selected because the sampling data sets being compared were not independent.

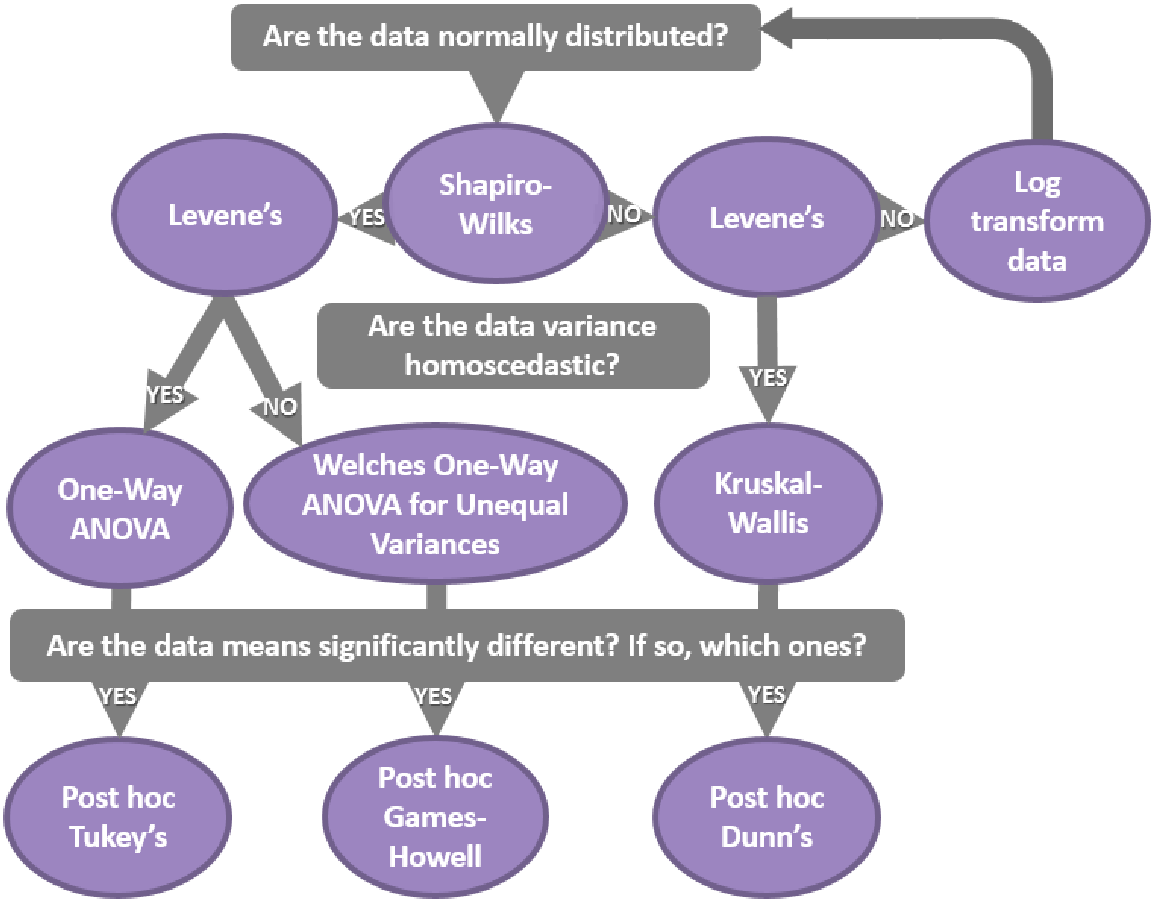

Figure 7: Statistical test decision tree for small sample sizes (n<50)

5.6 Soil Organic Carbon Dynamics

Soil organic carbon concentrations represent the ongoing balance between soil organic matter inputs via photosynthesis and losses via respiration (Follett, 2006). Numerous soil chemical, physical, and biological factors influence SOC dynamics and interactions between these factors effect SOC stocks to varying degrees across spatial and temporal scales. The complexity of SOC dynamics is one reason why these SOC sequestration processes are not fully understood and why main drivers of SOC dynamics shift according to site conditions. The following sections (1) summarize key findings of the study, (2) includes background information on select limiting, reducing and determining co-factors of SOC dynamics, and (3) discusses opportunities to improve the statistical power, statistical certainty, and cost effectiveness of future Willow + Biosolids baseline field studies. Figure 8 highlights the key limiting, reducing and determining co-factors of SOC dynamics categories in Willows + Biosolids systems.

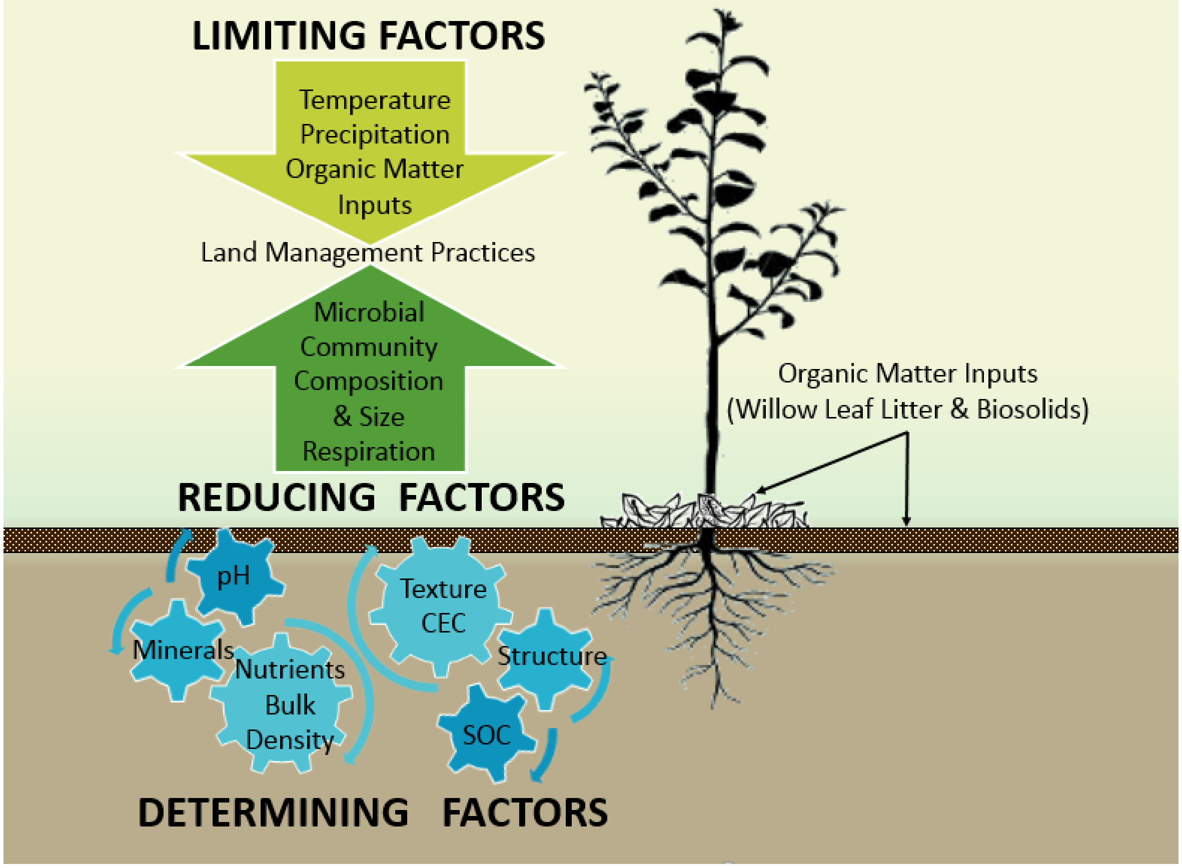

Figure 8: Schematic of limiting, reducing and determining co-factors of SOC dynamics in Willow + Biosolids systems

5.7 Limiting Factors of Soil Organic Carbon Sequestration

Limiting co-factors of SOC sequestration such as climate conditions and vegetation communities control the rate of in-situ carbon inputs via biomass production (Lemus & Lal, 2005). Hobley & Willgoose, (2010) found that climate is the primary driver of SOC dynamics at the surface soil layers (SOC stocks decrease with increases in temperature and increase with precipitation rates) and the influence of clay content on SOC dynamics increases with depth.

Climate

During the year prior to and over the course of the demonstration Project operations, some climatic records (Natural Regions Committee, 2006) suggested that the demonstration Project experienced mild drought conditions, while other records indicate that the soil moisture conditions were relatively normal (Alberta Agriculture and Forestry, 2019). Over the 2012-2018 period, the demonstration Project region experienced slightly cooler temperatures (-0.2°C) and substantially less precipitation (-28%) than is typical for the Foothills Fescue Natural Subregion (see Table A 1). Despite the relatively low precipitation rates for the Natural Subregion, the standardized precipitation index (SPI) maps published by Alberta Agriculture and Forestry (2019) indicated that the demonstration Project site location predominantly experienced near normal moisture conditions between 2012 and 2018. Although the potentially drier soil conditions may have inhibited willow establishment and biomass production early on during the project, the well-developed root system and vegetation shaded soil of the 5-year-old plantation will better protect the plantation for impacts of future drought conditions.

Agriculture Soil Capability

In terms of the soils biomass productivity potential, the agricultural soil capability of the local region was categorized as ‘2M’ - meaning that the site has slight limitations for small grain agricultural crops due to moisture deficiencies (Alberta Forestry and Agriculture, 2016). The Soil Research Institute, (1976) categorized the site as ‘poor’ due to soil moisture limitations and low pH (<5.5 pH).

Soil Carbon Inputs

Carbon inputs within the Study Area include 45 Mg ha^{-1} of dewatered biosolids that were 27% organic carbon (dry weight) (see Table B 7) and vegetation biomass inputs from the root and leaf litter of the crop and willow vegetation covers. Willow biomass production (root and shoot) rates can vary substantially based on the, climate, soil conditions, and physiological characteristics of each willow cultivar (Amichev et al., 2012; Cunniff et al., 2015; Garten et al., 2011). Cornell University (2017) reported that the average willow plantation in North America produces 11.3 Mg $ha^{-1}yr^{-1}$ of dry biomass, while a four year study conducted in Quebec, (Zan et al., 2001) found that willows can generate approximately 1.71 Mg ha^{-1} yr^{-1} of above ground biomass and 1.25 Mg ha^{-1} yr^{-1} of below ground biomass on marginal lands. In a willow biomass production and allocation study conducted by Rytter (2001), the root biomass the *Salix viminalis* from year 1 to year 3 shifted from 25%-30% to 10%-20% of the total biomass. Although this estimate would be substantially higher if fine root biomass were included in the calculation (Cunniff et al., 2015). Data on willow fine root biomass turn-over is not readily available because measuring it is technically difficult and labor intensive (Cunniff et al., 2015). In comparison to the Rytter (2001) study, SYLVIS (J. Lavery, personal communication, March 15, 2019) reported that the mean willow biomass harvest rates for the Milbrook cultivar at the demonstration Project was 19.4 ±3.3 Mg ha^{-1} (n=4) of dry biomass mass which equates to an annual rate of 6.5 ± 1.1 Mg ha^{-1} yr^{-1} of dry biomass during the first five years of operation. Assuming that Milbrook cultivar root-shoot ratio physiology is similar to the *Salix viminalis*, this would equate to an estimated total of 1.94-3.88 Mg ha^{-1} of dry below ground willow biomass after three years of growth. Table 5 provides a high-level summary of the estimated soil organic carbon inputs based on soil organic matter inputs from the biosolids and the willow biomass.

Table 5: High-level estimate of organic carbon inputs in the Willow + Biosolids Area of the Demonstration Project Site.

Soil Organic Matter Inputs	Proportion of Organic Carbon Within Organic Matter Inputs (Dry Mass)	Estimated Organic Carbon Inputs (Dry Mass)
2013 Biosolids Land-Spreading Application	24 Mg ha^{-1} at 27% Carbon	6.48 Mg C ha^{-1}
2013- 2016 Estimated Willow Leaf Litter Production	Unknown	NA
2013-2016 Estimated Study Area Willow Root Litter Production	10%-20% (Rytter, 2001) of Willow Shoot Production (19.4 ±3.3 Mg ha^{-1})	1.94-3.88 Mg ha^{-1}
2016 Biosolids Land-Spreading Application	21 Mg Dry Tons ha^{-1} at 27% Carbon	5.67 Mg C ha^{-1}
2016-2018 Leaf Litter Production	Unknown	NA
Total Estimate of Known Carbon Inputs (2013-2016)		**14.09 -16.03 Mg C ha^{-1}**

5.8 Determining Factors of Soil Organic Carbon Sequestration

Determining factors of SOC stocks and SOC sequestration rates are represented by the physical and chemical constraints of a soil system such as the total volume and the concentration of chemical bonding sites available to store and protect SOC compounds from SOC decomposition agents (Lemus & Lal, 2005).

Percent Soil Organic Carbon

Within the Study Area the mean percent soil organic carbon (%SOC) content ranged from 1.64 ± 0.04 % (Control) to 1.88 ± 0.08% (C+BS) at the 0-15 cm depth interval, and from 1.04 ± 0.04% (C+BS) to 1.41 ± 0.09 % (W+BS) at the 15-30 cm depth interval. The mean SOC stocks and standard error of the mean bars (SEM) for each sampling location and depth interval are presented in Figure 9.

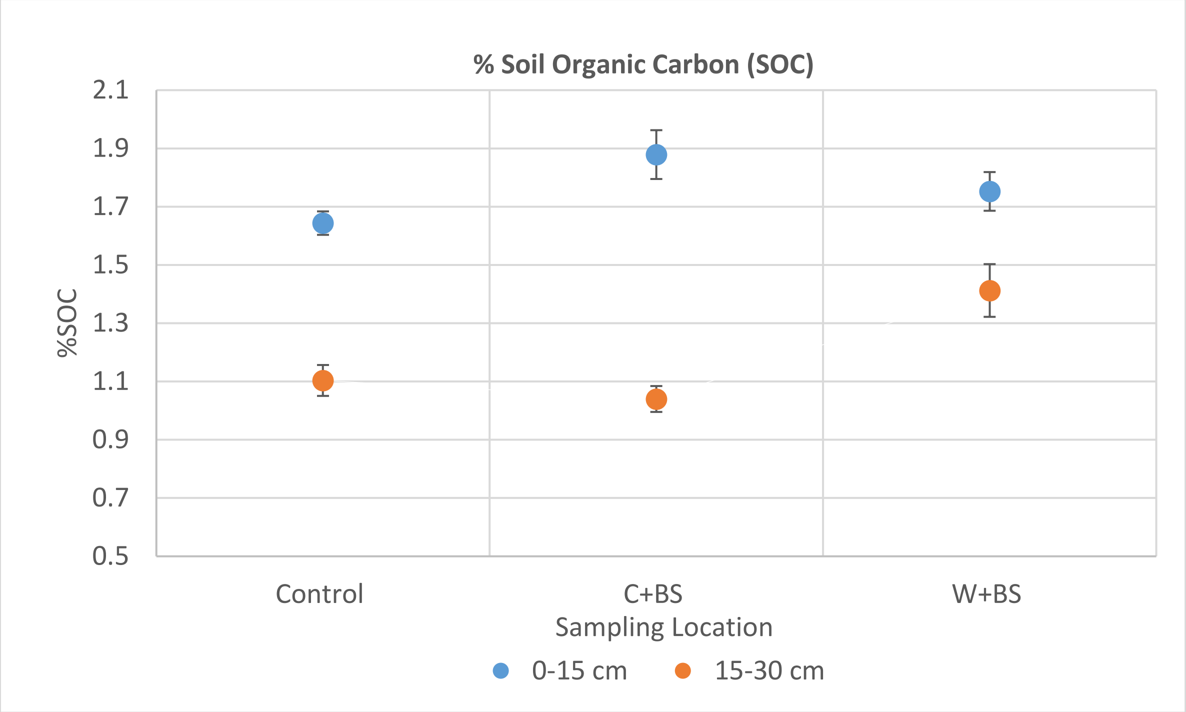

Figure 9: Mean percent (%) Soil Organic Carbon and standard error of the mean bars for each sampling location and sampling depth interval combination.

When testing the parametric assumptions of the %SOC data, the data from the 0-15 cm sampling depth intervals passed the normal distribution test (Shapiro-Wilk test; p=0.05) and failed the variance of the residuals homogeneous test (Levene's test; p=0.05). Therefore, the Welches ANOVA was used to compare the means of %SOC content between sampling locations at the 0-15 cm depth interval. The Welches ANOVA indicated that at least one significant difference was detected between the mean %SOC content data between two or more sampling locations at the 0- 15 cm (df = 43.68, F = 3.51, p <0.05). A post hoc pairwise comparison of the data conducted using the Games-Howell test. The results indicated that the mean %SOC content at the C+BS sampling location was significantly higher than both the Control sampling location (p=0.05). The pairwise comparisons of %SOC content results from the 0-15 cm depth interval are presented in Table 6.

When testing the parametric assumptions of the %SOC data of the 15-30 cm sampling depth interval, the data failed both the normal distribution test (Shapiro-Wilk test; p=0.05) and the variance of the residuals homogeneous test (Levene's test; p=0.05). Therefore, the data was log(10) transformed and reanalyzed. When testing the parametric assumptions of the log(10) transformed %SOC data, the data failed the normal distribution test (Shapiro-Wilk test; p=0.05) and passed the variance of the residuals homogeneous test (Levene's test; p=0.05). Therefore, the Kruskal-Wallis test was used to compare the means of %SOC content between sampling locations at the 15-30 cm depth interval. The Kruskal-Wallis analysis indicated that at least one significant difference was detected within the log(10) %SOC content data between two or more sampling locations (df = 2, H= 11.195, p=<0.05). A pairwise comparison of the %SOC content within the Kruskal-Wallis outcomes was conducted using the post hoc Dunn's Test adjusted with the Holm-Bonferroni correction. The pairwise comparison results (Table 7) indicated that the mean %SOC content at the W+BS sampling location was significantly higher (p=0.05) than both the Control, and C+BS sampling locations at the 15-30 cm depth interval.

Table 6: Pairwise comparison of mean %SOC content between sampling locations at the 0-15 cm depth interval using the post hoc Games-Howell test (p = 0.05) within the Welches ANOVA outcomes (bold text highlights significant outcomes)

Pair wise Comparisons of Sampling Locations	df	t	p
W+BS vs Control	**39**	**2.95**	**0.01**
Control vs C+BS	47	0.92	0.63
W+BS vs C+BS	**35**	**3.69**	**<0.01**

Table 7: Pairwise comparisons of mean log(10)%SOC content results between sampling locations at the 15-30 cm sampling depth interval within the Kruskal-Wallis outcomes using a post hoc Dunn's Test (p = 0.05). Results were adjusted with the Holm-Bonferroni correction for detecting significant differences (bold text highlights significant outcomes)

Pairwise Comparisons of Sampling Locations	Z	P_{adj}
Control vs W+BS	**-2.52**	**0.04**
Control vs C+BS	-0.65	1.00
W+BS vs C+BS	**-3.17**	**0.004**

Soil Bulk Density

The potential for soil to store SOC per unit volume of soil increases with soil bulk density (Lemus & Lal 2005). However trade-offs occur at soil bulk densities around 1.4 g/cm^3 to 1.6 g/cm^3 when vegetation growth and associated soil carbon inputs are restricted (Chaudhari, Ahire, Ahire, Chkravarty, & Maity, 2013). Within the Study Area, the mean soil bulk density did not exceed 1.4 g/cm^3. Across the Study Area, the mean soil bulk density ranged between 1.13 g/cm^3 (C+BS) to 1.25 g/cm^3 (Control) at the 0-15 cm sampling depth interval and 1.28 g/cm^3 (C+BS) to 1.38 g/cm^3 (Control) at the 15-30 cm sampling depth interval. The mean soil bulk density and SEM for each sampling depth interval and sampling location are presented in Figure 10. The Study Area soil bulk density results were consistent with typical soil bulk densities associated with the Midnapore, Rockyview and Delacour soil series (Government of Canada, 2013) (See Table A 2 and Table A 3).

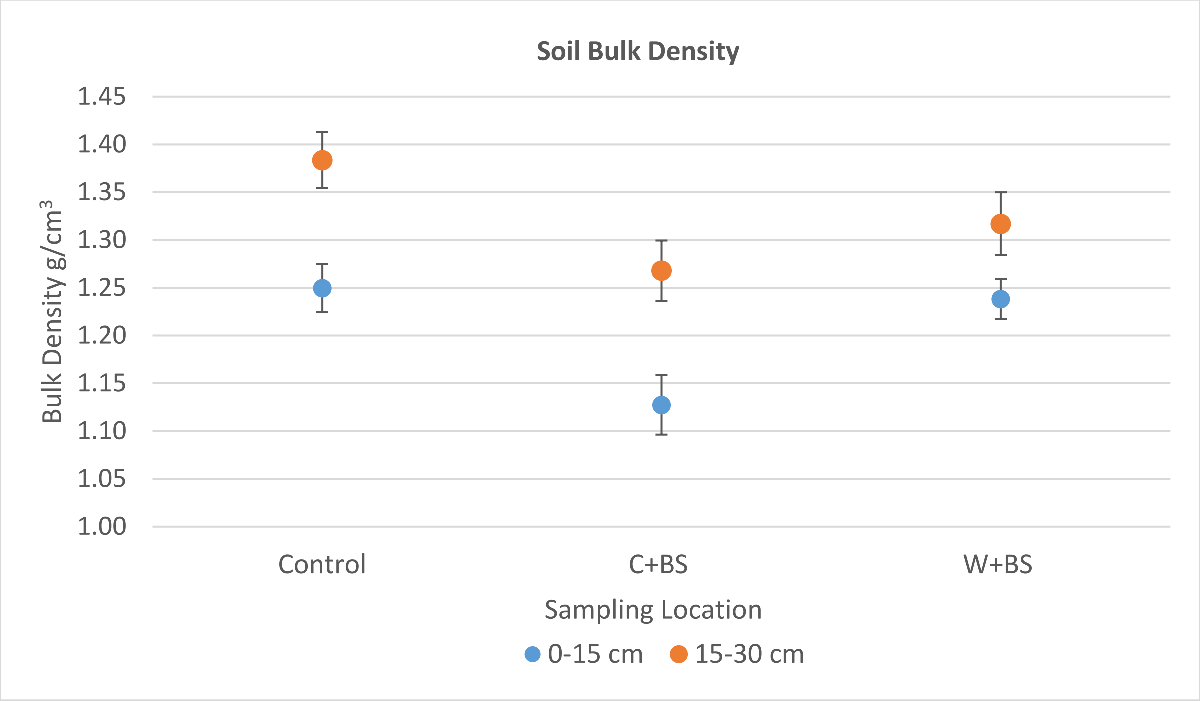

Figure 10: Soil bulk density (g/cm3) mean and standard error of the mean bars for each sampling location and depth interval combination

When testing the parametric assumptions of the soil bulk density data, the data from both sampling depth intervals failed the normal distribution test (Shapiro-Wilk test; p=0.05) and passed the variance of the residuals homogeneous test (Levene's test; p=0.05). Therefore, the non-parametric Kruskal-Wallis analysis was used to compare the median soil bulk density data between sampling locations at each depth interval. The Kruskal-Wallis analysis indicated that at least one significant difference was detected within the median soil bulk density data between two or more sampling locations at both the 0- 15 cm (df= 72 H= 13.79, p=<0.01) and the 15-30 cm (df= 72 H= 6.66, p= 0.03) sampling depth intervals. A pairwise comparison of the median soil bulk densities within the Kruskal-Wallis outcomes was conducted using the post hoc Dunn's Test adjusted with the Holm-Bonferroni correction. The pairwise comparison results indicated that the median soil bulk density at the C+BS sampling location was significantly lower (p=0.05) than the median densities at both the Control and W+BS sampling locations at the 0-15 cm depth interval. At the 15-30 cm depth interval, the pairwise comparisons using the post hoc Dunn's Test indicated that the median soil bulk density at the C+BS sampling location was significantly lower than the Control sampling location (p=0.05). The pairwise comparisons of median soil bulk density results are presented in Table 8.

