

# Using HoloS 4 to Model Regenerative Agricultural Practices for Carbon Sequestration

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**Abstract:** Carbon sequestration has the potential to be an important part of sustainable agriculture through regenerative agriculture. A pilot study was initiated in 2025 to sample soil organic carbon, nitrogen (total nitrogen), and bulk density in farms located in southern Ontario, Canada. The study area identified for the pilot was in Brant and Norfolk Counties (Ontario, Canada). This pilot endeavored to collect field data and use modeling to predict soil organic carbon gains of using regenerative agriculture. Therefore, the software was deployed to test for different scenarios where positive soil organic carbon change could occur, linked to management practices. Two farms were included in the study, and their agricultural practices were modeled in HoloS 4 to determine the potential for carbon sequestration given some changes. Soil texture differed at each location, comprising sandy loam, clay loam, and sand in three fields, although the farms incorporated similar best management practices for the pilot study. Since these farms were already using reduced tillage or no-tillage, legume-based cover crops (forage radishes, crimson clover, and hairy vetch) were recommended for best management practices along with a turkey compost amendment and half nitrogen fertilizer application for corn. For the two Gray Brown Luvisols (Canadian System of Soil Classification) deployed in this pilot, texture had important implications for modeled soil organic carbon content in sandy and clayey soils. This resulted in different soil organic carbon content in these soils, with greater amounts (3.69–5.33 Mg CO<sub>2</sub>e/ha per year) measured and modeled in the clayey soil.

**Keywords:** Greenhouse gas/ GHG modeling; GHG accounting; climate change mitigation; soil carbon capture and storage; agricultural soils; Ontario soils

## 1. Introduction

Regenerative agriculture focuses on improving soil health through reduced disturbance to soil structure and enhanced biodiversity to promote nutrient retention in soils as well as resilience to climate change. It is considered to be farming that is both productive and sustainable (Dent & Boincean, 2021). Such farming avoids harming the environment, while building soils, landscapes, and communities. The movement toward regenerative agriculture involves the use of best management practices (BMPs) (Jaworski et al., 2024), entailing:

- (1) reduced soil disturbance;
- (2) improved crop diversity;
- (3) year-round soil coverage;
- (4) year-round living roots; and
- (5) integrated livestock.

According to Obregon et al. (2023), a process-oriented approach to regenerative agriculture involves no/low external inputs (e.g., fertilizers, etc.), use of on-farm inputs (e.g., manure, compost), rotational livestock grazing, reduced tillage, agroforestry, crop rotations, and

perennial ground cover (e.g., living mulches, cover crops). More closed (regenerative) systems would retain nutrients within and avoid being leaky (Pearson, 2007). These would counter current systems, which overconsume resources and contribute to climate change by relying on fossil fuels and contaminating groundwater supplies.

Any management practice change instigated toward regenerative agriculture is not immediately measurable, as soil organic carbon (SOC) takes time to build. Authors have noted as much as 6–10 years for no-tillage and rotational complexity, with a systemic equilibrium reached only after 15–20 years under constant weather conditions (Khangura et al., 2023). Nevertheless, by reducing fuel-intensive tillage, it is possible to retain soil organic matter (SOM) while reducing greenhouse gas (GHG) emissions, improving soil structure, and reducing the potential for soil erosion (Singh, 2023).

For coarse- and medium-textured soils, including legumes in crop rotations has led to more positive SOC change under no-tillage agriculture (Mondal et al., 2023). The contribution of no-tillage to mitigating global anthropogenic GHG emissions can substantially offset 17–58% of emissions from agriculture (Mondal et al., 2023). No-tillage chiefly affects the surface soil within 0–5 cm depth, augmenting SOC by 38% at this depth compared to 6% at 5–10 cm depth and producing no change beyond 10 cm (Mondal et al., 2023). Accordingly, climate is another factor to consider, with temperate climates having nearly twice the positive change in SOC at 0–5 cm depth.

The overarching ambition of this paper is to present

modeled results of different scenarios of some regenerative agricultural practices for a 3-year pilot study in southern Ontario (Canada). It is anticipated that integrating regenerative management practices will enhance SOC compared to measured baseline, and that this positive change will accrue during the term of the pilot study. These outcomes are compared to the published literature and are indicative of the carbon sequestration potential of different agricultural management practices at the annual–decadal scale.

## 2. Materials and Methods

Two existing farms, located in Norfolk and Brant Counties (southern Ontario, Canada), were selected to identify ways to make them more regenerative. These large farms already employed BMPs (to varying degrees) and were open to suggestions for improved carbon sequestration (Table 1). For example, they already employed no-tillage and had 3-year crop rotations in place. All three fields tested represent soils in a temperate (humid, continental) climate. Soil type was the one variable between the field sites, which comprised Gray Brown Luvisols (Canadian System of Soil Classification) with textures of sandy loam, clay loam, and sand. In Holos 4, climate data were downloaded from NASA by selecting the locational polygon where the farm is situated. For the farms in the pilot study, this was the Norfolk Sand Plain, which is part of the Mixedwood Plains ecozone.

**Table 1.** Farming practices and regenerative agriculture recommendations to sequester carbon for two farms sampled in the pilot study.

Farm	Practice	Recommendation
C	YEAR 1 (Corn)	
	200 lb fertilizer applied in the spring replant and side-dressed in crop; 50% no-tillage on corn; sometimes plant rye (cover crop) after corn and beans	<ul style="list-style-type: none"> <li>• (Already using no-tillage)</li> </ul>
	YEAR 2 (Soybeans)	<ul style="list-style-type: none"> <li>• Use blended leguminous cover crops</li> </ul>
M	100 lb fertilizer applied in the spring replant and side-dressed in crop; no-tillage	<ul style="list-style-type: none"> <li>• Turkey compost for corn</li> <li>• ½ nitrogen fertilizer application rate for corn</li> </ul>
	YEAR 3 (1/3 Corn/Soybeans/Wheat)	
M	15 lb fertilizer applied in the spring replant and side-dressed in crop; no-tillage on soybeans and wheat	
	YEAR 1 (Corn)	
	No roundup/ herbicide applied	<ul style="list-style-type: none"> <li>• Reduced tillage to no-tillage</li> </ul>
	Fall deep tillage	<ul style="list-style-type: none"> <li>• Use blended leguminous cover crops</li> </ul>
M	Spring/Summer sprays herbicide – Acuron	<ul style="list-style-type: none"> <li>• Turkey compost for corn</li> <li>• ½ nitrogen fertilizer application rate for corn</li> </ul>
	Corn is planted vertical till (minimal tillage)	

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Plant corn no-till

First application of 180 actual lb/acre nitrogen fertilizer before broadcasting corn

Note: 30 lb/acre side-dressed in crop

Second application of 180 lb/acre nitrogen fertilizer in the crop

YEAR 2 (Soybeans)

Corn is harvested in the fall, and stalks are left over the winter

Spring/ Summer, no nitrogen fertilizer applied to soybeans

Soybeans planted no-till in the spring

YEAR 3 (Winter Wheat)

In the fall, winter wheat is planted no-till over the winter

Spring/ Summer nitrogen fertilizer (110 lb/acre) applied to winter wheat

Winter wheat harvest in July

Wheat straw left on the ground

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## 2.1. Holos 4

The suggested practices encourage the build-up of SOC in these soils and thereby represent measurable carbon sequestration, while reducing N<sub>2</sub>O and CH<sub>4</sub> emissions. Holos 4 is a government-issued software, developed by Agriculture and Agri-Food Canada, meant for the use of farmers to improve their GHG modeling and estimates and to support decision-making at the whole-farm scale for individual farms. This tool enables testing potential mitigation strategies for reducing emissions, including the implementation of BMPs.

According to the Government of Canada website (<https://agriculture.canada.ca/en/agricultural-production/holos>), the Holos model estimates GHG emissions as well as changes in SOC. Its development has been honed for enhancing environmental sustainability among Canadian farming systems. It allows for testing, validating, and providing sustainability attributes for Canadian agricultural production systems. Accordingly, the main purpose of this software application is "...to test possible ways of reducing GHG emissions and increasing soil C stocks by exploring the effects of different management practices."

Net emissions are output based on information entered for the individual or model farm. Estimates of CO<sub>2</sub>, N<sub>2</sub>O, and CH<sub>4</sub> are released in CO<sub>2</sub> equivalents (CO<sub>2</sub>e) based on farm sources, including enteric fermentation and manure management, cropping systems, and energy use. The model comprises 36 commonly grown annual and perennial crops, summer fallow, native rangeland, tame pasture; and eight types of tree plantings, along with beef, dairy, sheep, swine, poultry, and other livestock operations. It relies on extensive climate, soil, and agronomic

databases for regional data to estimate GHGs and SOC for the entire farm to help users identify ways to reduce farming emissions.