Table 8: Pairwise comparisons of median soil bulk density results between sampling locations at each sampling depth interval within the Kruskal-Wallis outcomes using a post hoc Dunn's Test (p = 0.05). Results were adjusted with the Holm-Bonferroni correction for detecting significant differences (bold text highlights significant outcomes)

Pairwise Comparisons of Sampling Locations	Sampling Depth (cm)	Z	P_{adj}
Control vs W+BS	0-15	0.67	0.50
Control vs C+BS	**0-15**	**-3.50**	**<0.01**
W+BS vs C+BS	**0-15**	**-2.83**	**<0.01**
Control vs W+BS	15-30	-1.12	0.30
Control vs C+BS	**15-30**	**-2.57**	**0.03**
W+BS vs C+BS	15-30	1.45	0.26

Across the 15-30 cm depth interval, the significantly lower median soil bulk density at C+BS sampling location compared to the other sampling sites likely inhibited the SOC stock potential by suppressing the total mass of SOC that could be sequestered within the volume of soil sampled.

Soil bulk density tends to increase with depth due to shifts in organic matter content, porosity and compaction (Chaudhari et al., 2013). As expected, the non-parametric Wilcox signed rank test results indicated that the median soil bulk densities at the 15-30 cm depth interval were significantly higher (p=0.05) than the median soil bulk densities at the 0-15 cm depth interval at the Control and the C+BS sampling locations. The results for comparing median soil bulk densities across sampling locations between sampling depths using the Wilcox signed rank are presented in Table 9.

Table 9: Wilcoxon signed rank test (p = 0.05) results comparing the median soil bulk density results between the sampling depth intervals at each sampling location (bold text highlights significant outcomes

Sampling locations	n	V	p
Control	**25**	**27.5**	**<0.01**
C+BS	**25**	**39.5**	**<0.01**
W+BS	25	94.5	0.11

Although the median soil bulk density at the 15-30 cm depth interval of the W+BS sampling location was lower than the 0-15 cm depth interval, the difference was not significant. The lack of significant differences in soil bulk density detected between sampling depth intervals at the W+BS sampling location was potentially caused by a combination of factors including; an increase in bulk density 0-15 cm layers bulk density resulting from 5 years of no-till (Rytter, 2016; Stahlman et al., 2009), and a decrease in soil bulk density at the 15-30 cm layers bulk density resulting from willow roots penetrating the soil matrix (Kahle, Hildebrand, Baum, & Boelcke, 2007; Lemus & Lal, 2005; Lockwell et al., 2012), or a sample size that was too small to detect a difference.

Soil Organic Carbon Stocks

The carbon sequestration potential of a new SOC sink project site depends on the initial SOC stock relative to the SOC stock saturation i.e. steady-state potential of the current land management practice (Thamo & Pannell, 2016) (see Figure 1). Considering that agriculture has depleted North America's native prairie SOC stocks by approximately 25%-50% (Alberta Agriculture and Forestry, 2001; Lemus & Lal, 2005; Post & Kwon, 2000), and it takes approximately 20-40 years for SOC sink projects to reach SOC stock saturation (Corsi et al., 2012; Government of Alberta, 2012; Sommer & Bossio, 2014; West & Post, 2002), it is unlikely that SOC saturation will present a SOC sequestration constraint within Study Area during the next 10-15 years (Sommer & Bossio 2014; Corsi et al., 2012; West & Post, 2002; Government of Alberta, 2012). Within the Study Area the mean SOC stocks ranged from 30.8 ± 1.0 Mg C ha^{-1} (Control) to 32.6 ± 1.4 Mg C ha^{-1} (W+BS) at the 0-15 cm depth interval, and from 19.8 ± 0.9 Mg C ha^{-1} (C+BS) to 27.5 ± 1.6 Mg C ha^{-1} (W+BS) at the 15-30 cm depth interval. The mean SOC stocks and standard error of the mean bars (SEM) for each sampling location and depth interval are presented in Figure 11.The estimated increase in total SOC stocks across both sampling depths (0-15 cm and 15-30 cm) from 2013 to 2018 between the Control and the W+BS sampling locations was 7.02 ± 1.6 Mg C ha^{-1} which equates to approximately 50% of the total estimated sum of carbon inputs listed in Table 5.

When converting sequestered SOC stocks (Mg C ha^{-1}) to carbon offsets (Mg CO$_2$e ha^{-1}) using a conversion factor of 3.66 (relative difference between the mass of elemental carbon and the mass of CO$_2$) the estimated carbon offset potential of the demonstration Project after 5 years of operation was 25.7 ± 5.9 Mg CO$_2$e ha^{-1}. However as discussed below the differences in SOC stocks between sampling locations at the 0-15 cm were not significantly different and therefore further research is necessary to confirm these results.

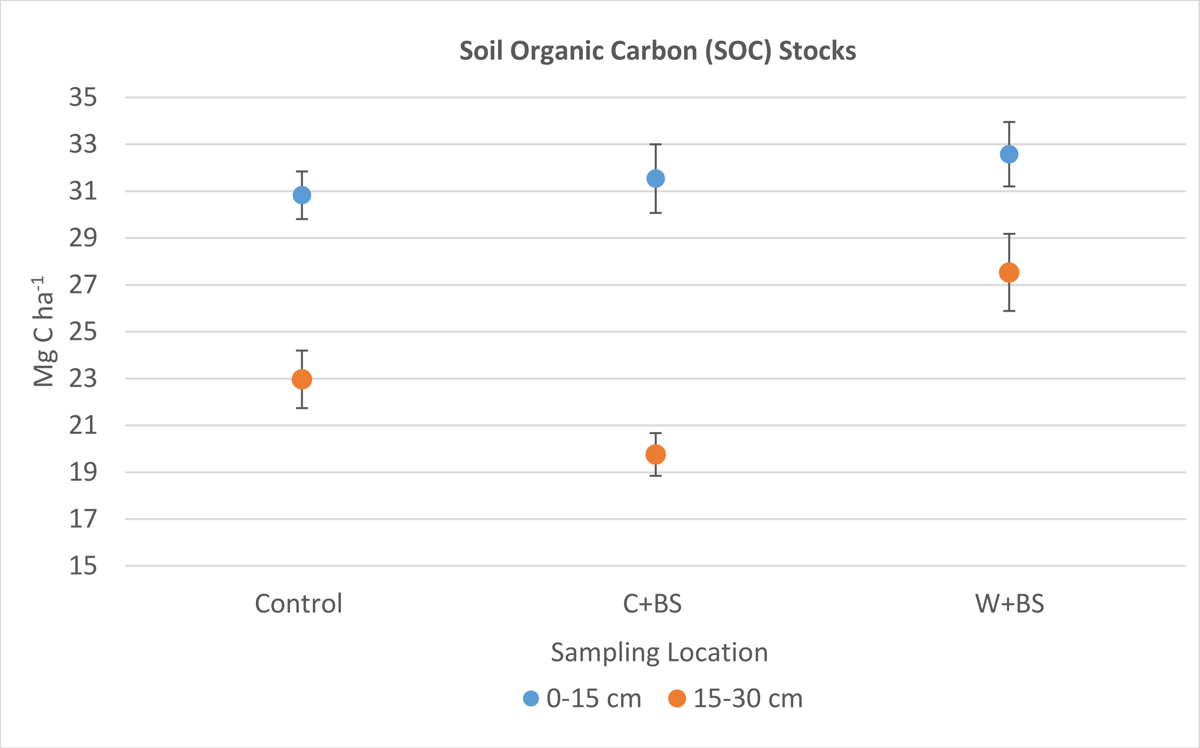

Figure 11: SOC stock (Mg C ha^{-1}) mean and standard error of the mean bars for each sampling location and sampling depth interval combination

When testing the parametric assumptions of the SOC stocks (Mg C ha^{-1}) the sampling data from both depth intervals (0-15 cm and 15-30 cm) were normally distributed (Shapiro-Wilk test; $p=0.05$), and only the 0-15 cm depth interval indicated that the variances of the residuals were homogeneous (Levene's test; $p=0.05$). Therefore, the Analysis of Variance (ANOVA) was used to compare the mean SOC stocks between sampling locations at the 0-15 cm depth interval and the Welches ANOVA was used to compare the means of SOC stocks between sampling locations at the 15-30 cm depth interval. The ANOVA indicated that no significant differences in SOC stock means were detected between sampling location SOC stock means at the 0-15 cm depth interval ($df = 72$, $F = 0.46$, $p = 0.63$). The Welch's ANOVA indicated that at least one significant difference in SOC stock means between sampling locations was detected at the 15-30 cm depth interval ($df = 72$, $F = 8.85$, $p <0.01$). A post hoc pairwise comparison of the mean SOC stocks at the 15-30 depth interval was conducted using the Games-Howell test. The results indicated that the mean SOC stocks at W+BS sampling location were significantly higher than both the C+BS and the Control sampling locations ($p=0.10$) at the 15-30 cm depth interval. The pairwise comparisons of mean SOC stocks at the 15-30 cm depth interval are presented in Table 10.

Table 10: Pairwise comparison of mean SOC stocks between sampling locations at the 15-30 cm depth interval using the post hoc Games-Howell test (p = 0.10) within the Welches ANOVA outcomes (bold text highlights significant outcomes)

Pair wise Comparisons of Sampling Locations	t	p
W+BS vs Control	**2.2**	**0.08**
Control vs C+BS	2.0	0.11
W+BS vs C+BS	**4.1**	**<0.01**

Because significant difference in bulk density were detected between sampling locations at each sampling depth, questions arises on whether soil bulk density or %SOC content was responsible for driving or inhibiting differences detected in SOC stocks between sampling locations. Figure 12 presents the measured SOC stocks at the Study site with arrows indicating which sampling location results had significantly different %SOC and soil bulk density relative to one another at each sampling depth. These findings suggest that differences in soil bulk density between sampling locations at the 0-15 cm sampling depth potentially supressed the SOC stock outcomes at the C+BS sampling location relative to the Control and the W+BS sampling locations. However, these results do not indicate that differences in soil bulk density supressed or amplified the Control and W+BS SOC stocks relative to one another. At the 15-30 cm sampling depth interval, it appeared that the significantly higher %SOC content at the W+BS sampling location relative to the other two sampling sites was the main driver of SOC stocks and that any potential amplification or suppression of SOC stocks resulting from differences in soil bulk density should only be considered when assessing relative SOC stock differences between the Control and C+BS sampling locations.

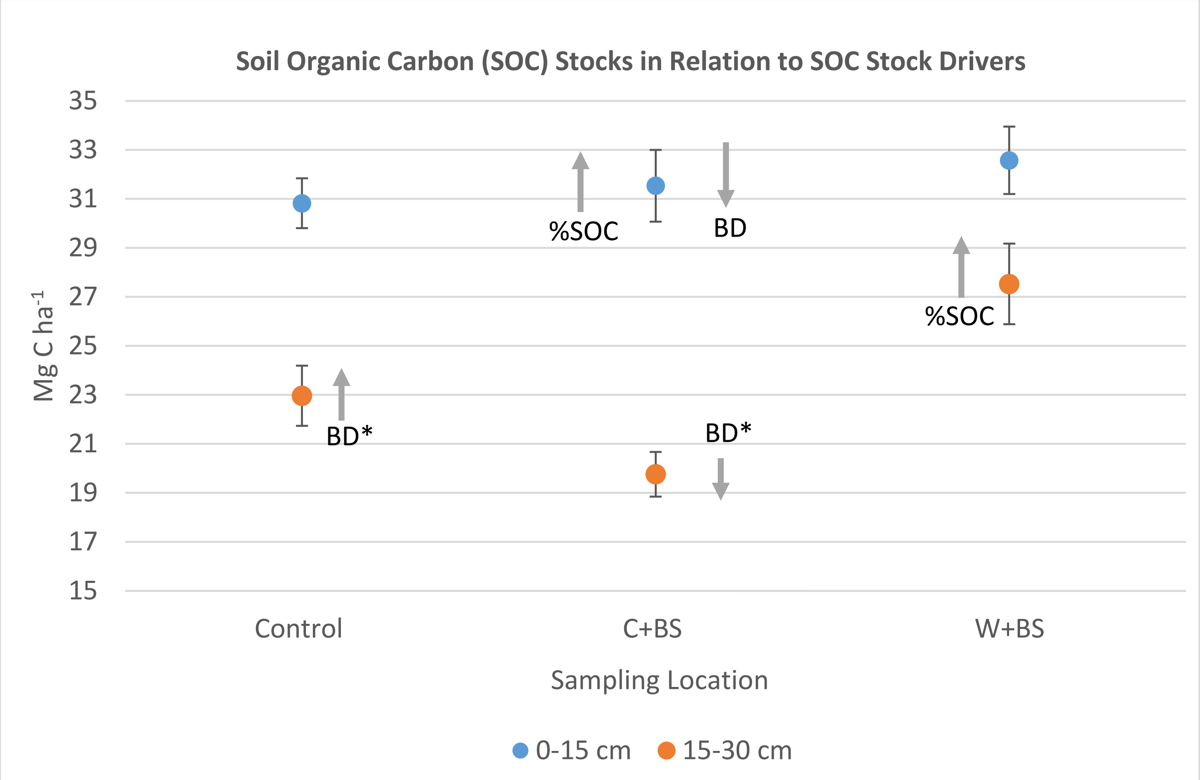

Figure 12: Potential influence of significant differences in percent soil organic carbon (%SOC) content and soil bulk density (BD) between sampling locations that may have affected the SOC stock outcomes. Note: *indicates that significant differences in BD at the 15-30 cm depth interval only occurred between the Control and the C+BS sampling locations and not the W+BS sampling location.

Due to the inconsistent results reported within the literature for similarly designed SOC monitoring studies at local and global scales, the inconsistency of results between the 0-15 cm sampling depth and the 15-30 cm sampling depth intervals were anticipated. Out of the previous SOC sequestration studies reviewed (see section 3.6), the closest analog for this study based on biophysical and climatic conditions was the VandenBygaart, Gregorich, & Angers (2003) study of 12 no-till projects located across western Canada. This meta-study analysis reported a SOC sequestration rate of 10.61± 4.76 Mg CO_2e ha^{-1} yr^{-1} for 11.4 ± 1.5-year-old willow plantations at sampling depths ranging from 10-45 cm. Because the average age of the studies included in this Canadian-based meta-analysis were approximately twice as old as this five-year-old study, the outcomes of this no-till agriculture meta-analysis may be used as a benchmark for projecting future SOC stock trends at the Study Area. Moreover, the sampling depths of benchmark studies should also be considered. Studies with deeper sampling depths are expected to have higher SOC stock estimates than studies with shallower sampling depths.

Based on SOC sink project age and recommended management practice (RMP) treatments applied, two potentially more comparable SOC monitoring studies for the current demonstration Project conditions include a three year old willow plantation located in Southwestern Quebec (Zan, Fyles, Girouard, & Samson, 2001) and a five year old willow plantation study located in Sweden (Rytter, Rytter, & Högbom, 2015). The SOC sequestration rates of these projects ranged from no significant difference (sampling depth 30 cm) (Rytter, Rytter, & Högbom, 2015) to 0.15 Mg CO_2e ha^{-1} yr^{-1} (sampling depth 60 cm) (Zan, Fyles, Girouard, & Samson 2001). Remarkably, despite large variability and differences in the physical and chemical conditions of the soil properties influencing SOC stocks and SOC sequestration rates between sampling locations at the Study Area, detectible differences in SOC stocks between the Control and the W+BS sampling locations were still observed at this early stage in the demonstration Project and were substantially larger (3.3 ± 1.2 Mg CO_2e ha^{-1} yr^{-1}) than reported in similarly aged willow plantation studies. The higher SOC sequestration/ CO_2e offset results from this study relative to the other willow plantation studies indicates the SOC sequestration benefits of including carbon and nutrient rich biosolids into willow plantation systems.

Soil SOC stocks generally decrease with depth due to a decrease in biological activity (Don, Schumacher, Scherer-Lorenzen, Scholten, & Schulze, 2007; Franzluebbers, 2005; Olson & Al-Kaisi, 2015). The non-parametric Wilcoxon signed rank test was used to compare median SOC stocks between sampling depth intervals at each sampling location. As expected, the study results indicated that median SOC stocks decreased significantly with depth (p=0.10) at all three sampling locations within the Study Area. The Wilcoxon signed rank test results comparing median SOC stocks across sampling depths at each sampling location are presented in Table 11.

Table 11: Wilcoxon signed rank test (p = 0.10) results for comparing the median SOC stocks (Mg C ha-1) between the sampling depths at each sampling location (bold text highlights significant outcomes)

Sampling Locations	V	p
Control	**302**	**<0.01**
C+BS	**325**	**<0.01**
W+BS	**232**	**0.06**

Notably, the difference in median SOC stocks between sampling depth intervals was the least pronounced at the W+BS sampling location (p=0.06). This outcome is potentially a result of the willow root litter releasing more carbon compounds into the 15-30 cm depth interval relative to the agricultural crop root litter production at the Control and C+BS sampling locations (Hu et al., 2016; Olson & Al-Kaisi, 2015). After investigating SOC contributions of willow root and leaf litter, Hu et al., (2016) concluded that willow root litter is the main driver of SOC sequestration on marginal agricultural land afforested with willow. Hu et al., (2016) suggests that fine root litter contributes more to SOC sequestration and stabilization than leaf litter because root exudes support soil aggregate formation which physically protects SOC compounds from decomposition agents. Root litter also has a higher proportion of recalcitrant carbon compounds (e.g. lignin) that are more resistant to decomposition than the more labile leaf litter compounds Hu et al., (2016). This differential SOC stock contribution from root and leaf litter could provide some explanation into why significantly higher median SOC stocks were detected at the W+BS sampling location compared to the other sampling locations at the 15-30 cm depth interval.

Soil pH

Since pH is influenced by soil mineral cation concentrations (e.g. calcium and magnesium), soil pH is a strong predictor for SOC stabilization mechanisms (microbial activity, vegetation growth, soil aggregate structure, etc.) (Rowley et al., 2018). Neutral pH ranges (6.5 to 7) are optimal for biomass production and cation exchange capacity, while strongly to very strongly acidic soil conditions tend to suppress SOC Stocks and sequestration rates by reducing bio-available cation concentrations, inhibiting vegetation growth, and suppressing soil microbial activity (Alberta Agriculture and Food, 2008 pg. 42-44).

Within the Study Area, the mean soil pH levels ranged from 4.91 (very strongly acidic) to 5.31 (strongly acidic) at the 0-15 cm depth interval and 5.34 (strongly acidic) to 6.03 (slightly acidic) at the 15-30 cm depth interval (Alberta Agriculture Food and Rural Development, 2002). In general, the Study Area mean pH results were more acidic than are typical for the soil series associated with this location; Midnapore (pH = 6.9), Rockyview (pH = 7.4), Delacour (pH = 6.0) (See Table A 2 and Table A 3). The study results for the mean and standard error of the mean of the soil pH for each sampling location and sampling depth interval combination are presented in Figure 133.