### 2.1.1. Model Parameters

The data were input into Holos 4 on November 1, 2025. Available components in the model include land management, such as crop rotation and any details for livestock and infrastructure (e.g., anaerobic digestion). The crop rotation in Table 1 was added to land management, specifying the year (starting 2025 with the corn rotation in both cases and subsequently followed in 2026 by soybeans and finally wheat in 2027), and 1 ha was selected to get results on a per-hectare basis for the outputted annual data. The options for crops are as follows:

- Annual:
  - Oilseeds: camelina, canola, flax, mustard, oilseeds, soybeans
  - Other field crops: berries and grapes, other field crops, safflower, sunflower seed, tomato, vegetables
  - Pulse crops: beans (dry field), chickpeas, dry/field peas, lentils, pulse crops
  - Root crop: potatoes, sugar beets
  - Silage: barley silage, grass silage, oat silage, silage corn, triticale silage, wheat silage

- Small grain cereals: barley, buckwheat, canary seed, Fall rye, grain corn, mixed grains, oats, small grain cereals, sorghum, triticale, under-sown barley, wheat
- Fallow: fallow
- Perennial: forage for seed, rangeland (native), seeded grassland, tame grass, tame legume, tame mixed (grass/legume).

The fertilizer application rate was based on an NPK (nitrophosphate) application. No-tillage was selected for the sandy soils, and the harvest method was used as a cash crop. Reduced tillage was chosen for the clayey soil, since it is heavier than the others and requires deep tillage in the fall. Turkey compost was applied once during the pilot study in the corn year in mid-October 2025. It was input into the software as a manure application based on imported solid storage poultry solid spread (no tillage) with an application rate of 5 Mt/acre (12355.27 kg/ha) for sandy soils and 2 Mt/acre (4942.11 kg/ha) for clayey soils. Cover crops, including forage radish, crimson clover, and hairy vetch, were planted each Fall for 3 years. They were terminated using glyphosate in the spring before seeding began. The application rate for cover crops was 25 lb/acre (28.02 kg/ha). As for residues, straw and roots were fully returned to the soil

Holos 4 adopts the Canadian System of Soil Classification. Farm C is in Norfolk County and comprises Plainfield sand (Gray Brown Luvisol) that is coarse, namely sand with 1% clay and 97% sand, and well-drained. It is situated in the Norfolk Sand Plain in the Mixedwood Plains (ecodistrict 568, polygon 568009). For Farm M, the soil zone represents Gobles clay (also Gray Brown Luvisol) that is fine-textured and imperfectly drained. This soil comprises 29% clay and 19% sand, representing a silt clay loam. Farm M likewise represents the ecozone Mixedwood Plains, but is part of a different SLC polygon, pertaining to the Mount Elgin Ridges (ecodistrict 565, polygon 565008).

### 2.1.2. SOC Modeling

Model calibration is performed when data are loaded for specific polygons using climatic data from NASA – if these data are not available, then SLR data are deployed

instead. Holos 4’s SOC modeling is based on the Inter-governmental Panel on Climate Change (IPCC) Tier 2 methodology adapted for Canadian farming systems. It uses specific Canada-based emission factors for N<sub>2</sub>O from land. The model also integrates the Introductory Carbon Balance Model (ICBM) to simulate soil carbon changes. These model the flow of carbon into and out of soil by tracking carbon inputs and outputs in different soil pools.

The ICBM is a two-pool model comprising a young carbon (Y) pool and a humified soil carbon (H) pool. It uses specific turnover rates (decomposition rates) for the two pools to calculate SOC changes annually based on climate and residues. Daily precipitation (428 mm/year during the growing season), temperature, and potential evapotranspiration (621 mm/year during the growing season) that drive decomposition and turnover rates of these carbon pools were integrated from the onset (based on polygon selection linked to climate data). For example, an active pool decay rate (ka) of 1, a slow pool decay rate (ks) of 0.1255–0.1444, and a passive pool decay rate (ps) of 0.0041–0.0048 were used in the modeling for Farm C. These respective rates were 1 (ka), 0.1449–0.2647 (ks), and 0.0042–0.0048 (ps) for Farm M.

Carbon modeling in Holos 4 allows for the simulation of different management practices, such as tillage (conventional, reduced, no-tillage), crop types, and pasture, on SOC. The baseline was set according to the data input for current management practices. Recommendations (set out in Table 1) were the same for these farms, including: (1) going from reduced tillage to no-tillage (for M), (2) halving the nitrogen fertilizer used for corn, (3) using turkey compost at the beginning of the 3-year rotation during the corn phase, and (4) the annual addition of legume-rich mixtures of cover crops entailing equal portions of forage radish, crimson clover, and hairy vetch. The central hypothesis is that these regenerative agricultural practices will enrich soils with SOC.