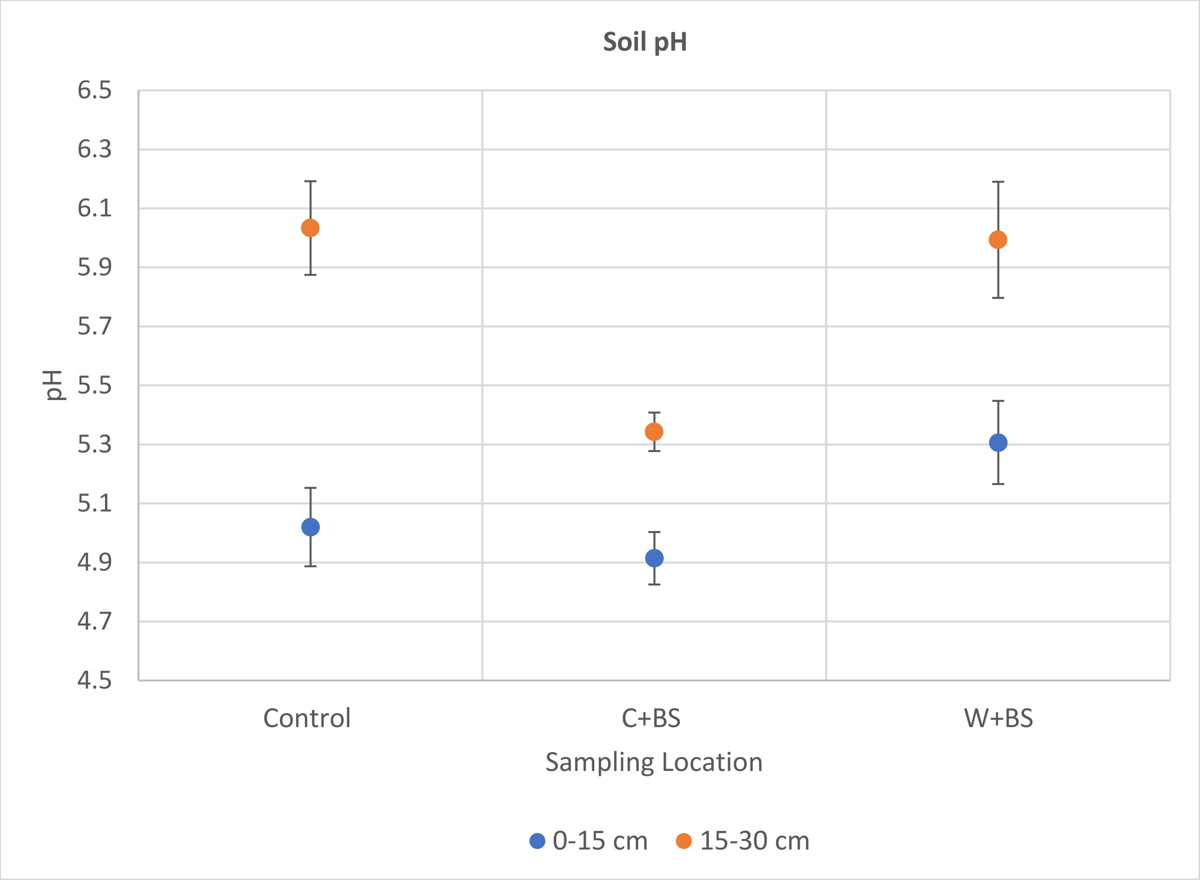

Figure 13: Soil pH mean and standard error bars for each sampling location and sampling depth interval

When testing the parametric assumptions of the pH data, the 0-15 cm depth interval data failed the normal distribution test (Shapiro-Wilk test; p=0.05) and passed the variance of the residuals homogeneous test (Levene's test; p=0.05); while the 15-30 cm depth interval passed the normal distribution test and failed the variance of the residuals homogeneous test. Therefore, the non-parametric Kruskal-Wallis was used to compare the median soil pH between sampling locations at the 0-15 cm depth interval and the Welches ANOVA was used to compare the mean soil pH between sampling locations at the 15-30 cm.

The Kruskal-Wallis indicated that there were no significant differences in median soil pH between sampling locations at the 0-15 cm sampling depth interval (df= 41, H=4.55, p = 0.10). The Welches ANOVA indicated that there was at least one significant difference in mean soil pH between two or more sampling locations at the 15-30 cm depth interval (df = 22, F=11.34, p<0.01).

The post hoc pairwise comparison of the 15-30 cm depth interval data using the Games-Howell test indicated that the mean soil pH at the C+BS sampling location was significantly lower both the Control and the W+BS sampling locations (p<0.05). The results of the pairwise comparison of mean soil pH means within the Welches ANOVA results at the 15-30 cm using a post hoc Games-Howell test (p = 0.05) are presented in Table 12.

Table 12: Pairwise comparison of the mean pH results at each sampling location across the 15-30 cm sampling depth interval using the post hoc Games-Howell test (p = 0.05) within the Welches ANOVA results (bold text highlights significant outcomes)

Pairwise Comparisons of Sampling Locations	Sampling Depth (cm)	t	df	p
Control vs W+BS	15-30	0.16	27	0.99
Control vs C+BS	**15-30**	**4.02**	**19**	**<0.01**
W+BS vs C+BS	**15-30**	**3.14**	**17**	**0.02**

Because soil pH is a key driver of SOC sequestration and retention rates, the significantly lower pH levels at the 15-30 cm depth interval of C+BS sampling location likely suppressed the sites SOC sequestration and retention potential relative to the other two sampling locations. Therefore, it was unclear if the significantly lower SOC stocks at the 15-30 cm sampling depth interval of the C+BS sampling location were a result of the land management practice at this location and depth or because of the relatively low pH levels.

Soil Texture

Out of the three main soil fractions (sand, silt, and clay), clay has the greatest positive influence on SOC sequestration rates and retention periods due to its relatively high mineral concentrations (Lal, 2002; McClean et al., 2015; Zhao, Sun, Zhang, Yang, & Drury, 2006). Likewise, sandy soils tend to have more variable SOC stocks and shorter SOC retention periods due to their lower mineral concentrations (Necpálová et al., 2014; Wijesekara et al., 2017). The soil within the Study Area was sandy loam with average clay contents ranging between 9%-12% which were approximately 10%-15% lower than typical clay content ranges for the Midnapore, Rockyview, and Delacour soil series present within the Study Area (see Table A 2 and Table A 3) (Government of Canada, 2013).

Figure 14 and Figure 15 present the soil texture measured at each sampling location and the 0-15 cm and the 15-30 cm depth interval respectively as a percentage of the mean percent (%) weight composition and standard deviation bars of the sand (50 µm - 2 mm), silt (2 µm - 50 µm) and clay (<2 µm) soil fractions. Standard deviations bars were selected instead of standard error of the mean bars because the variations of percent soil fractions were too small for the standard error bars to be visible when graphed.

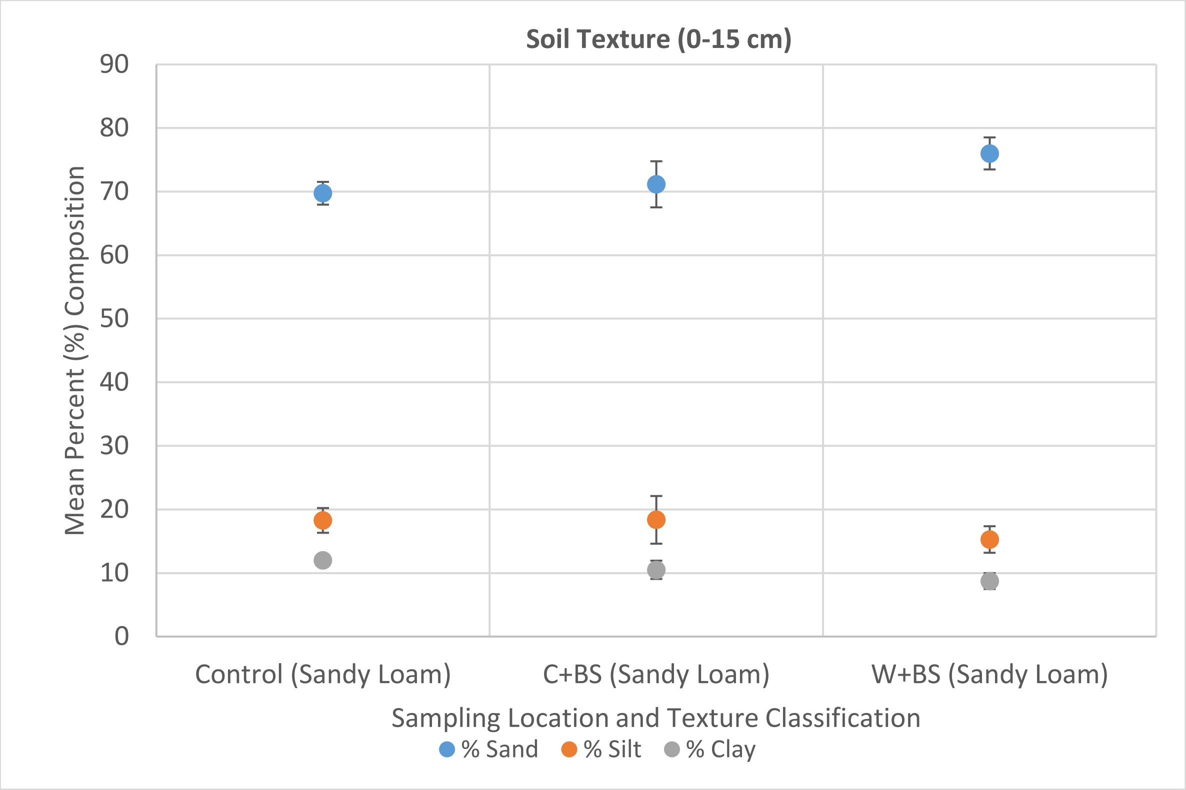

Figure 14: Soil classification and texture at the 0-15 cm depth interval of each sampling location represented by the mean percent (%) composition of each soil fraction (sand, silt, and clay) and standard deviation bars

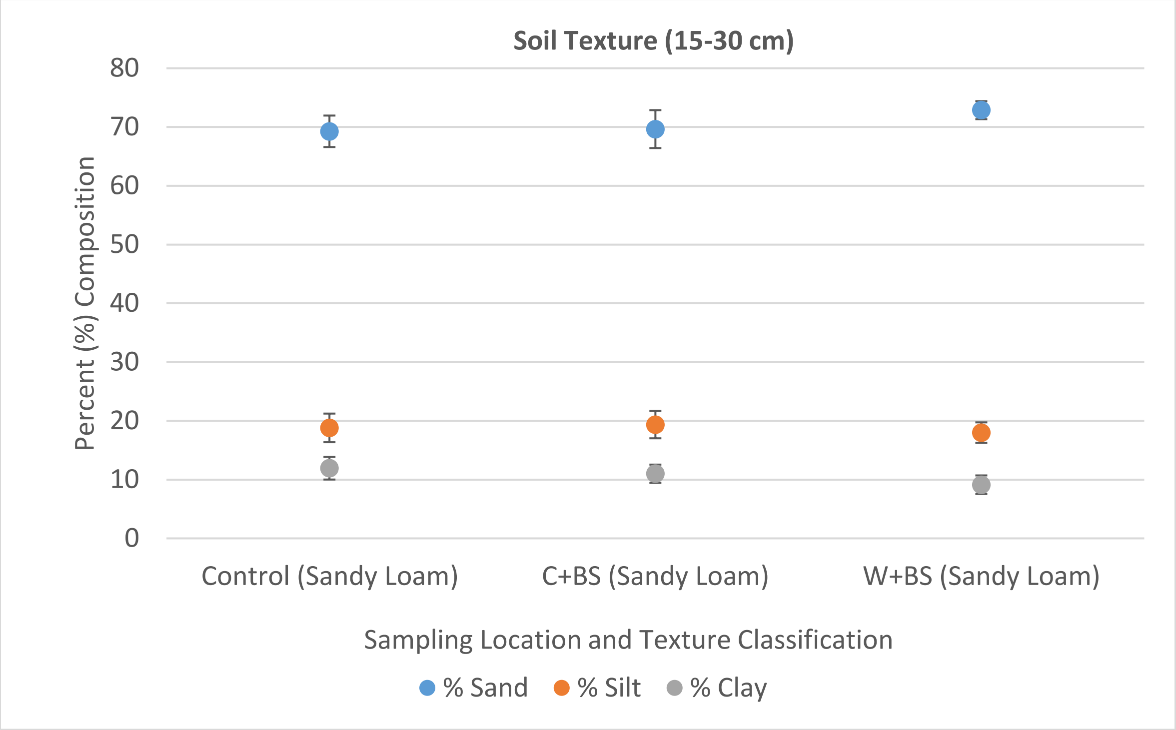

Figure 15: Soil classification and texture at the 15-30 cm depth interval of each sampling location represented by the mean percent (%) composition of each soil fraction (sand, silt, and clay) and standard deviation bars

When testing the parametric assumptions of the soil clay content data, both depth intervals (0-15 cm and 15-30 cm) failed the normal distribution test (Shapiro-Wilk; p=0.05) and passed the variance of the residuals homogeneous test (Levene's test; p=0.05). Therefore, the non-parametric Kruskal-Wallis analysis was used to compare the median percent clay content between sampling locations at each depth interval. The Kruskal-Wallis indicated that at least one significant difference was detected within the median percent clay content results between two or more sampling locations at both the 0-15 cm (χ^2= 26.5, df = 2, p < 0.01) and the 15-30 cm (χ^2= 16.6, df = 2, p = < 0.01) sampling depth intervals. Therefore, a pairwise comparison between sampling locations across each sampling depth interval was conducted using a post hoc Dunn's Test adjusted with the Holm-Bonferroni correction. The pairwise comparisons of mean percent clay content across sampling depth intervals and between sampling locations are presented in Table 13.

Table 13: Pairwise comparisons of mean percent clay content across sampling depth intervals and between sampling locations within the Kruskal-Wallis outcomes using a post hoc Dunn's Test (p = 0.05) results adjusted with the Holm-Bonferroni correction (bold text highlights significant outcomes)

Pairwise Comparisons of Sampling Locations	Sampling depth (cm)	Z	p_{adj}
Control vs W+BS	**0-15**	**5.15**	**<0.001**
Control vs C+BS	**0-15**	**-2.42**	**0.023**
W+BS vs C+BS	**0-15**	**2.64**	**0.047**
Control vs W+BS	**15-30**	**4.05**	**<0.001**
Control vs C+BS	15-30	-1.62	0.31
W+BS vs C+BS	15-30	2.36	0.06

Within the Study Area, the W+BS sampling location exhibited the significantly lower median percent clay content than both the C+BS and the Control sampling locations at the 0-15 cm depth interval, and the Control sampling location at the 15-30 cm depth interval (p=0.05). Because clay content is a key driver of SOC sequestration and retention rates, the lower clay content at the W+BS sampling location may have suppressed the W+BS SOC sequestration and retention potential relative to the Control and C+BS sampling locations.

Soil Minerals

Soil minerals, especially calcium and magnesium cations, play an important role in SOC sequestration and stabilization through the development of organo-mineral complexes (Grigal & Berguson, 1998; Heim, Wehrli, Eugster, & Schmidt, 2009; Merino, Nannipieri, & Matus, 2015). These organo-mineral complexes support SOC compound stabilization by providing both physical and chemical protected from microorganism degradation. Soil mineral concentrations are influenced by several biophysical, chemical, and land management factors including; slope position, parent material, pH, soil drainage patterns, clay content, and soil amendments (Heim et al., 2009; Jodral-Segado, Navarro-Alarcón, De La Serrana, & López-Martínez, 2006).

Soil minerals were not analyzed during this study. However SYLVIS collected the soil mineral (calcium, magnesium, iron, and sodium) and soil chemistry (pH, cation exchange capacity) data during routine environmental monitoring events and the soil data for immediately before (2013) the first biosolids application and a year after (2017) the biosolids application are presented in Appendix B (Table B 8 and Table B 9).Appendix B (Table B8) The SYLVIS environmental monitoring results indicated that the Study Area soils are non-calcareous which is consistent with regional soil studies (Alberta Forestry and Agriculture, 2016). The Study Area magnesium concentrations were within the range of values (60-300 mg/Kg) which is categorized as medium by Horneck, Sullivan, Owen, & Hart, (2011). Regional maps of relative exchangeable calcium and magnesium concentrations in Alberta soils were not available. The canola council of Canada (2017) indicates that calcium and magnesium deficiencies are rare in Alberta soils, although calcium deficiencies are possible in soils with strong acidity and sandy textures.

Cation Exchange Capacity

Cation Exchange Capacity (CEC) is an estimate of soil's capacity to retain and release positively charged ions such as calcium ($Ca^{2+),}$ magnesium (Mg^{2+}), potassium (K^{+}), sodium (Na^{+}), hydrogen (H^{+}) and aluminum (Al^{3+}) and is an indicator of soil fertility (Alberta Agriculture and Food, 2008 pg. 49). Soils with high CEC tend to have high clay and organic matter content while with soils with low CEC tend to have high sand and low organic content (Alberta Agriculture and Food, 2008 pg. 109). The CEC was not measured during this study, however the SYLVIS 2013 environmental monitoring results (n=4)Appendix B (Table B8) show that the average CEC within the Study Area was on the high end of the typical CEC range (5-15 meq/100g) for sandy loams in Alberta (Alberta Agriculture and Food, 2008 pg. 109). The CEC does not drive SOC sequestration, however it is a helpful indicator of a site's potential to sequester and stabilize SOC stocks.

5.9 Reducing Factors of Soil Organic Carbon Sequestration

Reducing factors are land management practices and site conditions which degrade or deplete SOC stocks by accelerating SOC decomposition rates (Smith et al., 2012). Any land management practice or site condition that increases exposure of SOC compounds to oxidative or decomposition agents will reduce SOC stocks. Soil microbial communities are key regulators of SOC dynamics and their influence on SOC sequestration and respiration rates depends on how often they are disturbed, how well they are protected from predators and decomposition agents, and what types of organic matter they have access to consume (Six, Frey, Thiet, & Batten, 2006).

Microbial community composition was not monitored during this study or during the historic SYLVIS environmental monitoring events. However, because microbial communities are responsible for the partitioning of fresh organic matter into carbon dioxide and SOC compounds, future monitoring of microbial activity and community composition in conjunction with SOC monitoring could provide valuable insights into the optimal conditions for SOC sequestration in Willow +

Biosolids systems. If effective, monitoring of fungal and bacterial community composition could also be used as a strategy to signal optimal timing of more intensive SOC stock monitoring events.

Tillage Practices

Soil disturbance is the primary mechanism of SOC depletion in agricultural systems (Post & Kwon, 2000). When soil aggregates are broken apart during soil disturbance events e.g. cultivation, harrowing, etc., the newly exposed SOC compounds become more susceptible to oxidation and microbial degradation (Smith et al., 2012). As little as one pass of cultivation can negate several years of SOC sequestration. As indicated in section 4.2, reduced tillage was practised at the C+BS sampling location and deep tillage was practised at the W+BS sampling location. During demonstration Project operations, the C+BS sampling location received reduced tillage annually and received numerous agriculture machinery passes during drill seeding and applications of fertilizer and pesticides as presented in Appendix B.

Tillage practices influence the composition of the microbial community, which in turn influences SOC sequestration and respiration rates. Regularly tilled soils of agricultural systems favor bacterial dominated microbial communities because the soil disturbances disrupt and break down fungal communities. No-till and agroforestry systems are believed to favor fungal dominated microbial communities (Lockwell et al., 2012) and sequester more SOC because fungal cells have a higher carbon to nitrogen (C:N) ratios than bacteria cells and fungal hypha support micro-aggregates formation which physically protect SOC compounds from decomposing agents (Six et al., 2006; Torri et al., 2014). Although there is still some debate on how other soil factors including chemical, physical, and biological characteristics play a role in how the microbial community compositions respond to tillage and agroforestry practices (Hydbom, 2017).

Soil Organic Matter Inputs

In some circumstances the addition of fresh organic matter to soil has been linked to temporary SOC stock losses (Corsi et al., 2012; Fontaine, Bardoux, Abbadie, & Mariotti, 2004; R. M. Rytter, 2016; Stockmann et al., 2013) that can last for several years (Pacaldo, Volk, & Briggs, 2013b). This phenomenon is known as "soil priming" and it is caused by an exponential surge in bacterial dominated microbial activity resulting from a rapid increase in labile organic matter (McClean et al., 2015). After the fresh labile organic matter is consumed, the exponentially larger microbial community begins consuming older and less labile compounds within the SOC stocks. The mechanisms controlling soil priming events occurring after influxes in labile organic matter are not fully understood, but it is presumed that priming events are largely dependent on the physio-chemical and biological conditions of the soil (McClean et al., 2015; Merino et al., 2015).

Future studies of microbial community composition in Willow + Biosolids systems would provide valuable insights into the optimal forms and rates of biosolids inputs that support SOC sequestration processes. Because willow/agroforestry systems generally favor fungal dominated microbial communities and biosolids are known to increase the proportion of soil bacteria (Cogger, Bary, Kennedy, & Fortuna, 2013), the competing community composition pressures could potentially explain unexpected periods of SOC stock losses or low sequestration rates during the Willow + Biosolids system project lifespan. Although biosolids landspreading has been linked to increased SOC sequestration rates, this outcome may largely depend on the relative application rates of biosolids land-spreading events. If biosolid application rates are too high, a rapid shift towards bacterial dominated microbial communities could reduce total SOC stocks. For example Jin, Johnson, Haney, & Arnold, (2011) reported that following an 8 year study of annual biosolids application SOC mineralization rates were significantly higher at sites which received a total of 45 Mg ha^{-1} or more of Class B biosolids. Meanwhile, SOC mineralization rates remained stable at biosolid application rates below 45 Mg ha^{-1}.

With regards to the Study Area, the significantly lower SOC stocks at the 15-30 cm depth interval of the C+BS sampling location relative to the Control and the W+BS sampling locations could be related to soil priming because willow roots were not available to absorb the excess biosolid nutrients and produce willow root exudes that were more resistant to decomposition than the labile biosolids carbon compounds.

5.10 Summary of SOC stocks and SOC dynamics Co-factor Results

The study data suggest that biosolids land-spreading application and willow plantation land management practices had insignificant to weakly positive effects on the SOC stocks within the Project Area. Because significant differences in the SOC dynamic cofactors between sampling locations were also detected, it was unclear to what degree the SOC stock levels were influenced by differences in land management practices and by differences in soil physio-chemical conditions across the Study Area. More time is required to conclusively assess the influence of Willow + Biosolids systems on SOC stocks. Based on the general consensus within the research community that SOC sink projects require a minimum of 10 years to generate conclusive results, it is recommended that the next intensive SOC stock monitoring event at the Study Area/demonstration Project site occur after 2023. This longer time period is expected to allow for the SOC sequestration effects of land management practices to override minor differences in the physio-chemical soil conditions between sampling locations.

Based on the SOC stock and SOC dynamics co-factors analyzed and considered in this study, provides a high-level overview of the net number of limiting, determining, and reducing factors identified at each sampling location within the Study Area. This table also highlights where soil conditions were not homogeneous between sampling locations which violates the assumption of the space-for-time study design. It is also important to note that only notes the net number of positive or negative influences on SOC dynamics considered between sampling locations and does not account for relative differences in strength between influencing factors.

Table 14: Summary of relative influences of limiting, determining and reducing factors on SOC stocks compared between Control, C+BS, and W+BS sampling locations

	Control	C+BS	W+BS	Notes
Legend: o = Negligible Influence; ? = Unknown Influence; - = Negative Influence; + = Positive Influence				
Limiting Factors				
Perennial Small Seed Crops vs. Annual Crops (Root and Leaf Litter Production)	o	o	+	The Milbrook willow cultivar at the W+BS sampling location produced an unknown mass of leaf litter and an estimated total of 1.94-3.88 Mg ha^{-1} of root litter during the first five years of project implementation. The root and leaf litter production of small grain agricultural crops are assumed to be negligible by comparison.
Determining Factors				
Baseline SOC Stocks	+	+	+	All the sampling locations within the Study Area are presumed to have started with the same level of depleted SOC stocks prior to demonstration Project implementation because of being subject to the same agricultural practices for several decades.
Bulk Density 0-15 cm	o	-	o	Across the 0-15 cm depth interval, the mean soil bulk density at the C+BS sampling location was significantly lower than the mean soil bulk densities at the Control and the W+BS sampling locations (p=0.05) (See section 5.8)
Bulk Density 15-30 cm	+	-	o	Across the 15-30 cm depth interval, the mean soil bulk density at the C+BS sampling location was significantly lower than the mean soil bulk density at the Control sampling location (p=0.05) (See section 5.8)

Table 14 continued: Summary of relative influences of limiting, determining and reducing factors on SOC stocks compared between Control, C+BS, and W+BS sampling locations

	Control	C+BS	W+BS	Notes
Legend: o = Negligible Influence; ? = Unknown Influence; - = Negative Influence; + = Positive Influence				
Soil pH 0-15 cm	o	o	o	Across the 0-15 cm depth interval, no significant differences in mean soil pH were detected between the three sampling locations (p=0.05) (see section 5.8)
Soil pH 15-30 cm	o	-	o	Across the 15-30 cm depth interval, the mean soil pH at the C+BS sampling location was significantly lower Control and the W+BS sampling locations (p=0.05) (see section 5.8)
Soil Texture 0-15 cm	+ +	o	-	Across the 0-15 cm depth interval, the mean percent (%) clay content at the W+BS sampling location was significantly lower than the Control and the C+BS sampling locations, and the mean %clay content at the C+BS sampling location was significantly lower than the mean %clay content at the Control sampling location (p=0.05) (See section 5.8)
Soil Texture 15-30 cm	+	o	-	Across the 15-30 cm depth interval, the mean percent % clay content at the W+BS sampling location was significantly lower than the mean % clay content at the Control sampling location (see section 5.8)
Soil Minerals 0-15 cm, 15-30 cm	?	?	?	Currently, there is insufficient data to compare soil mineral concentration data between sampling location
Cation Exchange Capacity (CEC) 0-15 cm, 15-30 cm	?	?	?	Currently, there is insufficient data to compare CEC data between sampling location.

Table 14 continued: Summary of relative influences of limiting, determining and reducing factors on SOC stocks compared between Control, C+BS, and W+BS sampling locations

	Control	C+BS	W+BS	Notes
Legend: o = Negligible Influence; ? = Unknown Influence; - = Negative Influence; + = Positive Influence				
Reducing Factors				
Site Preparation (2013) i.e. Biosolids Incorporation, and Deep Till	-	-	- -	During site preparation the Control and C+BS sampling location received reduced till and biosolids incorporation, while the W+BS sampling location received deep till and biosolids incorporation (see section 4.2)
Tillage Practices (2014-2018)	-	-	o	The W+BS sampling location was the only site where no-till agriculture was practiced
Soil Priming Induced by Biosolids Inputs	o	?	?	More research is required to evaluate the influence of biosolids on SOC dynamics and the circumstances in which biosolids land-spreading application rates or methods could shift SOC dynamics between net sequestration and respiration.
Net balance of the number of positive and negative factors influencing SOC stocks	**+4**	**-4**	**-2**	These results indicate that the Control sampling location had substantially better environmental conditions for stabilizing sequestered SOC than both of the W+BS and the C+BS sampling locations.

Based on the net balance results tabulated in , it appears that soil physio-chemical conditions put it at a SOC sequestration disadvantage relative to the Control and the C+BS sampling locations. Therefore, if the soil physio-chemical conditions had been more consistent across the Study Area, this study would have likely reported higher relative SOC stocks at the W+BS and the C+BS sampling locations compared to the Control sampling location.

Section 4—Synthesis

Chapter 6

Discussion, Recommendations, and Conclusions

6.1 Recommendations for Future Willow + Biosolids Project Field Study Design and Data Collection Methodologies

In order for Alberta soil-based carbon offset credits to become a viable trading commodity, SOC quantification protocols must satisfy stringent, methodological, statistical, auditing, and economic standards to earn market confidence (Poudyal, Siry, & Bowker, 2011). To reconcile these often competing data quality (validation and verification) and cost management objectives, SOC quantification protocols should be strategic in selecting study designs, biophysical parameters, soil analysis methods, and project locations to optimize study design statistical power and cost ratios. Quantification protocol study designs should also be (1) standardized enough to be universally reproducible, (2) flexible enough to accommodate Alberta's range of biophysical and climatic conditions, and (3) simple enough for landowners or other stakeholders to conduct easily.

Another important and potentially overlooked consideration for developing soil-based carbon offset quantification protocols are the perceptions, cultural norms, and expectations of the land owners considering adoption of COP21 RMPs to earn carbon offset credits. To develop successful soil carbon offset policy incentives that increase the "carbon farming" land base, SOC offset policies and marketing programs should be mindful of the various potential cultural, technical, and administrative barriers that could impede RMP adoption (Olander et al., 2011).

The following sections summarize the key field study design considerations, challenges, opportunities in need of attention before the environmental and societal value of developing and implementing a customized carbon offset protocol for Willow + Biosolids systems can be comprehensively assessed.

6.2 Field Study Designs for Measuring Soil Organic Carbon Stocks

One of the primary environmental barriers to cost effectively measuring SOC stocks accurately is the natural spatial and temporal variation that occurs across biogeophysical and climatic conditions that influences SOC sequestration rates and stability (Heim et al., 2009; Necpálová et al., 2014). To overcome this challenge, SOC stock monitoring study decisions should focus on systematically partitioning the SOC stock noise caused by natural biophysical variation from the SOC stock signals induced by new RMP SOC sequestration land use treatments. By separating SOC stock signals from noise and minimizing introduced systematic (i.e. sampling) error, the sample size (i.e. the cost) necessary to satisfy the carbon offset market's data verification and validation standards decreases.

Study design formats have a strong influence on how variances in SOC stocks and co-factors are controlled and evaluated (Heim et al., 2009; Putten & Knippers, 2010). Because the demonstration Project was already in operation at the time of this study; a mensurative space-for-time substitution (chronosequence) experiment design was the only option available to investigate the effects of biosolids land-spreading application and willow plantations on soil organic carbon (SOC) stocks within the Study Area. Although the arrangement for sampling locations relative to one another was not ideal for controlling natural environmental variation across the Study Area, it was the best approach given the pre-existing land management practices that were established by SYLVIS in 2013.

The space-for-time study design is standard for most SOC monitoring studies at the farm scale (Smith et al., 2012). This paired study design approach uses the differences measured in SOC stocks between the treatment and the control sampling locations to represent the net SOC sequestration effects of the RMP treatments (Smith et al., 2012). The advantage of the space-for-time study design is its ability to maintain relative differences in SOC stocks between sampling locations regardless of regional SOC stock gains or losses trends caused by annual climatic variation (Smith et al., 2012; Olander et al., 2011). The disadvantage of the space-for-time study design for monitoring SOC stock dynamics is its assumption that all spatial variability of SOC stocks and co-factors are equivalent at all sampling locations prior to RMP implementation. Given the naturally high variability of SOC stocks and its co-factors (i.e. soil texture, pH, mineral content, etc.), this assumption is difficult to satisfy under most natural conditions (Don et al., 2007; Jandl et al., 2014; Johnson & Miyanishi, 2008) – as was demonstrated during this study. The space-for-time study design increases the risk of falsely under or over estimating the effects of RPM SOC sequestration treatments when natural biophysical variation is not controlled or accounted for (Maillard et al., 2017).

The time-for-time study design is a common alternative to the space-for-time study design (Blois et al., 2013) and is generally used for SOC stock process-modelling studies (Olander & Haugen-Kozyra, 2012). The time-for-time study design establishes a SOC stock base-line prior RMP treatment applications and then repeatedly measures the same locations within the Study Area at regular time intervals. The advantages and disadvantages of the time-for-time study design and the space-for-time study design are reciprocal (Olander et al., 2011). The repeated measures approach of the time-for-time study design minimizes natural variation, however the potential effects of annual climate fluctuations on SOC stocks are not accounted for. Therefore some advocate for a hybrid study design approach to counteract the spatial, temporal, and climatic variation limitations of these individual study design approaches (Carter & Gregorich, 2006 pg. 50; Hooten, Wikle, Sheriff, & Rushin, 2009; Maillard et al., 2017). A hybrid study design approach also allows for more versatile analysis of the data and application of the results (Smith et al., 2012).

For future Willow +Biosolids systems SOC monitoring, the comparative mensurative design used in this study could be taken a step further with a manipulative study design such as the randomized complete block design to gain more detailed insights into the effects of different biosolid application rates and different willow cultivar varieties on SOC sequestration rates. The inclusion of a time-for-time feature within a study design allows for the application of SOC process modelling (i.e. CENTURY model) which may be used to (1) predict time frames for which changes in soil stocks will likely be detected (Necpálová et al., 2014), and (2) develop SOC sequestration coefficients based on biogeoclimatic regions similar to the Alberta conservation agriculture (no-till) quantification protocol. Once SOC sequestration coefficients are established for Willow + Biosolids systems, the SOC sink project management costs would substantially decrease because only the land management practices would require verification rather than the SOC stocks themselves (Olander & Haugen-Kozyra, 2012 pg. 29).

One prominent time-for-time SOC monitoring protocol deployed in Alberta is the Prairie Soil Carbon Balance Project which uses micro-plots (e.g. 4 m x 7 m, or 2 m x 5 m) with permanent electronic GPS markers. The GPS marker allow for precise positioning of annual soil sample positions to ensure that repeat measurement locations are adjacent but not overlapping as shown in Figure 16 (Carter & Gregorich, 2006 pg. 57). One option for a hybrid study design approach would be to use this staggered micro-plot sampling plan design in a space-for-time design to control for both spatial and climatic variation.

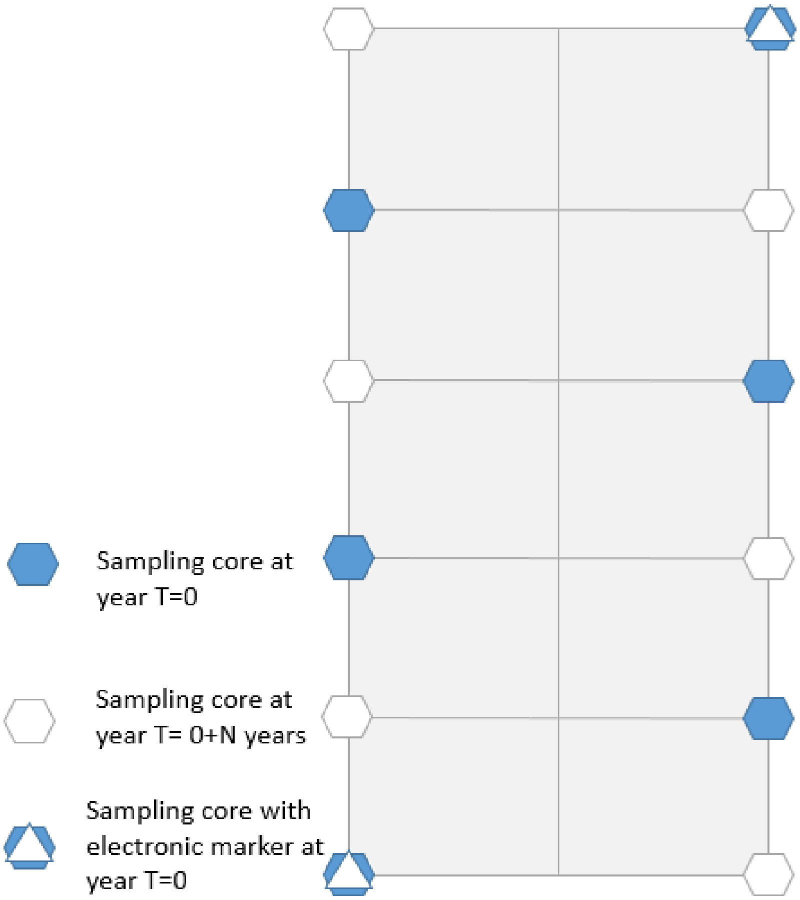

Figure 16: Example of a repeat measures sampling design plan based on the Prairie Soil Carbon Balance Project (adapted from Carter & Gregorich, 2006)

6.3 Strategies for Controlling Soil Organic Carbon Stock Variation

Enhancing the ability to measure and quantify SOC stock variance is vital for improving the accuracy of SOC stocks change detection and the reliability of soil carbon offset calculations. Millard et al., (2017) found that the combination of spatial scale (the size of a sampling plot) and soil profile depth accounts for approximately 44% of the variability measured in SOC stocks. Spatial variability of SOC stocks is a function of several spatio-temporal biophysical factors such as, soil type, soil texture, moisture, climate, vegetation community, land management etc. (Carter & Gregorich, 2006 pg. 39) and is generally assumed to increase with distance between sampling points or locations (Millard et al., 2017). Stratification of the biophysical factors that influence SOC stocks is considered to be one of the most efficient strategies for partitioning SOC stock signals from natural variation noise and improving the sensitivity of SOC monitoring studies to detect SOC stock changes (Carter & Gregorich, 2006; Conant & Paustian, 2002; Karunaratne, Bishop, Odeh, Baldock, & Marchant, 2014).

The Study Area of this study was stratified by vegetation cover type (willow vs. small grain crops), tillage practices (reduced till vs. no-till), and biosolids land application treatment (treatment vs no-treatment), and soil sampling depth intervals (0-15 cm, 15-30 cm). Based on background literature reviews and landowner records, the Study Area was presumed to have been subject to the same historical land management practices and exhibited relatively uniform biophysical characteristics based on prior to implementation of the study. To validate this assumption, soil parameters representing key SOC dynamics co-factors (pH, density, texture, %SOC) were tested. Section 5.10 *Summary of SOC stocks and SOC dynamics Co-factor Results* identifies where these assumptions fell short.

6.4 Soil Sampling Depth Considerations

After spatial variability, soil depth is the second largest source of SOC stock variability (Millard et al., 2017). To control SOC stock variability introduced by soil sampling depth, shorter depth intervals ranging from 5-20 cm in length are recommended (Don et al., 2007; Olson & Al-Kaisi, 2015). Because SOC stocks tend to decline exponentially with soil depth (Franzluebbers, 2005), SOC monitoring studies generally favor shallow soil sampling depth intervals (<20 cm) (Harrison, Footen, Harrison, Footen, & Strahm, 2011) to capture the highest proportion of SOC stock information across a sampling area for the lowest sampling cost. This shallow soil sampling strategy is endorsed by the international panel on climate change (IPCC), a leader in climate change research, which recommends a soil sampling depth of 30 cm for SOC monitoring (IPCC, 2014). Yet many (Carter & Gregorich, 2006 pg. 56; Hobley & Willgoose, 2010; Jandl et al., 2014; Olson & Al-Kaisi, 2015; Zang et al., 2018) argue that too much SOC stock information is lost when shallow sampling methods are applied and that it is more important to collect more accurate data from deeper soil depth intervals at fewer sampling sites than to collect more precise data from a higher number of shallow soil sampling sites. To illustrate, Harrison et al., (2011), reported that 27-77% more SOC stock was accounted for when sampling depths measured 80 cm or deeper. Harrison et al., (2011) and Olson & Al-Kaisi (2015) report that the conclusions of SOC stock studies have changed (in terms of both gains and losses) when SOC stock data from deeper soil sampling intervals were included in the analysis. Therefore, sampling depth should be included with all SOC stock reports.

When choosing a SOC stock sampling depth interval, it is important to consider the root physiology of the vegetation community occupying the sampling location and to ensure that the sampling depth accounts for the entire root zone (Hansen, 1993; Olson & Al-Kaisi, 2015; Rytter, 2016). Because Hu et al., (2016) has found that root rather than leaf litter is the primary driver of SOC sequestration in marginal soils of willow systems, sampling across the root zone is important for accurate SOC stock estimates of Willow + Biosolids systems. Hu et al., (2016) study also found that SOC sequestered from root litter was more stable than SOC sequestered from leaf litter because it contained more recalcitrant compounds, was less exposed to aerobic decomposition, and the fine roots enhanced aggregate formation (which provides SOC compounds with more physiochemical protection).

The study used sampling depth intervals of 0-15 cm and 15-30 cm to align with the IPCC protocols and historic environmental monitoring events conducted by SYLVIS environmental monitoring data. However, for future Willow + Biosolids systems SOC stock monitoring studies it is recommended that the sampling depth protocol include depth intervals that are a maximum of 15 cm and total sampling depths are minimum of 60 cm (Carter & Gregorich, 2006 pg.56) and preferably 100 cm (Zang et al., 2018). Because there is inconsistency within SOC stock research literature and SOC stock monitoring protocols (Maillard et al., 2017) soil sampling depths should be reported with estimate total SOC stocks and SOC sequestration rates.

6.5 Soil Bulk Density Methods

Soil density is a key parameter of SOC stock estimates and therefore it is important that soil density measures are conducted as precisely and as accurately as possible. Most soil monitoring protocols use a soil fixed volumetric approach to measure soil density because of methodological ease, (Mackenzie, 2011). Mackenzie, (2011) and Olander et al., (2011) argue that the fixed volume soil bulk density approach is not appropriate for multi-year paired-study assessments of SOC stocks; especially when soil bulk density altering land management practices (i.e. tillage, addition/removal of large volumes of material) or soil physio-chemical characteristics (swelling or shrinking) are involved. Therefore Carter & Gregorich, (2006 pg. 59), Olander et al., (2011), and Goidts, Van Wesemael, & Crucifix, (2009) recommend using the mass equivalent approach to bulk density measurements because it significantly reduces vertical variability of SOC stock measurements by ensuring that the same mass i.e. same “population” of soil particles are being compared between soil sampling locations. The mass equivalent bulk approach is also a more appropriate method to use when SOC sink project sites are subject to bulk density altering land management practices (e.g. tillage, biosolids landspreading applications). Additionally, Harrison et al., (2011) recommends that soil bulk density and %SOC concentration analysis be conducted on the same sample. During the study, the soil bulk density and %SOC analysis was conducted on separate adjacent soil samples because the laboratory conducting the soil chemistry analysis did not use volumetric or fixed mass soil bulk density analysis methods. For future monitoring of Willow + Biosolids systems, it is recommended that the fixed mass bulk soil density method be applied and that %SOC analysis be conducted on the same sample.

6.6 Sample Size Selection

The sample size required to detect a specified change in SOC stocks is dependent on the size of the Study Area, the background SOC stocks, the SOC stock variance, and the desired degree of statistical certainty (Conant & Paustian, 2002). To estimate the sample size necessary to measure SOC stocks at the sampling locations with a 95% certainty within ±10% of the mean, an ad hoc power analysis was conducted using the Aynekulu et al., (2011) samples size formula and a SOC stock coefficient of variance (CV) of 27% based historic environmental monitoring data (2013-2017) collected by SYLVIS. Based on this sample size formula and the SYLVIS CV results, a sample size of 23 was recommended and a sample size of 25 was chosen to improve statistical power. When comparing to other sample size assessment studies, a sample size of 25 appeared appropriate. Pennock, (2004) recommended a sample size of 17 for a SOC stock coefficient of variation of 20%, a certainty of 0.95, and a power of 0.8; while Conant & Paustian (2002) estimated that a sample size of 14-28 was enough to detect a SOC stock change of 2.3 Mg C ha^{-1} with %CV ranging from 12 to 19%.

The study SOC Stock CV results were similar to the SYLVIS results and ranged from 20-30% across all of the sampling locations and depth intervals. The Study Area SOC stock CV results reported by SYLVIS and the study were also consistent with the VandenBygaart (2003) regional meta-analysis study of 12 SOC stock monitoring studies located across western Canada which reported an average SOC stock %CV of 28 ± 7%. Although the %CV used in the ad hoc power analysis was similar to the post hoc power analysis, the study post hoc power analysis using the Aynekulu et al., (2011) formula (Equation 2) recommends a sample size 4 times larger (n=106) than the ad hoc power analysis for $\beta = 0.80$ and SOC stock CV=30%. This discrepancy is due to the relatively large differences in sampling area sizes between the study sampling plan and the SYLVIS environmental monitoring sampling plan. The available sampling area for which the W+BS sampling plot location could be randomly positioned was only 5% (84 m^2) of the size of the sampling area from which one of the SYLVIS environmental monitoring samples could be collected (1620 m^2). Although the SOC stock CV results for both sampling programs were similar, the relative spatial variation Study Area is much higher than the SYLVIS environmental monitoring data when sampling area was considered. The difference in soil sampling techniques is one potential explanation for the SYLVIS environmental monitoring data exhibiting relatively low spatial variability compared to the study data. The study used the point sample methods, while the SYLVIS environmental monitoring program used a composite (six sub-samples) sampling method; a practise known to reduce sampling variation relative to the point sampling method Study (Carter & Gregorich, 2006 pg. 34; Goidts et al., 2009).

To compare means and variation of measured SOC stock co-factors (pH, soil texture) a sample size of 15 was selected. Because these soil parameters typically have relatively low to moderate cofactor of variation values (<15%) (Carter & Gregorich, 2006), the smaller sample size was considered sufficient according to Carter & Gregorich (2006) recommended sample size chart based on CV, desired confidence and desired relative error.

6.7 Selection of Study Statistical Certainty Parameters

The main objective of this study was to determine if significant differences in SOC stocks can be detected between a control and two variations of COP21 RMP treatments for the purpose of assessing soil-based carbon emissions offset potential of Willow + Biosolids systems. Because it typically takes SOC sink projects 7-10 years to generate statistically significant results (Saby et al., 2008; Smith, 2004), the likelihood of this study detecting significant differences in SOC stocks between sampling location at the demonstration Project site which was five years old at the time of the study was possible but relatively low. Because the findings of this study will likely be used to assess the merits of continued investigation into the soil-based carbon emissions offset credits potential of Willow + Biosolids systems, it was considered more important to avoid committing a Type II statistical error by using a stringent p value of 0.05 than to avoid a Type I statistical error with a less stringent p value of 0.10. Therefore, this study used a p value of 0.10 to compare SOC stocks between sampling locations. It is important to note that if and when the demonstration Project site is monitored for soil-based carbon emissions offset credit calculations, future studies should occur after the demonstration Project is at least 10 years old and should be accurate and precise enough to calculate SOC stocks within 0.5 Mg C ha^{-1} of the mean 95% of the time (Paragon, 2006).