### 3. Results

The results are reported for the sandy and clayey soils in Farms C and M, respectively. Based on average yield data for the assignment method, yield details appear in Table 2. Grain corn had the greatest yield on both farms; however, soybean and wheat yields differed in these farms. Adding cover crops as part of the recommended BMPs doubled the yield amounts.

**Table 2.** Yield assessment based on average yields (wet weight).

Texture	Year	Crop	Crop Residues (kg/ha)	Baseline Yield (kg/ha)	BMP Yield (kg/ha)
	2025	Grain corn	184.44	7549.5	15074.82
Sandy	2026	Soybeans	141.11	5961.14	11943.90
	2027	Wheat	155.88	5495.66	10993.88
	2025	Grain corn	59.39	7154.57	7154.57
Clayey	2026	Soybeans	31.18	5112.35	10224.7
	2027	Wheat	88.19	6696.28	13392.56

The results in Tables 3 and 4 indicate different performance for the variety of crops planted in these rotations. Grain corn and soybeans have the greatest subtotals and the most released GHG emissions. In both cases, the

BMPs reduced mostly upstream CO<sub>2</sub> emissions, which can be attributed to the production of animal inputs (CO<sub>2</sub> losses from fertilizer, herbicide production).

**Table 3.** Annual emissions results for the 3-year rotations based on the baseline (kg/ha).

Texture	Emission Source	Direct N <sub>2</sub> O	Indirect N <sub>2</sub> O	Farm Energy	Upstream CO <sub>2</sub>	Subtotal
Sandy	Grain corn, 2025	0.454	0.264	133.000	257.796	391.514
	Soybeans, 2026	0.814	0.183	188.256	128.915	318.168
	Wheat, 2027	0.537	0.121	176.350	19.32	196.328
	<i>Total:</i>	<i>1.805</i>	<i>0.568</i>	<i>497.606</i>	<i>406.031</i>	<i>906.010</i>
Clayey	Grain corn, 2025	36.814	1.951	190.069	515.660	744.494
	Soybeans, 2026	6.574	0.261	168.069	141.787	316.691
	Wheat, 2027	3.494	0.162	190.283	0	193.939
	<i>Total:</i>	<i>46.882</i>	<i>2.374</i>	<i>548.421</i>	<i>657.447</i>	<i>1255.124</i>

**Table 4.** Annual emissions results for the 3-year rotations based on BMPs (kg/ha).

Texture	Emission Source	Direct N <sub>2</sub> O	Indirect N <sub>2</sub> O	Farm Energy	Upstream CO <sub>2</sub>	Subtotal
Sandy	Grain corn, 2025	0.335	0.195	133.000	128.915	262.446
	Soybeans, 2026	0.814	0.184	188.256	128.915	318.169
	Wheat, 2027	0.537	0.121	176.35	19.320	196.328
	<i>Total:</i>	<i>1.686</i>	<i>0.500</i>	<i>497.606</i>	<i>277.150</i>	<i>776.942</i>
Clayey	Grain corn, 2025	33.658	1.825	190.069	257.830	483.382
	Soybeans, 2026	6.574	0.261	168.069	141.787	316.691
	Wheat, 2027	3.494	0.162	190.283	0	193.939
	<i>Total:</i>	<i>43.726</i>	<i>2.248</i>	<i>548.421</i>	<i>399.617</i>	<i>994.012</i>

**3.1. Field Samples**

Baseline measurements were made in mid-October 2025 at both farms. This involved collecting cores to a 30-cm depth from the sites. Since a paired design was deployed, two samples were extracted from each field toward the middle of the research plots. These 3 plots each measured ~2 ha for a total coverage of ~6 ha, with 2/3rds of this representing sandy soils, while the remaining 1/3 was for clayey soil.

The soil samples were submitted to Brookside Laboratories, Inc. for soil tests outputting data on bulk density,

organic carbon, and nitrogen (Table 5). Evidently, the clayey soil had a lower bulk density, which, according to Bezkorowajnyj et al. (1993) should be <1.10 and <1.60 g/cm<sup>3</sup> for sandy soils. Root growth can be restricted at bulk densities >1.47–1.80 g/cm<sup>3</sup> (Bezkorowajnyj et al., 1993), reducing water infiltration and constraining the amount of oxygen available for crop roots. As for organic carbon, <1% is comparable to low levels evident in arid and semiarid soils. The amount of total nitrogen (TN) is likewise low, with <50 kg N/ha apparent for continuous cropping.

**Table 5.** Soil sample cores (baseline) test results for the pilot study farms.

Texture	Texture	Bulk Density (g/cm <sup>3</sup> )		SOC (Mg C/ha)		TN (kg N/ha)	
		Treatment	Control	Treatment	Control	Treatment	Control
Farm C	Sandy	1.34	1.30	25.20	22.50	62.8	54.9
Farm M	Clayey	1.08	1.07	30.30	43.80	66.1	93.0

Farm M	Sandy	1.25	1.27	26.40	22.80	109.9	112.1
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These laboratory data indicate the greater bulk density of sandy soils than that of clayey soil. Moreover, the clayey soil has more SOC than the sandy soils. However, TN depends on the farm, with Farm M having higher levels of TN measured in both fields.

### 3.2. SOC Modeling Outcomes

All recommendations show improvements over the baseline for a soil depth of 0–30 cm (Table 6). This is especially notable for soybeans and wheat in the clayey soil. Grain corn performs better at sequestering carbon in clayey soil than at the sandy site. In total, there is potential

to boost SOC to 8.188 Mg C/ha for these farms, which amounts to 29.968 Mg CO<sub>2</sub>e/ha during the pilot trial period. Given the uncertainty range between 25 and 48% determined by comparing measured and modeled SOC, it is anticipated that across the 3 years of the pilot study as much as 4.258 and 6.140 Mg C/ha can be secured in these soils. This estimated carbon sequestration will be compared with published studies in the next section. Using turkey compost contributed to manuring the fields, and enhanced soil health. In time, it should help to reduce the bulk density and augment SOM, especially affecting the clayey soil, as evident for the other BMPs.

**Table 6.** Soil carbon change from the baseline to encompassing the BMP recommendations.

Texture	Year	Crop	ΔSOC (Mg C/ha)	Uncertainty (Mg C/ha)
	2025	Grain corn	1.127	0.282–0.541
Sandy	2026	Soybeans	0.619	0.155–0.297
	2027	Wheat	0.638	0.160–0.306
Total:			2.384	0.597–1.144
	2025	Grain corn	2.408	0.602–1.156
Clayey	2026	Soybeans	1.513	0.378–0.726
	2027	Wheat	1.883	0.471–0.904
Total:			5.804	1.451–2.786

### 3.3. Advantages and Disadvantages

Collecting field data with modeling is a top-tier “gold standard” (Tier 3) data collection strategy adopted by carbon crediting standards (e.g., Verra), known to produce site-specific data derived from direct measurements rather than solely relying on regional averages (Thornbush & Govind, 2025). By integrating modeling to supplement these preliminary (baseline) data in this study, it was possible to create different emission scenarios and estimate SOC accrual during the span of the pilot trial. Using the baseline (field) data allows for the derivation of an error range estimate that is specific to the study sites. Using an integrated experimental (test) versus control design permitted testing all BMPs together, although their separate contributions were not determined. However, samples were collected from the same landscape components (topographic positions) and represent comparable data between the test and control plots. Notable differences between treatments and controls, however, were evident, especially for Farm M (clayey soil) for SOC and TN.

## 4. Discussion

It has been reported that as much as 11.712 Mg CO<sub>2</sub>e per year of SOC enhancement can occur due to targeted management practices in Canadian croplands (Ashton et al., 2023). A significant role can be attributed to crop rotation as well as tillage and nitrogen application rate in the achievement of these SOC gains. In this study, BMPs have included promoting the use of reduced tillage or no-

tillage over intensive tillage for these large farms. Furthermore, adding organic amendments like manure (turkey compost) improves soil health by influencing soil carbon and nitrogen dynamics (Bhowmik et al., 2017). According to these authors, mixed compost treatment can increase SOC by 30% compared to broiler litter and especially pasture treatments. Predicted annual SOC changes ranging between 0.110–1.236 Mg CO<sub>2</sub>e/ha have been reported for varied tillage and residue management systems (He et al., 2021). Diverse rotations, particularly those including legumes and canola, are known to enhance SOC sequestration more than wheat-based systems.

### 4.1. Other Models

Modeling using DayCent for southern Ontario, Canada has shown high rates of carbon sequestration in the first 20–30 years under switchgrass and miscanthus as warm-season perennial C4 grasses (Jarecki et al., 2020); accordingly, carbon sequestration subsequently stabilized after 50–60 years. Their predicted annual SOC sequestration rate for switchgrass was 0.951 Mg CO<sub>2</sub>e/ha and 3.441 Mg CO<sub>2</sub>e/ha for miscanthus, based on moderate warming scenarios.

Modeling in Century applied to long-term SOC dynamics in agroforestry alley crops and sole crop systems in Costa Rica and Canada showed SOC increase in those systems over 100 years (Oelbermann & Voroney, 2011). Accordingly, sole cropping systems showed SOC decline. The Century model underestimated alley cropping SOC, which the authors attributed to changes in soil bulk

density with SOM inputs.