Because homogeneous biophysical conditions were a key assumption of space-for-time studies, a more stringent p value of 0.05 was chosen to compare the co-factor parameters between sampling locations.

6.8 The Economics of Soil Carbon Farming in Willow + Biosolids Systems

To be competitive in the carbon offset trading market, Smith (2004) suggests that SOC based carbon offset protocols have the capacity to measure SOC stock within 0.5 Mg ha^{-1} of the mean 95% of the time, generate statistically significant results within five years. Additionally, Paragon (2006) recommends that the total soil monitoring costs sum to less than 20% of the SOC sink's total carbon offset credit value. Given that the market value for carbon offsets has doubled since 2007 ($15/Mg CO_2e to $30/Mg CO_2e) and that key elements of the Keoma demonstration Project overlap with currently approved offset protocols (Conservation Cropping, Biofuel Production and Usage, and Energy Generation from the Combustion of Biomass Waste), a pre-existing protocol (Afforestation), and a previously submitted protocols (Customized Agricultural Soil Carbon Sink Protocol for Greenhouse Gas Emission Reductions and/or Removals), there is strong indication that the development and utilization of a customized carbon emissions offset protocol for Willow + Biosolids systems is feasible.

However, the economic success of a SOC sink project largely depends on the value of carbon offsets, the sites SOC sequestration potential, and the homogeneity of the SOC stocks, i.e. the sample sizes required to accurately and precisely detect SOC stock changes at a project site within a given period. Evidently, unless the value of emissions offset credits continue to rise, there are a limited number of locations within Alberta where the soil conditions are favorable enough for carbon farming to be economically viable under the Alberta Emissions Offset System. To move forward in protocol development, trade-offs and improvements are necessary in the areas of statistical performance expectations, SOC analysis technology performance, and SOC monitoring study design approaches. The following sections explore SOC monitoring study design considerations and emerging SOC monitoring technologies opportunities in more detail.

Relationships between Coefficient of Variation, Sample Size, and Minimum Detectible Differences

The minimum detectible difference of a SOC stock data depends not only the variability of the data set but also on the relative size of the mean SOC stock difference in proportion to the total SOC stock pool. As SOC stocks increase via SOC sequestration, larger absolute changes in SOC stocks are required to detect relative percent changes in SOC stocks. Based on the background SOC stocks measured at the Control sampling location (30.8 Mg C ha^{-1} at the 0-15 cm depth interval and 23.0 Mg C ha $^{-1}$ at the 15-30 cm depth interval), and the statistical power set at of $\beta = 0.80$ and certainty of 95%, the expected minimum detectable difference of this study with a sample size of 25 was >±3 Mg C ha^{-1} at the 0-15 cm depth interval and >±2 Mg C ha^{-1} at the 15-30 cm depth interval. At the 0-15 cm depth interval, the difference between the Control and the W+BS sampling locations was less than >±3 Mg C ha^{-1} (1.8 Mg C ha^{-1}) and therefore, if the W+BS treatments did influence SOC stock changes it would not have been detected. At the 15-30 cm depth interval, the difference between the Control and the W+BS sampling locations was 4.5 Mg C ha^{-1} and a significant difference ($p=0.1$) was detected. In comparison, based on a meta-analysis of 51 studies Millard et al., (2017) reported that a 10 ha Study Area with a depth interval of 0-30 cm, would require a minimum sample size of 50 to detect a 15% SOC stock change, while a sample size of 25 could detect a 22% SOC stock change with a 95% certainty. To expand further, Table 15 provides a high-level summary of the relationships between coefficient of variance (CV), sample size, and minimum detectable differenced (MDD) influence soil monitoring costs and carbon offset revenue potential. In many of these scenarios, (particularly when the desired minimum detectible differences are small and the time frames are short) the mass of sequestered SOC required just to cover the cost of the soil sampling collection and analysis may be physically impossible.

Table 15: Relationship between coefficient of variation, sample size and minimum detectable difference (adapted from figure 3.2 in Carter & Gregorich 2006)

Coefficient of Variation (CV)	Sample Size	Minimum Detectable Difference (MDD) (Mg C ha^{-1})	Estimated Time (years) to Sequester[b] MDD of SOC stocks (Mg C ha^{-1})	Estimated Cost of Soil Sample Collection Labor and Analysis[a] (For Each Soil Sampling Depth Interval)	Total Mass of Sequestered SOC (Mg C) per Sampling Depth Interval of a Given Sampling Area Necessary to Make Soil Sampling and Analysis Cost Effective[c]	
					At \$30 Mg CO_2e	At \$50 Mg CO_2e
25%	20	11	11	\$1980	90	54
	30	8.5	9	\$2970	135	81
	50	6.75	7	\$4950	225	135
	100	4.75	5	\$9900	451	270
20%	20	9	9	\$1980	90	54
	30	7	7	\$2970	135	81
	50	6	6	\$4950	225	135
	100	4	4	\$9900	451	270

Assumptions:

[a] 0.5 hr/sample, \$110/hr labor, \$14/sample bulk density analysis, and \$30/sample %SOC analysis.

[b] Very high SOC sequestration rate of 1.0 Mg C ha^{-1} yr^{-1} (Goidts et al., 2009)

[c] To be cost effective soil sampling costs should be less than 20% of the value of the carbon emissions offset credits (Paragon, 2006) Excludes field sampling project management costs

6.9 Soil Organic Carbon Monitoring Time Scales

In addition to sample size and minimum detectible difference considerations, SOC monitoring study designers should also account for how much time is required to sequester minimum detectible differences in the SOC stocks of each project location (Necpálová et al., 2014) as the timing of producing significant results could impact access to future project funding. Given that regional governments generally operate on 4 to 5-year election and budgeting cycles (Smith, 2004), it is beneficial to design experiments with the statistical power to generating conclusive results within these time periods. Geochemical process models like CENTURY, could also assist with SOC sequestration forecasting and identify optimal scheduling periods for future SOC monitoring events (Albrecht & Alain, 2016).

As indicated in Table 15, sample sizes of 100 are generally required to detect SOC stock differences of <5 Mg C ha^{-1}. Assuming that the demonstration Project has a SOC stock CV of 25% and a very high SOC sequestration rate of 1.0 Mg C ha^{-1} yr^{-1} (Goidts et al., 2009) a minimum sample size of 100 would be necessary for the chance to satisfy a 5-year target. A more cost-effective scenario would be to wait until 2023 when the project is 10 years old and a sample size of 30 would be sufficient to detect than the minimum detectible difference of 8.5 Mg C ha^{-1} (assuming a SOC sequestration rate of 1.0 Mg C ha^{-1} yr^{-1}).

6.10 Future Study Design Recommendations for Willow + Biosolids Soil Carbon Offset Projects

Although there are still knowledge gaps within the fundamental mechanics of the ecological mechanisms driving SOC dynamics, continued improvements in field study designs and SOC analysis methods are helping to drive the science of SOC stock monitoring forward. With regards to optimizing the carbon offset earning potential for Willow + Biosolids systems, targeted research is required to explore the specific mechanics and conditions driving the SOC processes unique to these systems. By investing in comprehensive Willow + Biosolids SOC dynamics studies with broader sets of soil carbon monitoring parameters, future Willow +Biosolid SOC sequestration models may be better calibrated to the specific climatic, land management, biological, physical, chemical, and morphological properties of project locations. Once these Willow + Biosolids SOC dynamics models are developed, the cost of SOC stock monitoring decreases rapidly because only baseline studies of select parameters and a limited number of repeated measures samples would be necessary to calibrate and validate the Willow + Biosolids SOC dynamics process models. The following study design and soil monitoring parameter recommendations are intended to generate some of the data necessary to close some of the foundational knowledge gaps in characterizing SOC dynamics for Willow + Biosolids systems.

6.11 Detailed Baseline Study Design

Before implementation of willow and biosolids treatments, a site characterization study should be conducted at the proposed project location in a systematic grid pattern across the entire project area to stratify the site according to SOC dynamics co-factors. Study stratification factors could include; morphology, land use history, vegetation communities, parent material, soil classification, and soil chemistry results (soil texture, bulk density, pH, CEC, soil minerals, SOC stocks, total nitrogen). Then the error propagation method could be used to quantify the relative contribution of each measured variable on SOC stock variability and the experimental design can be stratified into spatial landscape units according to the top contributors of SOC stock variability (Goidts et al., 2009). The number and spatial pattern of stratified spatial land units could be used to identify the number and location of sampling locations for future Willow + Biosolids SOC dynamics studies.

6.12 Soil Organic Carbon Monitoring Study Design

Because there is limited research on the interactive effects of willows and biosolids on SOC stocks in marginal agricultural lands, and because space-for-time studies generally do not control for natural variances in soil physical and chemical conditions effectively, it is recommended that future Willow + Biosolids SOC dynamics studies us randomized complete block design with repeat measures. This way both spatial and temporal variability can be controlled and it will be easier to infer the influences of biosolids and willows on SOC stocks separately. This experimental design could also help to identify optimal biosolids land application rates and willow cultivar species. An example Willow +Biosolids complete block design data collection arrangement is presented in Figure 17 and an example of the field sampling block plan is presented in Figure 18. Within each block, the sampling plan could be arranged in a similar fashion to Figure 16 to allow for repeat sample analysis over time.

Willow Cultivar	Biosolids Application rate (Mg ha^{-1})	Block 1			Block 2			Block 3		
		time 1	time 2	time 3	time 1	time 2	time 3	time 1	time 2	time 3
Cultivated Field (No Willow Cultivar)	0									
	20									
	50									
Willow Cultivar 1	0									
	20									
	50									
Willow Cultivar 2	0									
	20									
	50									

Figure 17: Example block plot soil sampling plan with varying willow species and biosolids land application rates (adapted from Gumpertz 1993)

		Willow Treatment		
		No Willow Cultivar (W0)	Willow Cultivar 1 (W1)	Willow Cultivar 2 (W2)
Biosolids Treatments	No Biosolids (BS0)	BS0, W0	BS0,W1	BS0, W2
	Biosolids rate 1 (BS1)	BS1, W0	BS1,W1	BS1,W2
	Biosolids rate 2 (BS2)	BS2,W0	BS2,W1	BS2,W2

Figure 18: Example of a Willow + Biosolids field sampling block treatment plan

6.13 Broader Exploration of the Willow + Biosolids SOC dynamics

During the 10-year period that it generally takes to detect changes in total SOC stock, there are several opportunities to monitor dynamics of the more labile carbon fractions as they transition into more stable carbon compounds. By doing so, much can be learned about the relationship between short term labile carbon decomposition patterns and long term SOC sequestration and stabilization trends. The more immediate feedback generated by short-turn-over SOC parameters (Han et al., 2017) also provides opportunities to adjust land management practices (adaptive management) to improve SOC sequestration conditions within a few years rather than a decade. Potential adaptive management adjustments for Willow + Biosolids systems include; biosolids application and re-application rates, biosolids incorporation methods; willow harvesting periods; soil amendment types and, selection of neighboring willow cultivar species. The key to optimizing adaptive management strategies for Willow + Biosolids systems is to identify the best short term parameter surrogates to signal long term SOC sequestration and stabilization trends (Cogger et al., 2013).

6.14 Monitoring Labile Carbon Fractions

Examples of labile carbon pool parameters that may be used as indicators of SOC stock responses to land management practices includes; microbial biomass, light fraction carbon, and particulate organic carbon (Han et al., 2017). Monitoring of microbial communities could be a valuable indicator of SOC dynamics trends because the higher the ratio of fungus to bacteria, the more likely the soil system will be in a state of SOC sequestration (Cogger et al., 2013). Because adoption of Willows + Biosolids land management practices involves soil tillage for site prep and the addition of labile organic carbon from biosolids, the microbial community during the first few years is likely bacterial dominated would be at a higher risk of soil priming. For example Cogger et al., (2013), reported that bacterial/fungus ratios ranged from 2:1 to 4:1 after cumulative application of medium (34 Mg ha^{-1}) to high rates of biosolids (45 Mg ha^{-1}). However, as the willows establish after soil disturbance ceases, the microbial community will likely transition into a fungal dominated system (Six et al., 2006). Through monitoring microbial community fungus and bacteria composition, more informed decisions could be made on scheduling total SOC stock monitoring events.

6.15 Soil Organic Carbon Isotope Tracer Monitoring

The use of isotopic tracers such as δ^{13}C/ δ^{15}N ratios to fingerprint the flow carbon inputs sourced from biosolids through a soil system is also a useful technique for assessing how biosolids influence SOC sequestration and mineralization processes (Agostini,et al 2015). Wijesekara et al., (2017) used this isotopic tracer technique in tandem with soil respiration monitoring to measure in-situ carbon fluxes. Wijesekara et al., (2017) found evidence for the accumulation of biosolids residual carbon in non-labile carbon compounds at both sandy and clayey sites during the same year the study site received one treatment of 70 Mg ha^{-1} biosolids. In this study, biosolids were applied with horizontal disc rear discharge spreaders and were incorporated into surface soils (0-15 cm depth) using chisel ploughs.

6.16 Emerging Methods Soil Organic Carbon Analysis

Because of the high costs associated with soil sample collection and ex-situ SOC analysis, concerted efforts have been made to develop in-situ soil carbon analysis technologies that are less costly, more time efficient, more accurate, and provides better data resolution than conventional laboratory soil carbon analysis methods (Chatterjee et al., 2009). Infrared spectroscopy is one of the most prominent ex-situ SOC analysis technologies. This technology works by measuring how soils absorb and re-emit different wavelengths, to determine SOC content and other soil physiochemical properties including; soil water content, texture, cation exchange capacity, calcium and magnesium (exchangeable), total nitrogen (N), pH, concentration of metals, microbial size, and microbial activity (Paustian et al., 2017). When comparing the two methods, near-infrared (NIR) and mid-infrared (MIR), NIR can measure a wider range of soil carbon properties to monitor soil humification processes, however the MIR produces more accurate results (Soriano-Disla, Janik, Rossel, MacDonald, & McLaughlin, 2014). The major drawback of this technology that is preventing it from becoming a more widespread alternative to dry combustion laboratory analysis is the challenges with calibrating large datasets to the local soil conditions (Jandl et al., 2014; Paustian et al., 2017).

6.17 Conclusions

Climate change mitigation is a complicated issue requiring complex multi-disciplinary solutions. SOC sequestration is one of several promising strategies currently available to help overcome this global challenge. To incentivize and support the land management practices and economic systems that promote SOC sequestration, international collaboration across political, economic, scientific, and engineering sectors, practices, and communities is required.

Given that the United Conservative Party recently (April 2019) succeeded in forming a legislative majority in Alberta and stated their intention to dismantle Alberta's climate legislation (the *Climate Leadership Act* (2017)), it is unlikely that development of a custom Willow +Biosolids project emission offset quantification protocol through the Alberta Emissions Offset System will be possible in the near future. However, despite the more broadly regressive climate change mitigation policies of Alberta's newly elected government compared to their predecessors, alternative climate change mitigation pathways are available for Alberta's municipal government and industry organizations who do not favor these low social discount rate policies. For those individuals, municipal governments, and organizations in Alberta wishing to invest in a more sustainable future, global carbon offset markets provide one alternative technological and global societal network pathway for supporting sustainable development advancements being supressed by local government policies. Examples include; the Gold Standard (www.goldstandard.org); The Climate Trust (climatetrust.org), First Carbon Credits (firstcarboncredits.org)) and the United Nations Framework Convention on Climate Change Clean Development Mechanism.

Section 5—Supporting Information

References

Alberta Agriculture Food and Rural Development. (2002). Liming Acid Soils. Retrieved March 14, 2019, from https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex3684/$file/534-1.pdf?OpenElement

NOAA Earth System Research Laboratory ESRL (NOAA/ESRL). (2018). Recent Monthly Mean CO2 at Mauna Loa. Retrieved January 22, 2019, from http://www.esrl.noaa.gov/gmd/ccgg/trends/

Adegbidi, H. G., Briggs, R. D., Volk, T. A., White, E. H., & Abrahamson, L. P. (2003). Effect of organic amendments and slow-release nitrogen fertilizer on willow biomass production and soil chemical characteristics. *Biomass and Bioenergy*, *25*(4), 389–398. https://doi.org/10.1016/S0961-9534(03)00038-2

Agostini, F., Gregory, A. S., & Richter, G. M. (2015). Carbon Sequestration by Perennial Energy Crops: Is the Jury Still Out? *Bioenergy Research*, *8*, 1057–1080. https://doi.org/10.1007/s12155-014-9571-0

Alberta Agriculture and Food. (2008). *Nutrient Management Planning Guide*. (and L. U. Atta Atia, Phil Boehme, Dr. Jason Cathcart, Rob Dunn, Shawn Elgert, Lloyd Healy, Crystal Korth, Len Kryzanowski, Germar Lohstraeter, Dr. Ross McKenzie, Blaine Metzger, Jennifer Neden, Sheilah Nolan, Dr. Barry Olson, Matt Oryschak, Doon Pauly, Brian Sexton, Ed.). Edmonton, AB: Alberta Environmentally Sustainable Agriculture Program.

Alberta Agriculture and Forestry. (2001). Soil Organic Matter. https://doi.org/doi:10.1201/b11267-14

Alberta Agriculture and Forestry. (2019). 180-Day Standarized Precipitation index (SPI). Retrieved January 25, 2019, from https://agriculture.alberta.ca/acis/climate-maps.jsp

Alberta Forestry and Agriculture. (2016). Alberta Soil Information Viewer. Retrieved November 21, 2018, from https://soil.agric.gov.ab.ca/agrasidviewer/

Albrecht, G., & Alain, S. (2016). Methods for Measuring Greenhouse Gas Balances and Evaluating Mitigation Options in Smallholder Agriculture. In *Methods for Measuring Greenhouse Gas Balances and Evaluating Mitigation Options in Smallholder Agriculture* (pp. 135–162). https://doi.org/10.1007/978-3-319-29794-1

Amichev, B. Y., Kurz, W. A., Smyth, C., & Van Rees, K. (2012). The carbon implications of large-scale afforestation of agriculturally marginal land with short-rotation willow in Saskatchewan. *GCB Bioenergy*, *4*(1), 70–87. https://doi.org/10.1111/j.1757-1707.2011.01110.x

AROWRN. (2019). Alberta Rural Organic Waste to Resources Network. Retrieved March 25, 2019, from https://www.arowrn.ca/

Athamenh, B., Salem, N., Et-Zuraiqi, S., Suleiman, W., & Rusan, M. (2015). Combined land application of treated wastewater and biosolids enhances crop production and soil fertility. *Desalination and Water Treatment*, *53*(12), 3283–3294. https://doi.org/10.1080/19443994.2014.933037

Australia Government. (2014). *Carbon farming iniative soil sampling and analysis methods and guidelines*.

Aynekulu, E., Vagen, T.-G., Shephard, K. D., & Winowiecki, L. (2011). A Protocol for Modeling , Measurement and Monitoring Soil Carbon Stocks in Agricultural Landscapes. *World Agroforestry Centre (ICRAF) United*, (May), 26.

Blois, J. L., Williams, J. W., Fitzpatrick, M. C., Jackson, S. T., & Ferrier, S. (2013). Space can substitute for time in predicting climate-change effects on biodiversity. https://doi.org/10.5061/dryad.d5f1r.1

Bloom, A. A., Exbrayat, J.-F., van der Velde, I. R., Feng, L., & Williams, M. (2016). The decadal state of the terrestrial carbon cycle: Global retrievals of terrestrial carbon allocation, pools, and residence times. *Proceedings of the National Academy of Sciences*, *113*(5), 1285–1290. https://doi.org/10.1073/pnas.1515160113

Bowen, A. (2011). *The case for carbon pricing: policy brief. The Grantham Research Institute in Climate Change and the Environment; The Centre for Climate Change Economics and Policy* (Vol. 1). https://doi.org/10.1039/c3ta14042c

Brown, K., & Wherrett, A. (2014). Bulk Density-Measuring. https://doi.org/10.1016/j.eururo.2007.10.015"

Brown, S., Kurtz, K., Bary, A., & Cogger, C. (2011). Quantifying benefits associated with land application of organic residuals in washington state. *Environmental Science and Technology*, *45*(17), 7451–7458. https://doi.org/10.1021/es2010418

Canola Council of Canada. 2017. Managing Other Nutrients. Available at:https://www.canolacouncil.org/canola-encyclopedia/fertilizer-management/managing-other-nutrients/#calcium-supply-for-canola. Accessed on March 23, 2019.

Carter, M. R., & Gregorich, E. . (2006). *Soil Sampling and Methods of Analysis*. *Canadian Society of Soil Science* (2nd ed., Vol. 44). Taylor & Francis Group, LLC. https://doi.org/10.1017/S0014479708006546

Chatterjee, A., Lal, R., Wielopolski, L., Martin, M. Z., & Ebinger, M. H. (2009). Evaluation of different soil carbon determination methods. *Critical Reviews in Plant Sciences*, *28*, 164–178. https://doi.org/10.1080/07352680902776556

Chaudhari, P. R., Ahire, D. V, Ahire, V. D., Chkravarty, M., & Maity, S. (2013). Soil Bulk Density as related to Soil Texture , Organic Matter Content and available total Nutrients of. *International Journal of Scientific and Research Publications*, *3*(2), 1–8.

Cogger, C. G., Bary, A. I., Kennedy, A. C., & Fortuna, A.-M. (2013). Long-Term Crop and Soil Response to Biosolids Applications in Dryland Wheat. *Journal of Environment Quality*, *42*(6), 1872. https://doi.org/10.2134/jeq2013.05.0109

Conant, R.T; Smith, G.R.;Paustian, K. (2003). Spatial variability of soil carbon in forested and cultivated sites: implications for change detection. *Journal of Environmental Quality*, *32*, 278–286. https://doi.org/doi: 10.2134/jeq2003.2780

Conant, R. T., & Paustian, K. (2002). Spatial variability of soil organic carbon in grasslands: Implications for detecting change at different scales. *Environmental Pollution*, *116*, S127–S135. https://doi.org/10.1016/S0269-7491(01)00265-2

Cornell University. (2017). Willow energy crop information. Accessed on October 18, 2017. Available at: http://willow.cals.cornell.edu/.

Corsi, S., Friedrich, T., Pisante, M., & Sà, J. D. M. (2012). *Soil Organic Carbon Accumulation and Greenhouse Gas Emission Reductions from Conservation Agriculture: a literature review* (Vol. 16). Rome, Italy.