#### 4.2. Evaluating Holos 4 Outcomes

The model is sensitive to tillage and somewhat to nitrogen application rate and cover crops. This trend was also observed by Man et al. (2022), who likewise saw that tillage management had a stronger influence on soil carbon cycling and microbial dynamics than nitrogen fertilization. They explained that conservation tillage promotes the decomposition of specific SOM components through

alterations in microbial communities.

Published studies have shown more substantial emissions reductions for certain cover crops under no-tillage (Table 7). Estimated annual SOC change from the modeled results are comparable to the published findings, indicating positive change (carbon sequestration) that more than doubles in the clayey soil. The modeling in the current study conveys that soils associated with the rotation crops improved with use of the BMP recommendations (see Table 6), particularly in the clayey soil.

**Table 7.** Measured reductions (Mg CO<sub>2</sub>e/ha per year) for different crops in select publications.

Cropping System	Soil Type	Measured Depth (cm)	SOC Change	Source
Corn-soybeans, no-tillage	Silt loam	0–5	2.84	Deen & Katakai (2003)
Oats, winter cereal rye, oilseed radish & radish&rye mix <sup>1</sup>	Sandy loam	0–15	3.67–7.33	Chahal et al. (2020); Chahal & Van Eerd (2018, 2020)
Corn-soybeans, no-tillage	Clay loam	0–5	4.87	Shi et al. (2012)
Corn, soybeans, winter wheat, no-tillage	Clay loam	0–5	5.06	Van Eerd et al. (2014)
Corn, soybeans, wheat, no-tillage	Sandy	0–30	1.52–2.19	Current study (Farm C)
Corn, soybeans, wheat, reduced tillage	Clayey	0–30	3.69–5.33	Current study (Farm M)

<sup>1</sup> Cover crops

There are opposing trends evident in published studies, with one study (Shi et al., 2012) showing 1.12 CO<sub>2</sub>e added with no-tillage, and the study by Van Eerd et al. (2014) having a reduction of 0.20 CO<sub>2</sub>e for the top 5 cm of soil when no-tillage was deployed. The latter study, however, over the course of 11 and 15 years of no-tillage and crop rotations, measured more SOC and TN for no-till plots and in rotations that included winter wheat. Specifically, no-tillage had 14% more SOC than conventional tillage with moldboard plowing to a depth of 100 cm.

A metaanalysis by VandenBygaert et al. (2002) examining the impact of no-tillage on SOC stock changes in Ontario on Gray Brown Luvisols revealed significant variations in SOC sequestration rates. The estimated increase in SOC due to no-tillage in Ontario contributes an annual total of 13.542 Mt CO<sub>2</sub>e. However, there is much uncertainty around these estimates because of variability in initial SOC levels and the impact of management practices across sites.

#### 4.3. Measuring SOC Stock

Regenerative agriculture can be practiced in a variety of different ways. Authors (Jiménez-Ballesta et al., 2025) in a recent review have suggested that agroecological actions and processes are set to offer ecosystem services and functional soil quality as well as food quality. This emphasizes environmental health (soil health) and economic profits associated with farm operation, including livestock farming.

A report by the Rodale Institute (2014) based on data from farming systems and pasture trials from around the world indicated the sequestration of 37 Gt CO<sub>2</sub> per year for global pasture (12 Gt CO<sub>2</sub> per year in the US alone) if organic management practices were deployed as regenerative organic agriculture. In other countries, like for crops in Egypt, Iran, and Thailand, this represented 21, 21, and 32 Gt CO<sub>2</sub> per year, respectively (see their Figure 1, p. 8). Regenerative organic agriculture refrains from using

synthetic pesticides and other inputs (such as petroleum-based nitrogen fertilizer) and is designed to build soil health through practices such as cover crops, residue mulching, composting, crop rotation, and conservation tillage. Reduced use of mineral fertilizer production and diesel fuel energy consumption in farming are especially reduced with organic farming than conventional systems (p. 16). These conservation systems produce less N<sub>2</sub>O emissions from soils and are associated with less diesel fuel emissions, herbicides, and mineral fertilizer production (p. 17).

Studies have indicated a trade-off between yield and SOM in Northern farm soils. For instance, LaCanne and Lundgren (2018) found 29% less grain production over traditional corn production systems in the American Northern Plains. Nevertheless, profits were still 78% higher due to the particulate organic matter (POM) in soils (and inversely related to the soil’s bulk density; see their Figure 3, p. 6). They also discovered a 10-fold reduction in pests in insecticide-free corn fields where there were regenerative practices, indicating further advantages of reduced chemical pesticides. Their fertilizer costs were substantially reduced in regenerative fields (see their Figure 2, p. 5), as were irrigation costs.

The study by Congreves et al. (2014) summarized the SOC (Mg/ha) for Ontario soils, with their Table 1 denoting available (published and unpublished) data. It is evident from this that Luvisols are most represented in these studies. Therefore, their summary can inform the current study because of its focus on two (Gray Brown) Luvisols. Data are presented according to depth, and all data are within 120 cm of the surface. Most measurements are for a depth of 0–30 cm. Their data reveal increasing SOC with depth in these soils.

The following subsections examine the recent evidence of factors influencing SOC sequestration in agricultural soils. Sampling depth will affect measurements and

their comparability, especially since it is known that SOC stocks increase with depth beyond 30 cm in the soil profile (Rodale Institute, 2014). Additionally, promoting diversity through crop rotation can counteract nutrient depletion associated with continuously using specific crops. Conservation tillage is an important factor, since plowing destroys soil structure. No-tillage used with cover crops or crop rotations benefits SOC accumulation. Finally, retaining crop residues adds to nutrients entering the system, as added biomass from the surface.

#### 4.3.1. Depth

Depth is an important consideration. According to Blanco-Canqui et al. (2015), no-tillage boosts surface (0–5 cm) SOC, and combining it with deep-rooted cover crops can enhance SOC accumulation at depth. A study by Peng et al. (2024) evaluated SOC stock estimations from a fixed depth, which tends to overestimate SOC stocks – most notably for no-tillage with zone tillage systems – by ~15% compared to equivalent soil mass methods (e.g., ESMcubic\_spline method). No-till systems (with zone tillage) show a 22% higher SOC stock than conventional tillage at a depth of 0–30 cm (Peng et al., 2024).

A study that included Ontario sites in Elora (silt loam) and Woodslee (clay loam) found no-tillage to have higher SOC in surface layers of 16.51% and 47.62%, respectively, compared to traditional moldboard plowing (Yang, Drury, Wander, et al., 2008). However, the benefits of no-tillage diminished or reversed in deeper soil layers.

Long-term no-tillage resulted in SOC stratification in the top 20 cm of soil, meaning that more SOC was concentrated at the surface compared to deeper layers (Yang, Drury, Reynolds, et al., 2008). They attributed this to SOM accumulation at the surface due to minimal soil disturbance. During their study period between 1983 and 2004, there was SOC enrichment for no-till plots (compared to moldboard plowed plots) that ranged between 18 and 32% at 0–5 cm depth.

#### 4.3.2. Diversity

Another important consideration for SOC is that of grass cover crops that have slow-decomposing residues, which enhance soil carbon more effectively than legumes (Blanco-Canqui et al., 2015). For instance, a study by Campbell et al. (2007) showed an annual SOC gain of 1.614 Mg CO<sub>2</sub>e/ha for continuous wheat, while legume green manure rotations had annual gains of 1.204 Mg CO<sub>2</sub>e/ha. However, legumes are nitrogen-fixing and affect nitrogen levels in soils.

Mixtures are another factor to consider, since they provide greater biomass both above and below ground (Blanco-Canqui et al., 2015) and outperform monocrops. Measured carbon sequestration for above- and below-ground carbon in trees, soil carbon, soil respiration, and carbon leaching resulted in total carbon pools of 250.71–353.19 t CO<sub>2</sub>e/ha for intercropping systems compared to sole barley cropping with 250.71 t CO<sub>2</sub>e/ha (Peichl et al., 2006). The authors determined annual net carbon fluxes to be +4.026–48.312 t CO<sub>2</sub>e/ha in intercropping systems and -10.614 t CO<sub>2</sub>e/ha for sole cropping.

A metaanalysis by Warner et al. (2023) examined the

carbon sequestration potential of mixed-species planted forests compared to monocultures. They found that mixed-species forests store an average of 70% more above-ground carbon than monocultures. Accordingly, the highest over-yielding was observed in four species mixtures. Our pilot study included three species mixtures that differed from the main (cash) crop; for example, winter wheat was not deployed as a cover crop because the two farms already had wheat in rotation.

A study in southern Ontario by Thevathasan and Gordon (2004) conveyed the carbon sequestration potential in hybrid poplar-based intercropping systems, which was 4× higher than in conventional fields. According to them, tree-intercropping systems reduce fertilizer use and N<sub>2</sub>O emissions by ~0.003 Mg CO<sub>2</sub>e/ha annually. Their findings are supported by work from Quebec, likewise relaying that a hybrid poplar plantation had the highest carbon sequestration potential, followed by a hybrid poplar-hay intercrop, then grain corn, and last hay (Winans et al., 2015).