Cunniff, J., Purdy, S., Barraclough, T., Castle, M., Maddison, A., Jones, L., … Karp, A. (2015). High yeilding biomass genotype of willow (Salix spp.) show differences in below ground biomass allocation. *Biomass and Bioenergy*, *126*(80), 114–127. https://doi.org/.1037//0033-2909.I26.1.78

Dahlby, B., Ragan, C., Drummind, D., Frank, B., & St-Hiliaire, F. (2019). 10 Myths about carbon pricing in Canada. EcoFiscal Comission. Montreal.

Don, A., Schumacher, J., Scherer-Lorenzen, M., Scholten, T., & Schulze, E. D. (2007). Spatial and vertical variation of soil carbon at two grassland sites - Implications for measuring soil carbon stocks. *Geoderma*, *141*(3–4), 272–282. https://doi.org/10.1016/j.geoderma.2007.06.003

Environment and Climate Change Canada. (2017). Pricing Carbon Pollution: How it will work. Retrieved January 22, 2019, from https://www.canada.ca/en/environment-climate-change/news/2017/05/pricing_carbon_pollutionincanadahowitwillwork.html

Follett, R. F. (2006). Soil management concepts and carbon sequestration in cropland soils. *Progress in Natural Science: Materials International*, *16*(3). https://doi.org/10.1016/S0167-1987(01)00180-5

Fontaine, S., Bardoux, G., Abbadie, L., & Mariotti, A. (2004). Carbon input to soil may decrease soil carbon content. *Ecology Letters*, *7*(4), 314–320. https://doi.org/10.1111/j.1461-0248.2004.00579.x

Franzluebbers, A. J. (2005). Soil organic carbon sequestration and agricultural greenhouse gas emissions in the southeastern USA. *Soil and Tillage Research*, *83*, 120–147. https://doi.org/10.1016/j.still.2005.02.012

Garten, C. T., Wullschleger, S. D., & Classen, A. T. (2011). Review and model-based analysis of factors influencing soil carbon sequestration under hybrid poplar. *Biomass and Bioenergy*, *35*, 214–226. https://doi.org/10.1016/j.biombioe.2010.08.013

Goglio, P., Smith, W. N., Grant, B. B., Desjardins, R. L., McConkey, B. G., Campbell, C. A., & Nemecek, T. (2015). Accounting for soil carbon changes in agricultural life cycle assessment (LCA): A review. *Journal of Cleaner Production*, *104*, 23–39. https://doi.org/10.1016/j.jclepro.2015.05.040

Goidts, E., Van Wesemael, B., & Crucifix, M. (2009). Magnitude and sources of uncertainties in soil organic carbon (SOC) stock assessments at various scales. *European Journal of Soil Science*, *60*(5), 723–739. https://doi.org/10.1111/j.1365-2389.2009.01157.x

Google Earth (2018a). Study Area location including treatment sampling areas and treatment sampling locations. Imagery date: August 22, 2015. Location: 12 U 326303.00 m E 5675229.00 m N. Map scale: 1:5029200. Eye altitude: 2.7 km. Retrieved on November 19, 2018.

Google Earth (2018b). View of Study Area on July 16, 2011 two years prior to demonstration Project implementation. Imagery date: July 16, 2011. Location: 12 U 326303.00 m E 5675229.00 m N. Map scale: 1:5029200. Eye altitude: 2.7 km. Retrieved on November 19, 2018.

Google Earth (2018c). View of Study Area on November 11, 2011 two years prior to demonstration Project implementation. Imagery date: November 11, 2011. Location: 12 U 326303.00 m E 5675229.00 m N. Map scale: 1:5029200. Eye altitude: 2.7 km. Retrieved on November 19, 2018.

Google Earth (2019a). Study Area location relative to regional communities. Imagery date: October 18, 2018. Location: 12 U 321876 m E 5682234 m N. Map scale: 1:161176. Eye altitude: 59.03 km. Retrieved on February 21, 2019.

Google Earth (2019b). Study Area location relative to local secondary roads. Imagery date: July 12, 2018. Location: 12 U 327004 m E 5675318 m N. Map scale: 1:9424. Eye altitude: 3.46 km. Retrieved on February 21, 2019.

Government of Alberta. (2007). *Facts about climate change*. Retrieved March 15, 2019 from http://www.assembly.ab.ca/lao/library/egovdocs/2007/alen/158856.pdf .

Government of Alberta. (2012). *Quantification Protocol for Conservation Cropping*. Retrieved March 15, 2019 from https://extranet.gov.ab.ca/env/infocentre/info/library/8561.pdf.

Government of Alberta. (2018a). *Climate Leadership Plan - Implementation Plan 2018-19*. Retrieved from https://open.alberta.ca/dataset/da6433da-69b7-4d15-9123-01f76004f574/resource/b42b1f43-7b9d-483d-aa2a-6f9b4290d81e/download/clp_implementation_plan-jun07.pdf

Government of Alberta. (2018b). *Technical Guidance for Offset Protocol Development and Revision*.

Government of Canada. (2013). Soils of Alberta. Retrieved December 21, 2018, from http://sis.agr.gc.ca/cansis/soils/ab/soils.html

Government of Canada. (2017). Beiseker AGCM Alberta Monthy Data Report. Retrieved November 21, 2018, from http://climate.weather.gc.ca/historical_data/search_historic_data_e.html

Grigal, D. F., & Berguson, W. E. (1998). Soil carbon changes associated with short-rotation systems. *Biomass and Bioenergy*, *14*(4), 371–377. https://doi.org/10.1016/S0961-9534(97)10073-3

Grogan P., & Matthews, R. (2002). A modelling analysis of the potential for soil carbon sequestration under short rotation coppice willow bioenergy plantations. *Soil Use and Management*, *18*, 175–183. https://doi.org/10.1079/SUM2002119

Haak, D. E. (2007). *Soil Management Protocols and Projects for Greenhouse Gas Offsets in Canada*.

Halvorsen Okland, R. (2007). Wise Use of Statistical Tools in Ecological Field. *Folia Geobotanica*, *42*, 123–140.

Han, X., Zhao, F., Tong, X., Deng, J., Yang, G., Chen, L., & Kang, D. (2017). Understanding soil carbon sequestration following the afforestation of former arable land by physical fractionation. *Catena*, *150*, 317–327. https://doi.org/10.1016/j.catena.2016.11.027

Hansen, E. A. (1993). Soil carbon sequestration beneath hybrid poplar plantations in the North Central United States. *Biomass and Bioenergy*, *5*(6), 431–436. https://doi.org/10.1016/0961-9534(93)90038-6

Harrison, R. B., Footen, P. W., Harrison, R. B., Footen, P. W., & Strahm, B. D. (2011). and in Assessing Whole-Ecosystem Response to Management and, (June 2016).

Heim, A., Wehrli, L., Eugster, W., & Schmidt, M. W. I. (2009). Effects of sampling design on the probability to detect soil carbon stock changes at the Swiss CarboEurope site L??geren. *Geoderma*, *149*(3–4), 347–354. https://doi.org/10.1016/j.geoderma.2008.12.018

Heller, M. C., Keoleian, G. A., & Volk, T. A. (2003). Life cycle assessment of a willow bioenergy cropping system. *Biomass and Bioenergy*, *25*, 147–165. https://doi.org/10.1016/S0961-9534(02)00190-3

Hobley, E., & Willgoose, G. (2010). *Measuring soil organic carbon stocks – issues and consideratio*. *19th World Congress of Soil Science, Soil Solutions for a Changing World*. Brisbane, Australia. Retrieved from http://www.iuss.org/19th WCSS/Symposium/pdf/1794.pdf

Hooten, M. B., Wikle, C. K., Sheriff, S. L., & Rushin, J. W. (2009). Optimal spatio-temporal hybrid sampling designs for ecological monitoring. *Journal of Vegetation Science*, *20*(4), 639–649.

https://doi.org/10.1111/j.1654-1103.2009.01040.x

Horneck, D. A., Sullivan, D. M., Owen, J. S., & Hart, J. M. (2011). Soil test interpretation guide. *Oregon State University Extension Service*, (July), 1–12. https://doi.org/10.1017/CBO9781107415324.004

Hu, Y. L., Zeng, D. H., Ma, X. Q., & Chang, S. X. (2016). Root rather than leaf litter input drives soil carbon sequestration after afforestation on a marginal cropland. *Forest Ecology and Management*, *362*, 38–45. https://doi.org/10.1016/j.foreco.2015.11.048

Hydbom, S. (2017). *Tillage practices and their impact on soil organic carbon and the microbial community*. Lund. Retrieved from http://portal.research.lu.se/ws/files/34560477/Hydbom_thesis_kappa.pdf

Jandl, R., Rodeghiero, M., Martinez, C., Cotrufo, M. F., Bampa, F., van Wesemael, B., … Miglietta, F. (2014). Current status, uncertainty and future needs in soil organic carbon monitoring. *Science of the Total Environment*, *468–469*, 376–383. https://doi.org/10.1016/j.scitotenv.2013.08.026

IPCC. (2014). Climate Change 2014 Mitigation of Climate Change: Working Group III Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

IPCC. (2018). Global warming of 1.5°C: An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to eradicate poverty. Geneva, Switzerland. https://doi.org/10.1017/CBO9781107415324

Jin, V. L., Johnson, M. V. V., Haney, R. L., & Arnold, J. G. (2011). Potential carbon and nitrogen mineralization in soils from a perennial forage production system amended with class B biosolids. *Agriculture, Ecosystems and Environment*, *141*(3–4), 461–465. https://doi.org/10.1016/j.agee.2011.03.016

Jodral-Segado, A., Navarro-Alarcón, M., De La Serrana, H., & López-Martínez, M. (2006). Calcium and magnesium levels in agricultural soil and sewage sludge in an industrial area from southeastern Spain: Relationship with plant (Saccharum officinarum) disposition. *Soil and Sediment Contamination*, *15*, 367–377. https://doi.org/10.1080/15320380600751736

Johnson, E., & Miyanishi, K. (2008). Testing the assumptions of chronosequences in succession. *Ecology Letters*, *11*, 419–431. https://doi.org/10.1111/j.1461-0248.2008.01173.x

Kahle, P., Hildebrand, E., Baum, C., & Boelcke, B. (2007). Long-term effects of short rotation forestry with willows and poplar on soil properties. *Archives of Agronomy and Soil Science*, *53*(6), 673–682. https://doi.org/10.1080/03650340701648484

Karunaratne, S. B., Bishop, T. F. A., Odeh, I. O. ., Baldock, J. A., & Marchant, B. P. (2014). Estimating changes in soil organic carbon using legacy data as the baseline; issues, approaches and lessons to learn, 349–365.

Kaufmann, N., & Krause, E. (2016). *Putting a Price on Carbon: Reducing Emissions*. *World Resources Institute*. https://doi.org/10.3109/02841851003691979

Kirschbaum, M. U. F. (2000). Will changes in soil organic carbon act as a positive or negative feedback on global warming? *Biogeochemistry*, *48*(1), 21–51. https://doi.org/10.1023/A:1006238902976

Kravchenko, A. N., & Robertson, P. G. (2011). Whole-Profile Soil Carbon Stocks: The Danger of Assuming Too Much from Analyses of Too Little. *Soil Science Society of America Journal*, *75*(1), 235. https://doi.org/10.2136/sssaj2010.0076

Lal, R. (2002). Soil carbon dynamics in cropland and rangeland. *Environmental Pollution*, *116*(3), 353–362. https://doi.org/10.1016/S0269-7491(01)00211-1

Lal, R. (2005). Forest soils and carbon sequestration. *Forest Ecology and Management*, *220*, 242–258. https://doi.org/10.1016/j.foreco.2005.08.015

Lal, R. (2016). Beyond COP 21 : Potential and challenges of the “4 per Thousand” initiative. *Journal of Soil and Water Conservation*, *71*(1), 20–25. https://doi.org/10.2489/jswc.71.1.20A

Lavery, J. (SYLVIS Environmental), personal communication, March 15, 2019a. 2012-2017 land management practices at 07-026-25 W4M of *The City of Calgary Dewatered Biosolids Land Application Program – Willow Biomass and Marginal Land Reclamation Demonstration Project*.

Lavery, J. (SYLVIS Environmental), personal communication, March 15, 2019b. 2012-2017 biosolid landspreading application practices and rates at 07-026-25 W4M of *The City of Calgary Dewatered Biosolids Land Application Program – Willow Biomass and Marginal Land Reclamation Demonstration Project*.

Lavery, J. (SYLVIS Environmental), personal communication, March 15, 2019c. 2013 and 2016 data on the City of Calgary Shepard waste water treatment plant biosolid chemical analysis results used for land-spreading at *The City of Calgary Dewatered Biosolids Land Application Program – Willow Biomass and Marginal Land Reclamation Demonstration Project*.

Lavery, J. (SYLVIS Environmental), personal communication, March 15, 2019d. SYLVIS 2016 willow cultivar planting locations and willow biomass harvesting results from the SW 07-026-25 W4M location of *The City of Calgary Dewatered Biosolids Land Application Program – Willow Biomass and Marginal Land Reclamation Demonstration Project*.

Lavery, J. (SYLVIS Environmental), personal communication, March 15, 2019e. 2013 and 2016 data from the City of Calgary Shepard waste water treatment plant biosolid chemical analysis results use for landspreading at *The City of Calgary Dewatered Biosolids Land Application Program – Willow Biomass and Marginal Land Reclamation Demonstration Project*.

Lehmann, J., & Kleber, M. (2015). The contentious nature of soil organic matter. *Nature*, *528*, 61–68.

Lemus, R., & Lal, R. (2005). Bioenergy Crops and Carbon Sequestration. *Critical Reviews in Plant Sciences*, *24*(1), 1–21. https://doi.org/10.1080/07352680590910393

Lockwell, J., Guidi, W., & Labrecque, M. (2012). Soil carbon sequestration potential of willows in short-rotation coppice established on abandoned farm lands. *Plant and Soil*, *360*(1–2), 299–318. https://doi.org/10.1007/s11104-012-1251-2

Mackenzie, D. (2011). *Best Management Practices For Conservation of Reclamation Materials in the Mineable Oil Sands Region of Alberta*. *Direct*. https://doi.org/978-1-4601-0048-6

Maillard, É., McConkey, B. G., & Angers, D. A. (2017). Increased uncertainty in soil carbon stock measurement with spatial scale and sampling profile depth in world grasslands: A systematic analysis. *Agriculture, Ecosystems & Environment*, *236*, 268–276. https://doi.org/10.1016/j.agee.2016.11.024

McClean, G. J., Rowe, R. L., Heal, K. V., Cross, A., Bending, G. D., & Sohi, S. P. (2015). An empirical model approach for assessing soil organic carbon stock changes following biomass crop establishment in Britain. *Biomass and Bioenergy*, *83*, 141–151. https://doi.org/10.1016/j.biombioe.2015.09.005

Mcconkey, B., Liang, C., Padbury, G., Pennock, D., Lindwall, W., Canada, A., & Canada, E. (2000). *Measuring Soil Carbon Change on Cropland : The Prairie Soil Carbon Balance Project. Proceedings for the Climate Change Workshop*.

Merino, C., Nannipieri, P., & Matus, F. (2015). Soil carbon controlled by plant, microorganism and mineralogy interactions. *Journal of Soil Science and Plant Nutrition*, *15*(2), 321–332. https://doi.org/10.4067/S0718-95162015005000030

Michael A. Bashkin, & Dan Binkley. (1998). Changes in soil carbon following afforestation in Hawaii. *Ecology*, *79*(3), 828–833.

Natural Regions Commitee. (2006). *Natural Regions and Subregions of Alberta*. (D. J. Downing & W. W. Pettapiece, Eds.). Edmonton: Pub. No. T/852.

Nature. (2019). Significant debate. *Nature*, *567*, 283.

Necpálová, M., Anex, R. P., Kravchenko, A. N., Abendroth, L. J., Del Grosso, S. J., Dick, W. A., … Villamil, M. B. (2014). What does it take to detect a change in soil carbon stock? A regional comparison of minimum detectable difference and experiment duration in the north central United States. *Journal of Soil and Water Conservation*, *69*(6), 517–531. https://doi.org/10.2489/jswc.69.6.517

Oertel, C., Matschullat, J., Zurba, K., Zimmermann, F., & Erasmi, S. (2016). Greenhouse gas emissions from soils—A review. *Chemie Der Erde - Geochemistry*, *76*(3), 327–352. https://doi.org/10.1016/j.chemer.2016.04.002

Olander, L. P., & Haugen-Kozyra, K. (2012). Using biogeochemical process models to quantify greenhouse gas mitigation from agricultural management. *Climate Change Mitigation and Agriculture*, (March), 227–241. https://doi.org/10.4324/9780203144510

Olander, L. P., Haugen-kozyra, K., & Kravchenko, A. (2011). *Assessing Greenhouse Gas Mitigation Opportunities and Implementation Strategies for Agricultural Land Management in the United States*.

Olson, K. R., & Al-Kaisi, M. M. (2015). The importance of soil sampling depth for accurate account of soil organic carbon sequestration, storage, retention and loss. *Catena*, *125*, 33–37. https://doi.org/10.1016/j.catena.2014.10.004

Pacaldo, R. S., Volk, T. A., & Briggs, R. D. (2013a). Greenhouse Gas Potentials of Shrub Willow Biomass Crops Based on Below- and Aboveground Biomass Inventory Along a 19-Year Chronosequence. *Bioenergy Research*, *6*(1), 252–262. https://doi.org/10.1007/s12155-012-9250-y

Pacaldo, R. S., Volk, T. A., & Briggs, R. D. (2013b). No significant differences in soil organic carbon contents along a chronosequence of shrub willow biomass crop fields. *Biomass and Bioenergy*, *58*, 136–142. https://doi.org/10.1016/j.biombioe.2013.10.018

Paragon. (2006). *Draft Guide to Development of Customized Agricultural Soil Carbon Sink Protocol for Greenhouse Gas Emission Reductions and / or Removals under Canada ' s Offset System Prepared By : Quantification Methodology and Protocol Division Canada ' s Greenhouse Ga*. Edmonton, AB.

Paul, K. I., Polglase, P. J., Nyakuengama, J. G., & Khanna, P. K. (2002). Change in soil carbon following afforestation. *Forest Ecology and Management*, *168*, 241–257.

Paustian, K., Andrén, O., Janzen, H. H., Lal, R., Smith, P., Tian, G., … Woomer, P. L. (1997). Agricultural soils as a sink to mitigate CO $_2$ emissions. *Soil Use and Management*, *13*(s4), 230–244. https://doi.org/10.1111/j.1475-2743.1997.tb00594.x

Paustian, K., Collier, S., Baldock, J., Burgess, R., Creque, J., Delonge, M., … Grundy, M. (2017). Quantifying soil carbon measurement for agricultural soils management : A consensus view from science, (September), 1–24.

Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G. P., & Smith, P. (2016). Climate-smart soils. *Nature*, *532*, 49–57. https://doi.org/10.1038/nature17174

Pennock, D. J. (2004). Designing field studies in soil science. *Canadian Journal of Soil Science*, *84*(December 2003), 1–10. https://doi.org/10.4141/S03-039

Post, W. M., & Kwon, K. C. (2000). Soil carbon sequestration and land-use change: processes and potential. *Global Change Biology*, *6*, 317–327. https://doi.org/10.1007/s00704-011-0500-2

Poudyal, N. C., Siry, J. P., & Bowker, J. M. (2011). Quality of urban forest carbon credits. *Urban Forestry & Urban Greening*, *10*(3), 223–230. https://doi.org/10.1016/j.ufug.2011.05.005

Putten, B. Van, & Knippers, T. (2010). On Design and Statistical Analysis in Soil Treatment Experiments. *Soil Science*, *175*(11), 519–529. https://doi.org/10.1097/SS.0b013e3181fa2821

Quaye, A. K., & Volk, T. A. (2013). Biomass production and soil nutrients in organic and inorganic fertilized willow biomass production systems. *Biomass and Bioenergy*, *57*, 113–125. https://doi.org/10.1016/j.biombioe.2013.08.002

Raul Ponce-Hernandez, P. K., & Antoine, J. (2000). a Methodological Framework for the Assessment of Carbon Stocks and. *Data Base*, (Cdm), 1–17.

Rose, S., Spinks, N., & Canhoto, A. I. (2014). Tests for the assumption that a variable is normally distributed. In Management research: Applying the principles. (pp. 1–4).

Rowley, M. C., Grand, S., & Verrecchia, É. P. (2018). Calcium-mediated stabilisation of soil organic carbon. *Biogeochemistry*, *137*(1–2), 27–49. https://doi.org/10.1007/s10533-017-0410-1

Rytter, R. (2001). Biomass production and allocation , including root turnover , and annual N uptake in lysimeter-grown basket willows, *140*, 177–192.

Rytter, R. (2012). The potential of willow and poplar plantations as carbon sinks in Sweden. *Biomass and Bioenergy*, *36*, 86–95. https://doi.org/10.1016/j.biombioe.2011.10.012

Rytter, R. M. (2016). Afforestation of former agricultural land with Salicaceae species - Initial effects on soil organic carbon, mineral nutrients, C: N and pH. *Forest Ecology and Management*, *363*, 21–30. https://doi.org/10.1016/j.foreco.2015.12.026

Rytter, R., Rytter, L., & Högbom, L. (2015). Carbon sequestration in willow (Salix spp.) plantations on former arable land estimated by repeated field sampling and C budget calculation. *Biomass and Bioenergy*, *83*, 483–492. https://doi.org/10.1016/j.biombioe.2015.10.009

Saby, N. P. A., Bellamy, P. H., Morvan, X., Arrouays, D., Jones, R. J. A., Verheijen, F. G. A., … Simota, C. (2008). Will European soil-monitoring networks be able to detect changes in topsoil organic carbon content? *Global Change Biology*, *14*, 2432–2442. https://doi.org/10.1111/j.1365-2486.2008.01658.x

Schumacher, B. a. (2002). *Methods for the Determination of Total Organic Carbon in Soils and Sediments*. *Ecological Risk Assessment Support Center*. Las Vegas, NV. https://doi.org/http://epa.gov/esd/cmb/research/papers/bs116.pdf

Singh, K., Murphy, B. W., & Marchant, B. P. (2012). towards cost effective estimation of soil carbon stocks at the field scale. *Soil Research*, *50*, 672–684.

Six, J., Frey, S. D., Thiet, R. K., & Batten, K. M. (2006). Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems. *Soil Science Society of America Journal*, *70*(2), 555. https://doi.org/10.2136/sssaj2004.0347

Smith, P. (2004). How long before a change in soil organic carbon can be detected? *Global Change Biology*, *10*(11), 1878–1883. https://doi.org/10.1111/j.1365-2486.2004.00854.x

Smith, P., Davies, C. A., Ogle, S., Zanchi, G., Bellarby, J., Bird, N., … Braimoh, A. K. (2012). Towards an integrated global framework to assess the impacts of land use and management change on soil carbon: Current capability and future vision. *Global Change Biology*, *18*(7), 2089–2101. https://doi.org/10.1111/j.1365-2486.2012.02689.x

Soil Research Institute. (1976). Soil Capability for Agriculture. Retrieved January 25, 2019, from http://sis.agr.gc.ca/cansis/publications/maps/cli/1m/agr/cli_1m_agr_alberta.jpg

Sommer, R., & Bossio, D. (2014). Dynamics and climate change mitigation potential of soil organic carbon sequestration. *Journal of Environmental Management*, *144*, 83–87. https://doi.org/10.1016/j.jenvman.2014.05.017

Soriano-Disla, J. M., Janik, L. J., Viscarra Rossel, R. A., MacDonald, L. M., & McLaughlin, M. J. (2014). The performance of visible, near-, and mid-infrared reflectance spectroscopy for prediction of soil physical, chemical, and biological properties. *Applied Spectroscopy Reviews*, *49*(2), 139–186. https://doi.org/10.1080/05704928.2013.811081

Stahlman, P. W., Blanco-Canqui, H., Schlegel, A. J., Stone, L. R., Lyon, D. J., Vigil, M. F., … Mikha, M. M. (2009). No-till Induced Increase in Organic Carbon Reduces Maximum Bulk Density of Soils. *Soil Science Society of America Journal*, *73*(6), 1871. https://doi.org/10.2136/sssaj2008.0353

Stockmann, U., Adams, M. A., Crawford, J. W., Field, D. J., Henakaarchchi, N., Jenkins, M., … Zimmermann, M. (2013). The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agriculture, Ecosystems and Environment*, *164*, 80–99. https://doi.org/10.1016/j.agee.2012.10.001

Thamo, T., & Pannell, D. J. (2016). Challenges in developing effective policy for soil carbon sequestration: perspectives on additionality, leakage, and permanence. *Climate Policy*, *16*(8), 973–992. https://doi.org/10.1080/14693062.2015.1075372

Tian, G., Granato, T. C., Cox, A. E., Pietz, R. I., Carlson, C. R., & Abedin, Z. (2009). *Soil Carbon Sequestration Resulting from Long-Term Application of Biosolids for Land Reclamation*. *Journal of Environment Quality* (Vol. 38). https://doi.org/10.2134/jeq2007.0471

Tom Goddard, Karen Haugen-Kozyra, A. R. (2008). Session 3 . 1 : Climate Change Conservation Agriculture Protocols for Greenhouse Gas Offsets in a Working Carbon Market, 341–350.

Torri, S. I., Corrêa, R. S., & Renella, G. (2014). *Soil carbon sequestration resulting from biosolids application*. *Applied and Environmental Soil Science* (Vol. 2014). Hindawi Publishing Corporation. https://doi.org/10.1155/2014/821768

United Nations Environment Programme (UNEP). (2018). *Emissions Gap Report*. Nairobi, Kenya. Retrieved from http://www.un.org/Depts/Cartographic/english/htmain.htm

United States Department of Agriculture. (1998). Bulk Density/Moisture/Aeration - Soil Quality Kit. Retrieved January 25, 2019, from https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_053260.pdf

VandenBygaart, a. J., Gregorich, E. G., & Angers, D. a. (2003). Influence of agricultural management on soil organic carbon: A compendium and assessment of Canadian studies. *Canadian Journal of Soil Science*, *83*(4), 363–380. https://doi.org/10.4141/S03-009

VandenBygaart, A. J. (2006). Monitoring soil organic carbon stock changes in agricultural landscapes: Issues and a proposed approach. *Canadian Journal of Soil Science*, *86*, 451–463. https://doi.org/10.4141/S05-105

Walter, K., Don, A., & Flessa, H. (2015). No general soil carbon sequestration under Central European short rotation coppices. *GCB Bioenergy*, *7*, 727–740. https://doi.org/10.1111/gcbb.12177

Wasserstein, R. L., Schirm, A. L., & Lazar, N. A. (2019). Moving to a World Beyond " *p* < 0.05." *The American Statistician*, *73*(sup1), 1–19. https://doi.org/10.1080/00031305.2019.1583913

Watson, K. (2016). Field Handbook for the Soils of Western Canada Section 3 Soil Profile Description. Retrieved from http://sis.agr.gc.ca/cansis/taxa/cssc3/index.html.

West, T. O., & Marland, G. (2002). A synthesis of carbon sequestration, carbon emissions, and net carbon flux in agriculture: Comparing tillage practices in the United States. *Agriculture, Ecosystems and Environment*, *91*(1–3), 217–232. https://doi.org/10.1016/S0167-8809(01)00233-X

West, T. O., & Post, W. M. (2002). Soil Organic Carbon Sequestration Rates by Tillage and Crop Rotation. *Soil Science Society of America Journal*, *66*(6), 1930–1946. https://doi.org/10.2136/sssaj2002.1930

West, T. O., & Six, J. (2007). Considering the influence of sequestration duration and carbon saturation on estimates of soil carbon capacity. *Climatic Change*, *80*, 25–41. https://doi.org/10.1007/s10584-006-9173-8

Wijesekara, H., Bolan, N. S., Thangavel, R., Seshadri, B., Surapaneni, A., Saint, C., … Vithanage, M. (2017). The impact of biosolids application on organic carbon and carbon dioxide fluxes in soil. *Chemosphere*, *189*, 565–573. https://doi.org/10.1016/j.chemosphere.2017.09.090

World Bank and Ecofys. (2018). *State and Trends of Carbon Pricing 2018 (May)*. Washington, DC. https://doi.org/10.1596/978-1-4648-1218-7

World Business Council for Sustainable Development (WBCSD). (2017). *Why Carbon Pricing Matters: A guide for implementation*. https://doi.org/10.1108/978-1-78714-447-720171009

Xue, J., Kimberley, M. O., Ross, C., Gielen, G., Tremblay, L. A., Champeau, O., … Wang, H. (2015). Ecological impacts of long-term application of biosolids to a radiata pine plantation. *Science of the Total Environment*, *530–531*, 233–240. https://doi.org/10.1016/j.scitotenv.2015.05.096

Zan, C. S., Fyles, J. W., Girouard, P., & Samson, R. A. (2001). Carbon sequestration in perennial bioenergy, annual corn and uncultivated systems in southern Quebec. *Agriculture, Ecosystems and Environment*, *86*, 135–144. https://doi.org/10.1016/S0167-8809(00)00273-5

Zang, H., Blagodatskaya, E., Wen, Y., Xu, X., Dyckmans, J., & Kuzyakov, Y. (2018). Carbon sequestration and turnover in soil under the energy crop Miscanthus: repeated13C natural abundance approach and literature synthesis. *GCB Bioenergy*, *10*, 262–271. https://doi.org/10.1111/gcbb.12485

Zhao, L., Sun, Y., Zhang, X., Yang, X., & Drury, C. F. (2006). Soil organic carbon in clay and silt sized particles in Chinese mollisols: Relationship to the predicted capacity. *International Journal of Industrial Engineering and Management*, *7*(4), 143–152. https://doi.org/10.1016/j.geoderma.2005.04.026

Appendix A

Experimental Study — Supporting Information

Study Location

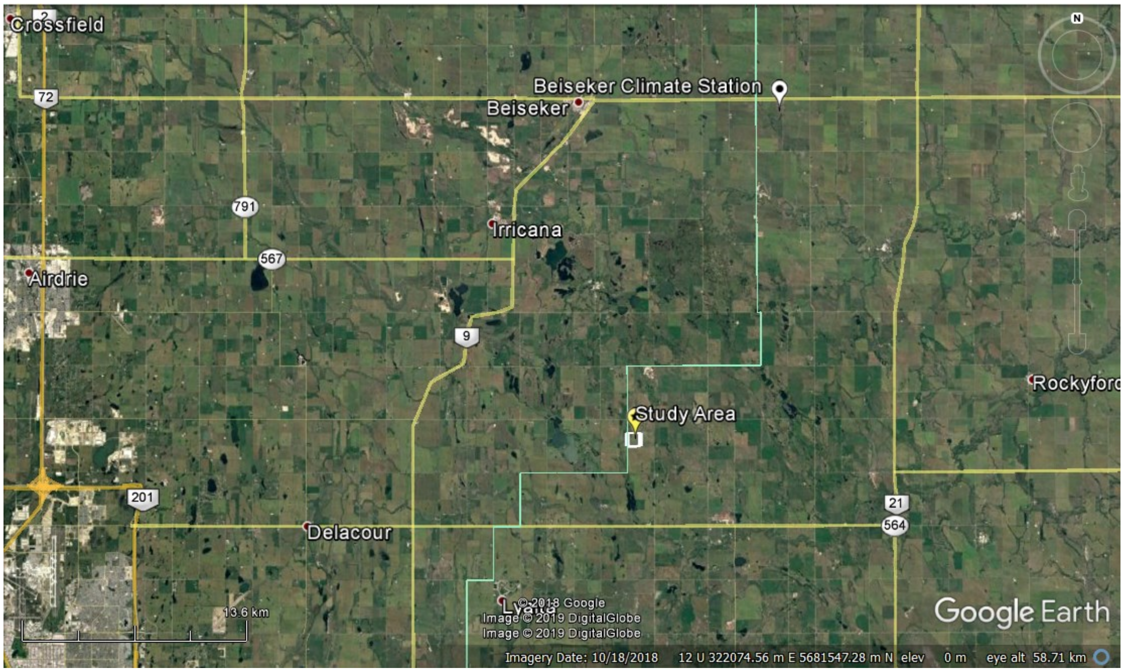

Figure A 1: Study Area location relative to regional communities (Google Earth, 2019a)

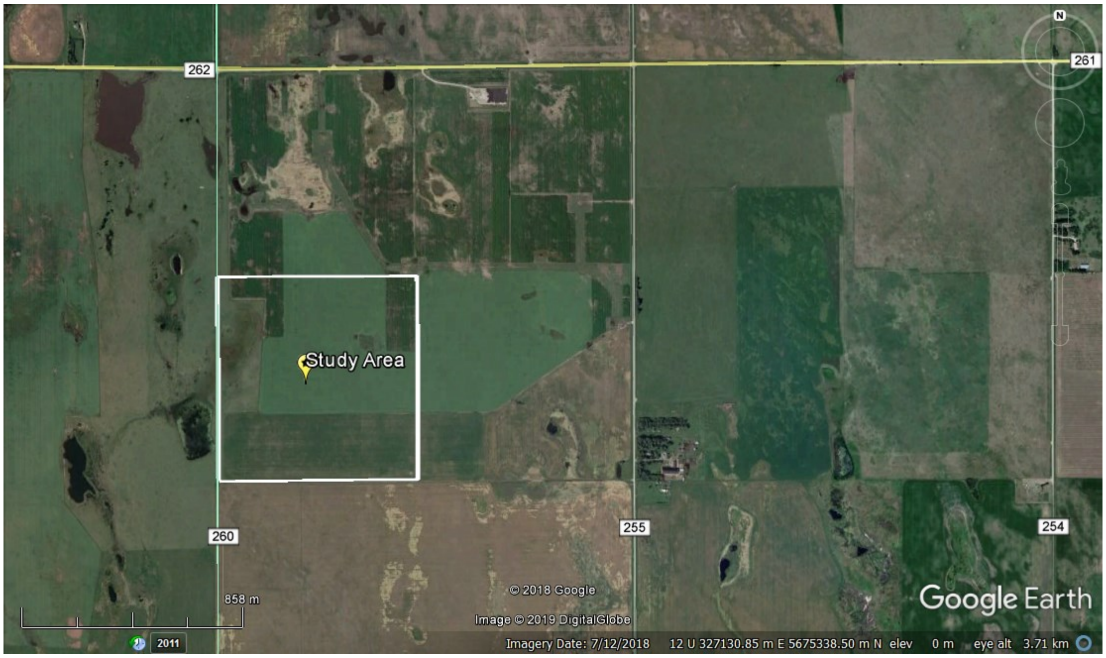

Figure A 2: Study Area location relative to local secondary roads (Google Earth, 2019b)

Climactic Conditions

Table A 1: Annual (2012-2018) Beiseker AGCM weather station data and average climate data and standard error of the mean for the Foothills Fescue Natural Subregion

Climate Parameters	Foothills Fescue Natural Subregion[a]	Annual Beiseker AGCM Climate Data[b]							
		Average (2012-2018)	2012	2013	2014	2015	2016	2017	2018
Mean Daily Mean Temperature (°C)	3.9	3.4 ± 0.2	3.3 ± 0.6	3.3 ± 0.6	2.9 ± 0.6	4.3 ± 0.5	4.6 ± 0.5	3.3 ± 0.6	1.9 ± 0.6
Mean Daily Max Temperature (°C)	16.3	10.8 ± 0.3	11.3 ± 0.7	10.7 ± 0.7	9.7 ± 0.7	12.0 ± 0.6	11.8 ± 0.6	10.8 ± 0.7	9.6 ± 0.7
Mean Daily Min Temperature (°C)	-9.7	-4.0 ± 0.2	-4.2 ± 0.6	-4.1 ± 0.6	-4.2 ± 0.6	-3.4 ± 0.5	-2.6 ± 0.5	-4.2 ± 0.6	-5.7 ± 0.6
Total Annual Precipitation (mm)	470	344 ± 19	274	362	350	357	423	349	292

References: [a] Natural Regions Commitee (2006), [b] Government of Canada (2017)

Regional Soil Characteristics

Table A 2: Soil physical properties modified from Soils of Alberta (Government of Canada, 2013)

Soil Series (Agriculture Version)	Depth (cm)	Bulk Density (g/cm^3)	Course Fragments (%)	Total Sand (%)	Total Silt (%)	Total Clay (%)	Dominant Sand Fraction
Midnapore	0-30	1.25	0	33	42	25	Fine
Rockyview	0-18	1.15	0	15	65	20	Fine
Rockyview	18-40	1.4	0	15	60	25	Fine
Delacour	0-20	1.15	20	24	52	24	Fine
Delacour	20-35	1.4	20	31	36	33	Fine

Table A 3: Soil chemical properties modified from Soils of Alberta (Government of Canada, 2013)

Soil Series (Agriculture Versions)	Depth (cm)	Soil Organic Matter (%)	pH in Calcium Chloride	Calcium Carbonate (%)
Midnapore	0-30	4	6.9	0
Rockyview	0-18	4	7.4	1
Rockyview	18-40	1.5	7.4	1
Delacour	0-20	4	6	0
Delacour	20-35	1.3	5.9	0

Historic Air Photos

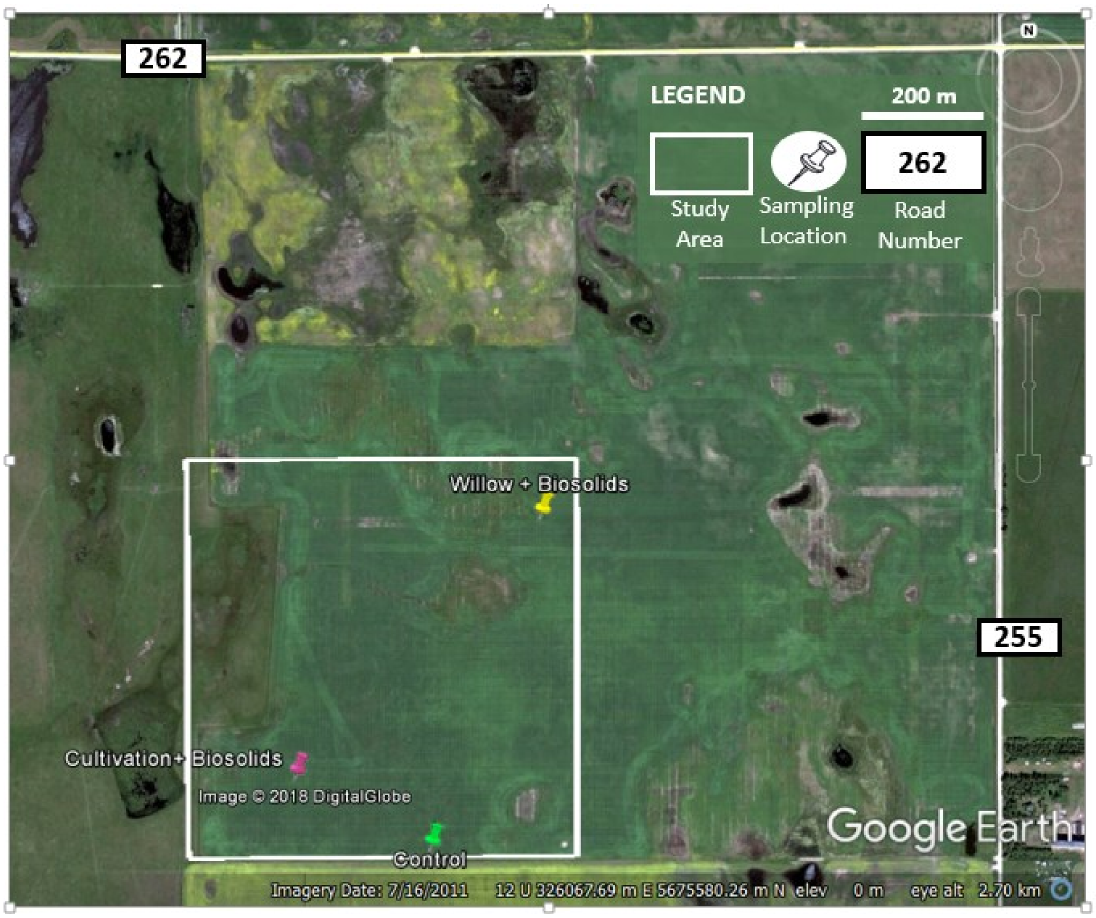

Figure A 3: View of Study Area on July 16, 2011 two years prior to demonstration Project implementation (Google Earth, 2018b)

Figure A 4: View of Study Area on November 11, 2011 two years prior to demonstration Project implementation (Google Earth, 2018c)

Appendix B

Historical Environmental Monitoring and Land Management Practices within the Study Area (2012-2017)

Table B 1: 2012 Agriculture Land Management Practices at all sampling locations within the Study Area

Activity	Product	Application or harvesting rate per acre
April 23, 2012		
Fertilizing	Nitrogen (18-0-0)	26.5 L
Fertilizing	Urea (46-0-0)	68 kg
Planting	GO Spring Wheat	45 kg
Fungicide	Tilt 250E (29820) (3)	100 L
Fungicide	Folicur 250 EW (298020) (3)	0.20 L
Herbicide	Barricade SG (29544) (2)	0.16 L
Herbicide	Roundup Transorb HC (28198) (9)	100 L
Herbicide	Simply Herbicide (28887) (2)	0.20 L
August 8, 2012		
Harvesting	GO Spring Wheat	39.34 bushel

Table B 2: 2013 Land Management Practices at the C+BS and the Control Sampling Locations within the Study Area

Activity	Product	Application or harvesting rate per acre (ac)
May 10, 2013		
Fertilizing	Phosphate canola 11-52-0	18 kg
Fertilizing	Ammonium sulphate	45 kg
Fertilizing	Urea (46-0-0)	33 kg
Herbicide	Roundup Transorb HC (28198) (9)	0.33 L
Planting	Canola Dupont D3153 (RR)	0.9 kg
Planting	Canola (RR Nexara 1012)	0.9 kg
June 4, 2013		
Fertilizer	Nitrogen (18-0-0)	11 L
Herbicide	R-T 540 Liquid Herbicide (28487) (9)	0.33 L
June 23, 2013		
Fertilizer	Nitrogen (18-0-0)	11 L
Herbicide	R-T 540 Liquid Herbicide (28487) (9)	0.33 L
October 7, 2013		
Harvesting	Canola (RR Nexara 1012)	24 bushels
Harvesting	Canola Dupont D3153 (RR)	34 bushels

Table B 3: 2014 Land Management Practices at the C+BS and the Control Sampling Locations within the Study Area

Activity	Product	Application or harvesting rate per acre (ac)
May 14, 2014		
Herbicide	Crush R plus	0.5 L
Herbicide	Amitrol	1.0 L
May 30, 2014		
Planting	Barley (Xena)	54 kg
Planting	Barley (Xena)	54 kg
June 30, 2014		
Herbicide	Turbocharge 23135	0.095 L
Fertilizer	Ammonium sulphate	0.5 L
Herbicide	Liquide Achieve SC	0.0125 case
Herbicide	Approve Herbicide (28124) (4)	0.20 L
November 3, 2014		
Harvesting	Barley (xena)	7.5 bushel

Table B 4: 2015 Land Management Practices at the C+BS and the Control Sampling Locations within the Study Area

Activity	Product	Application or harvesting rate per acre (ac)
April 27, 2015		
Herbicide	Vantage plus max (27615) (9)	0.75 L
May 15, 2015		
Planting	Oats (mustang)	36 kg
Planting	Wheat Spring (GO)	54 lbs
May 19, 2015		
Herbicide	Vantage Plus Max (27615) (9)	1.0 L
Planting	Canola (Liberty L130)	1.0 kg
June 6, 2015		
Herbicide	Simply Herbicide (28887) (2)	0.3 L
Herbicide	MPCA Ester 600 (29001) (4)	0.3 L

Table B 5: 2016 Land Management Practices at the C+BS and the Control Sampling Locations within the Study Area

May 05, 2016			
Herbicide	Maverick III Herbicide (28977) (9)	0.75 L/ac	0.75 L
May 08,2016			
Fertilizing	0-20-0-10	4.50 lb/ac	2.04 Kg
Planting	Canola (Liberty L252)	5.00 lb/ac	2.26 Kg
June 09,2016			
Herbicide	Centurion Herbicide (27598) (1)	0.01 case/ac	0.01 case
Herbicide	Liberty 150 SN Herbicide (28837) (10)	1.50 L/ac	1.50 L
September 09, 2016			
Herbicide	Vantage Plus Max (27615) (9)	0.75 L/ac	0.75 L
October 25, 2016			
Harvesting	Canola (Liberty L252)	9.10 bu/ac	9.10 bu

Table B 6: 2017 Land Management Practices at the C+BS and the Control Sampling Locations within the Study Area

May 10, 2017			
Fertilizing	18-0-0	2.00 US gal/ac	7.57 L
Fertilizing	Humates	5.00 US gal/ac	18.92 L
Fertilizing	Alpine g22	3.00 US gal/ac	11.35 L
Herbicide	PrePass Flex Herbicide (31259) (2)	8.00 US gal/ac	30.28 L
Herbicide	Vantage Plus Max (27615) (9)	0.60 L/ac	0.60 L
Planting	Hard red spring (cdc go)	110.00 lb/ac	49.89 Kilograms (kg)
June 15, 2017			
Adjuvant	Agral 90 (11809)	0.06 L/ac	0.06 L
Herbicide	Octain	0.45 L/ac	0.45 L
Herbicide	Simplicity GoDri	0.01 jug/ac	0.01 jug
July 04,2017			
Fertilizing	Humates	1.00 US gal/ac	3.78 L
Fertilizing	18-0-0	1.00 US gal/ac	3.78 L
Fungicide	Folicur 250 EW Fungicide (29820) (3)	0.02 jug/ac	0.02 jug
October 09, 2017			
Harvesting	Hard red spring (cdc go)	65.45 bu/ac	65.45 bu

Table B 7: Summary of the City of Calgary Shepard waste water treatment plant biosolid chemical analysis results used for land-spreading at the demonstration Project site in 2013 and 2016

Biosolids Chemical Parameters Analyzed	Units	2013 Mean Chemical Analysis Results (n=12)	2016 Mean Chemical Analysis Results (n=11)	% Change
Moisture Content	%	82	82	0
pH (1:2)	NA	7.4	7.7	4
Cation Exchange Capacity	meq/100g	80	60	-25
Organic Matter	% dry weight	53	54	-2
Total Organic Carbon	% dry weight	27	27	0
Available Nitrate	mg/kg	10	2	-80
Available Phosphorous	mg/kg	6467	4318	-33
Available Potassium	mg/kg	1878	1665	-11
Available Calcium	mg/kg	6063	3998	-34
Available Magnesium	mg/kg	2318	1534	-34
Available Sodium	mg/kg	841	630	-25
Available Iron	mg/kg	927	1053	14

References: J. Lavery (SYLVIS Environmental), personal communication, March 15, 2019c

Table B 8: Soil mineral concentration results from soil samples (0-15 cm) taken from within the Study Area during environmental monitoring events conducted by SYLVIS in prior to the demonstration Project installation (2013), and after two biosolids land-spreading application events (2017) totaling 45 Mg ha^{-1}

0-15 cm Soil Sampling Depth Interval		SYLVIS 2013 (n=4)		SYLVIS 2017 (n=4)	
Available Nutrients	Units	Mean	SEM	Mean	SEM
Calcium	mg/kg	1658	201	1505	146
Magnesium	mg/kg	246	37	240	19
Iron	mg/kg	89	7	113	8
Sodium	mg/kg	68	42	16	9
Soil Acidity					
pH		6.5	0.3	5.9	0.2
Cation Exchange Capacity	meq/100g	15.0	2.9	NA	NA

Note: NA= not available

Data provided by J. Lavery (SYLVIS Environmental), personal communication, March 15, 2019e

Table B 9: Soil mineral concentration results from soil samples (15 -30 cm) taken from within the Study Area during environmental monitoring events conducted by SYLVIS in prior to demonstration Project installation (2013), and after two biosolids land-spreading application events (2017) totaling 45 Mg ha^{-1}

15-30 cm Soil Sampling Depth Interval		SYLVIS 2013 (n=4)		SYLVIS 2017 (n=4)	
Available Nutrients	Units	Mean	SEM	Mean	SEM
Calcium	mg/kg	2158	349	1960	307
Magnesium	mg/kg	441	91	243	13
Iron	mg/kg	61	7	67	6
Sodium	mg/kg	215	24	118	14
Soil Acidity					
pH		7.2	0.2	6.6	0.5
Cation Exchange Capacity	meq/100g	12.5	2.5	NA	NA

Note: NA= not available
Data provided by J. Lavery (SYLVIS Environmental), personal communication, March 15, 2019e

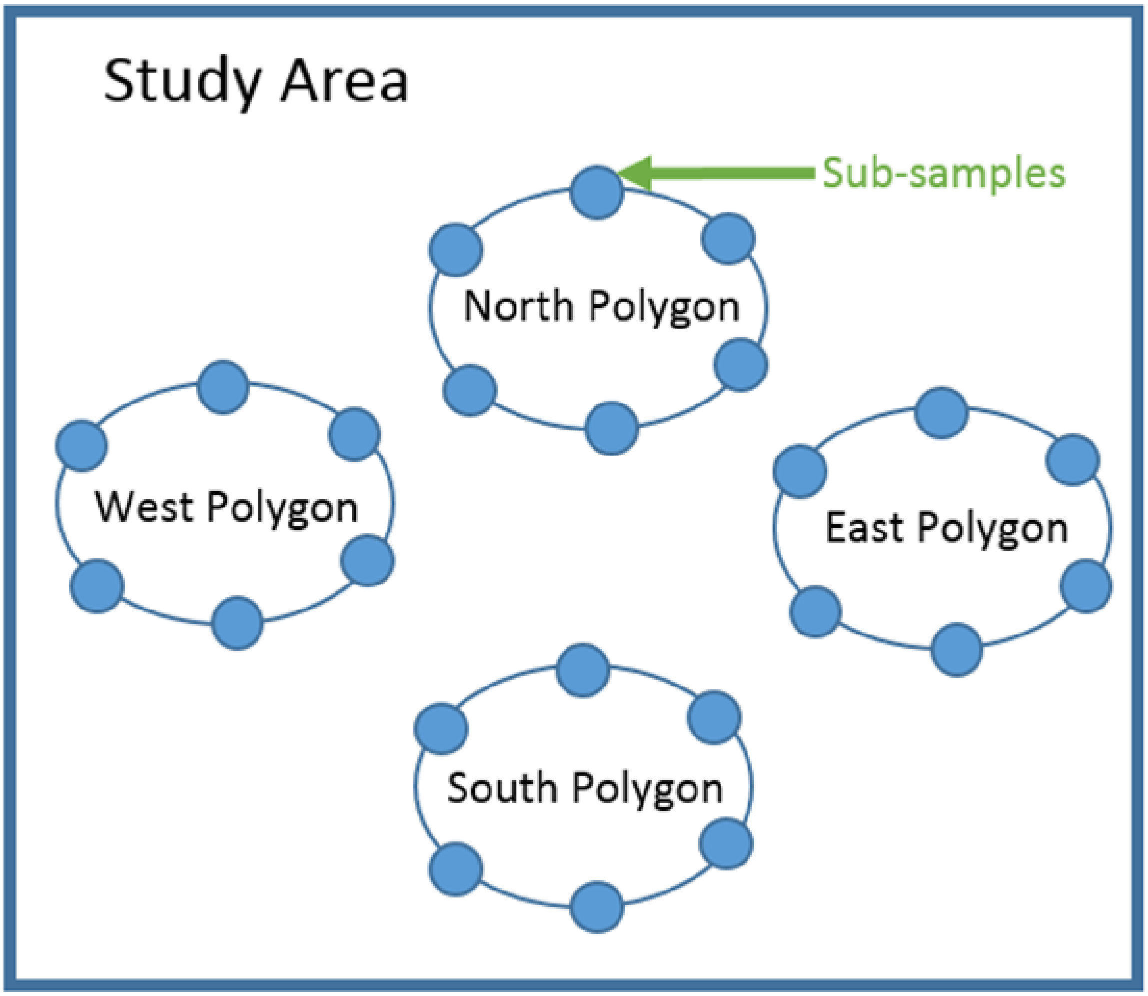

Figure B 1: General representation of *Alberta Guidelines for the Application of Municipal Wastewater Sludges to Agricultural Lands* (2001) soil sampling protocol implemented by SYLVIS (not to scale

Appendix C

EXOVA Laboratory Methodologies

DRYING SOIL SAMPLES

1. INTRODUCTION

Many analyses require that a soil sample be dried and ground prior to analysis. The soil is dried to the point where the moisture content is constant.

2. SCOPE

This method is applicable to all soil samples received in the lab where the analysis must be performed on a dried and ground sample.

3. SUPPORTING DOCUMENTS

3.1. WI PREP 010-10, *In-house Subsampling Procedures*
3.2 Despatch Oven Operations Manual

4. SAMPLE REQUIREMENTS

4.1. The holding time and quantity of sample required is dependent on the analyses requested. See individual test methods for holding times and quantities.
4.2. Sample collected in a container that prevents gross contamination is required.

SAMPLE GRINDING

1. SIGNIFICANCE AND SCOPE

The types of dried samples which are processed by way of grinding include, but are not limited to:

- agricultural soil
- environmental soil
- international soil
- sludge
- stone/sand
- waste
- feed

2. PRINCIPLE

Grinding samples prior to analysis increases the surface area of the sample, allowing more efficient reaction with the chemicals used during laboratory analysis. The type of sample received dictates the method(s) by which it must be ground. The resultant particle size is limited to 2mm width by sifting ground/crushed sample though 2mm sieve or other grillwork

3. INTERFERENCES

Rocks will not be ground in the manual grinder. Large rocks will be omitted by hand removal, or by passing the sample through a large sieve prior to grinding. Samples must be completely dried (according to WI PREP 007-10) prior to grinding for optimal results.

4. SAMPLE REQUIREMENTS

Dried sample: the amount of ground sample required is dependent on the analysis required. Usually, the final volume of sample ground will be about 400mL. This can weigh anywhere from 10g (of very light material) up to 800g (of dense material).

PARTICLE SIZE ANALYSIS OF SOIL BY HYDROMETER

1. METHOD

1.1 Reference Method

Martin R. Carter & E.G. Gregorich. Soil Sampling and Methods of Analysis, 2008. Method 55.3, *Hydrometer Method*; **Modified.**

1.2 Modifications

1. A dried and ground sample is used.
2. 50 g of sample is used rather than 40 g specified in the reference method.
3. 2 minutes initial mixing of the conditioned soil is used rather than 5 minutes specified in the reference method.
4. The reference method instructs to take the second hydrometer readings at 7 hours. In practice, readings are taken at 6 hours for environmental samples and 2 hours for agricultural samples.
5. The hydrometer reading varies considerably with temperature. This is compensated by using blank-corrected readings which eliminate the need for adjustment to standard temperature, notwithstanding the fact that most published versions of the hydrometer method (except Sheldrick and Wang, 1993) include one. Justification for ignoring any temperature correction came from tests with suspensions of fine silt and clay. In these tests, blank-corrected readings agreed well with weights of suspended solids (obtained by drying the suspensions) whether hydrometer readings were made at 15 or at 24 ℃. The temperature dependence of the raw reading ($\delta R/\delta T = -0.3$ g/L per ℃) parallels that of RL. The laboratory operates at virtually all times within the range 22 – 24 ℃. Although the hydrometer scale is calibrated by the manufacturer at 20 ℃, judging from the results of our calibration tests, any difference from 20 ℃ in the actual operating temperature has little or no effect on the direct relationship between R’ and the true density of suspensions. To ensure that R’ is accurate, RL must be read carefully, and the temperature of the blank and soil suspensions must be the same, or very nearly.

2. SIGNIFICANCE AND SCOPE

This method is valid for all soil types except certain organic soils with un-decomposed plant material such as peat. Un-decomposed organic material is less dense than mineral particles and has a slower rate of settling. However, the method is valid for soils with as much as 10 % or more of well-decomposed organic matter. Oil-contaminated soils must be pre-treated by washing with toluene to obtain a friable sample. Detection limits are not applicable to this method and the measurement limits of 0.4% for clay and 1.6% for sand are used. A measurement limit is not applicable to silt as it is a calculated value. The analytical range for clay is 0.5 to 100%, and for sand is 2.0 to 100%. The analytical range for silt is not applicable.

Associated LIMS methods:

- Particle Size Analysis – GS
- Particle Size Analysis – FS

3. PRINCIPLE

Particle size analysis is the measurement of the proportions by weight of primary soil particles of different size. This method relies on separating size-fractions in aqueous suspension by means of differing sedimentation rates. Size fractions are traditionally given as percentages of sand (> 50 microns), silt (2 – 50 microns) and clay (< 2 microns). Their relative amounts determine various properties of the whole soil, such as its bulk density, hydraulic conductivity and saturation percentage. These in turn are related to ease of cultivation, to drainage, compaction, erosion, crop production and suitability for landscaping, etc. Numerous guidelines stipulate particle size distribution criteria for specific soil uses.

The hydrometer method is a sedimentation procedure in which the density of a soil suspension is measured at various times. It is based upon “Stokes’ Law” which determines the relationship between the size of a particle (its equivalent spherical diameter) and its rate of settling in a liquid. Sand, silt and clay particles settle at different rates, the larger sand particles falling fastest. A slurry is made with a known mass of sample in a specific sample volume. Individual soil particles are separated from each other using first, a chemical dispersing agent in water and second, mechanical agitation. A hydrometer reading taken at 40 seconds after vigorously homogenization (and corrected for the weight of dispersing agent) includes the density (g/L) of clay and silt particles remaining in suspension. The change from the initial soil in suspension (50 g/L) represents the weight percentage of sand. A second reading after 6 h similarly indicates the weight percentage of clay. The weight percentage of silt is calculated as the balance required to make the total equal to 100%.

pH AND ELECTRICAL CONDUCTIVITY IN SOIL

1. METHOD

1.1 Reference Method

1. 1:2 extraction: J.A. McKeague. Manual on Soil Sampling and Methods of Analysis, 1978. Method 4.12, *1:2 Soil:Water Ratio*; **Modified.**

1.2 Modifications

1. Mixture allowed to settle for 15 minutes before measuring the pH and EC.
2. Depending on the service package requested, the EC value is multiplied by 2.06 to represent the equivalent of a saturated paste EC. This corresponds to the EC measurement recommended by McKeague in Table 10 (page 154).
3. A 5, 10, or 25 g scoop is used to sub-sample the dry and ground soil rather than weighing the portion.

2. SIGNIFICANCE AND SCOPE

2.1 This method is applicable to determining Electrical Conductivity (EC) and pH of extractions of all types of soil with the exception of soils containing oils which may interfere with the measurement by coating the probe. The electroconductivity (EC) can be used to assess soil salinity. The EC increases with increasing salt concentrations. The pH of a soil indicates whether it is acidic, neutral, or alkaline. A pH of 7 is neutral only for pure water at 25 °C. For soils, neutrality is typically in the range 6.2 – 7.3. Acidic soils have pH <6.1 and alkaline soils have pH >7.4. EC and pH analysis can be used to determine the suitability of the soil for plant growth. pH values are useful for estimating lime requirements.

2.2 For EC, the detection limit is 0.01 dS/m and the analytical range is 0.01 – 100 dS/m. The analytical range is 0.5 – 14 for pH.

Associated LIMS methods:

- pH and Conductivity in general soil 1:2
- pH in soil by 1:10 water extraction
- pH and Conductivity in farm soil

3. PRINCIPLE

3.1 The EC of a soil can be determined by measuring the electrical resistance in a soil-water mixture between two parallel electrodes. The EC is the inverse of the resistance. An EC electrode is placed directly in the soil-water mixture, and the EC reading is recorded. The EC result obtained from this method is then multiplied by 2.06 to simulate a saturated paste EC measurement. This conversion factor is used by Alberta Agriculture (1995).

3.2 Hydrogen ion activity is estimated in soil by mixing the soil with water in a 1:2 ratio and reading the pH of the soil/water suspension using a pH meter.

Total Nitrogen, Total Carbon, Inorganic Carbon By Combustion Method Summary

1. METHOD

1.1 **Reference Method**

- 1.1.1 Carbon in Soil: SSSA Book Series: 5, Methods of Soil Analysis Part 3 – Chemical Methods, 1996. Chapter 34, *Total Carbon, Organic Carbon and Organic Matter*, D.W. Nelson and L.E. Sommers. **Modified**
- 1.1.2 Nitrogen in Soil: SSSA Book Series: 5, Methods of Soil Analysis Part 3 – Chemical Methods, 1996. Chapter 37, *Nitrogen-Total*, J.M. Bremner. **Modified**
- 1.1.3 British Columbia Ministry of Environment, *Total Organic Carbon (TOC/FOC) in Soil/Sediment by Combustion PBM*, August 2014.

1.2 **Modifications**

- 1.2.1 Instrument conditions are set up specific to the LECO Truspec Analyzer
- 1.2.2 Reference standard soils supplied by LECO are used for calibration.

2. SIGNIFICANCE AND SCOPE

2.1 The analysis of Total Nitrogen and Total Sulfur content of composts and manures allows assessment of potential deficiencies and allows managed use a source of nutrients in soil.

2.2 The Total C in soils is the sum of the Organic C present in the soil organic matter fraction and the Inorganic C of the carbonate minerals. Organic C consists of cells of microorganisms, decomposing plant and animal residues, humus and highly carbonized forms of C such as charcoal, graphite and coal. It is present in all agricultural soils. Inorganic C is associated with the principal carbonate minerals present in the soil, primarily calcite and dolomite. In non-calcareous soils, the Total Carbon is equal to the Organic Carbon. In calcareous soils, the Organic Carbon can be estimated as the difference between the Total Carbon and the Inorganic Carbon.

2.3 This method is applicable to all dried and ground soils and composts. Based on an approximate 0.2 g sample size, the nitrogen reporting range is 0.02 to 47% N; the carbon reporting range is 0.02 to 99% C.

3. PRINCIPLE

3.1 The sample is weighed into a foil cup and inserted into a combustion chamber where the high temperature and flow of oxygen gas cause the sample to combust. The combustion process will convert any elemental carbon and nitrogen into CO_2, N_2 and NO_x.

3.2 The CO_2 gas absorbs IR radiation at specific wavelengths in amounts proportional to the level of CO_2 present.

3.3 The gases are passed through a TC (thermal conductivity) cell that detects differences in thermal conductivity. The cell consists of two pairs of matched filaments used in four legs of a Wheatstone bridge. Helium carrier gas is passed over one pair of filaments while the sample gas is passed over the other pair. The lower thermal conductivity of the nitrogen causes an imbalance in the Wheatstone bridge proportional to the concentration of the nitrogen present.

3.4 The dry combustion method is based on the oxidation of organic C and thermal decomposition of carbonate minerals in the induction furnace of the combustion analyzer. The CO_2 liberated by combustion is determined by infrared absorption to produce values for the samples for Total C.

3.5 Ignition of soils at 500 ˚C for two hours will burn off the Organic Carbon. The ignited soils are then analyzed for carbon to give Inorganic Carbon values. The Organic Carbon may then be calculated as the difference between Total Carbon and Inorganic Carbon.

4. SAMPLE REQUIREMENTS

4.1 Minimum sample volume: a minimum of 5 g of sample prepared
4.2 Sample container: glass or plastic
4.3 Transportation and storage conditions: Room temperature, samples are dried upon receipt
4.4 Holding time: 28 days
4.5 Chemical preservation: none
4.6 Sample pre-treatment: Samples are dried as soon as possible after receipt

Total Nitrogen, Total Carbon, Inorganic Carbon
By Combustion Method Summary

5. CALCULATION OF RESULTS

5.1 Standards correction calculation:

Corrected Result $R_c\,(\mathrm{wt\%}) = \dfrac{D_t \times R}{\overline{D}}$

Where D_t is the theoretical value of drift standard
R is the test result (*wt%*)
$\overline{D}$ is the mean measured value of the drift before and after

5.2 Instrument results are wt% with the sample weight being entered electronically. To correct to actual sample weight, in the case of sample mix-up.

$$R_c = \frac{R_i \times W_d}{W_s}$$

Where R_i is the instrument result (*wt%*)
W_d is the instrument entered weight
W_s is the actual sample weight (g)

5.3 Inorganic carbon can be calculated as in 16.2 with W_s the weight of *ignited* sample as entered electronically.

5.4 Inorganic carbon is corrected back to pre-ignited weight.

$$R_{IC}\,(\mathrm{wt\%}) = R_i \times \frac{(100 - LOI)}{100}$$

R_i is the instrument result (*wt%*) based on the weight of *ignited* sample as entered electronically

And where LOI is given

$$Loss\ on\ Ignition\,(LOI)\ wt\% = \frac{(m_d - m_a)}{(m_d - m_c)} \times 100$$

where m_d = oven dried weight *(g)*
m_a = ashed weight *(g)*
m_c = crucible weight *(g)*

5.4.1 Organic Carbon

$$Organic\ Carbon = Total\ Carbon - Inorganic\ Carbon$$

5.4.2 Organic Matter (by combustion)

$$Organic\ Matter = 2 \times Organic\ Carbon$$

Total Nitrogen, Total Carbon, Inorganic Carbon By Combustion Method Summary

6. QUALITY CONTROL AND DATA ACCEPTANCE

6.1 Quality Control Plan

AQC	Material	Frequency	Insertion	Criteria
Total carbon Blank	<60 mesh Sand	1 per batch		0.00 ± 0.05
Total nitrogen Blank	<60 mesh Sand	1 per batch		0.00 ± 0.03
Total carbon Duplicate	Samples	1 per batch, every 15 samples		Absolute Range of 0.1%, Relative Range of 6.0%.
Total nitrogen Duplicate	Samples	1 per batch, every 15 samples		Absolute Range of 0.1%, Relative Range of 2.5%.
SS-20XX Carbon	In-house soil standard	1 per batch, every 25 samples	Beginning & end of analysis	See LIMS
SS-20XX Nitrogen	In-house soil standard	1 per batch, every 25 samples	Beginning & end of analysis	See LIMS

6.1.1 One method blank is run within the batch, this is not the same as the initial pre run blanks. .

6.1.2 All data generated under an out of control condition shall be considered nonconforming data unless approved by the quality officer, team leader or manager and the reason for approval documented.

6.1.3 If an out of control condition is discovered, it is necessary to go back and reanalyze at least some of the samples run since the last in control analyses, unless the expressed approval of the quality officer, team leader or manager is obtained and the reason for the approval is documented.

Based on TM SOIL 008(8)-60 C, N by Combustion
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