In another study, a 25-year-old temperate tree-based intercropping system was compared to a conventional soybean monoculture. Total carbon pools ranged from 260.226–415.044 t CO<sub>2</sub>e/ha across tree species with net annual carbon fluxes of 4.029–7.686 t CO<sub>2</sub>e/ha (Wotherspoon et al., 2014). These authors found there to be significant CO<sub>2</sub> sequestration potential in intercropping systems compared to conventional agriculture.

Nevertheless, a study comparing mixed-species forest stands and their impact on above-ground carbon storage in boreal forests located in Ontario and Quebec concluded that species interactions in mixed stands may offset potential benefits, due to increased competition for resources (allelopathy), and that mixing species of jack pine, black spruce, and trembling aspen may not significantly enhance carbon sequestration compared to pure forest stands (Cavard et al., 2010). Evidently, the type of species combined needs careful deliberation, including knowing how cover crop species could affect cash crops.

#### 4.3.3. Tillage

Using no-tillage benefits SOC by slowing residue decomposition (Blanco-Canqui et al., 2015) compared to conventional tillage. Long-term studies over 25 years have shown that zero-tillage contributes 11–16% higher SOC storage in surface layers between 0 and 5 cm (Deen & Katakai, 2003). Focused on Gray Brown Luvisols in Ontario, the metaanalysis of no-tillage practices on SOC stock changes by VandenBygaert et al. (2004) estimated an increase in SOC from no-tillage of ~13.542 Mt CO<sub>2</sub>e/ha each year.

However, the study by Yang and Kay (2001), which investigated the effects of crop rotation and tillage on SOC sequestration in southern Ontario, found over a 20-year period that continuous alfalfa had the highest SOC concentration and continuous corn had lower concentrations. High SOC was also attributed to rotations of soybeans and winter wheat or barley interseeded with red clover and followed by corn. Furthermore, moldboard plowing and chisel plowing differently affected SOC distribution, with chisel plowing not significantly increasing SOC content over moldboard plowing. Overall, these authors advocated for including legumes in rotations and reducing tillage to enhance SOC sequestration.

No-tillage significantly affects soil bulk density, macroporosity, and SOM content in the upper 0–10 cm of soil (Kay & VandenBygaart, 2002). There was increased SOM and porosity under no-tillage compared to conventional tillage. Their research also encouraged long-term studies of >15 years to accurately predict changes and maximize carbon sequestration potential in agricultural soils under reduced tillage practices. This work is supported by long-term trends found by Meyeraurich et al. (2006), who observed increased macroporosity and SOM accumulation under no-till conditions, which promoted enhanced water infiltration and nutrient retention.

In a 36-year experiment in southern Ontario, total carbon for a 4-year crop rotation with different tillage treatments demonstrated that no-tillage consistently resulted in higher total carbon concentrations than conventional tillage across all rotations (Laamrani et al., 2020). Although, accordingly, the corn-corn-oats-barley rotation with red clover cover crops had the highest total carbon increase notable over a 20-year period.

Annually-cropped rotations sequester significantly more carbon (0.099–1.574 Mg CO<sub>2</sub>e annually) than bare fallow (McConkey, 2003). No-tillage systems outperform tilled systems by annually sequestering 0.245–1.874 Mg CO<sub>2</sub>e/y (McConkey, 2003). Therefore, eliminating tillage and bare fallow could result in annual SOC increases of ~1.098 Mg CO<sub>2</sub>e/ha in semiarid regions and 2.928 Mg CO<sub>2</sub>e/ha in subhumid climates (McConkey, 2003). These authors highlighted that SOC increases under no-tillage where there is clay soil, which according to them has greater potential for SOC gains as fine-textured soils under no-till management.

#### 4.3.4. Cover Crops

The use of cover crops increases microbial activity in soils (Chahal & Van Eerd, 2019), since these increase SOC in the medium- to long-term potentially due to carbon losses from GHG emissions, leaching, and erosion balances and gains from above- and below-ground biomass. An experiment conducted by Chahal and Van Eerd (2020) on sandy loam plots located in Ridgetown, Ontario found that cover crops and retaining crop residues significantly enhanced SOC and nitrogen storage. Oilseed radishes had the highest cover crop biomass of 4850 kg/ha.

Oilseed radishes have a significant above-ground biomass that contributes to high annual carbon inputs of 4.063 Mg CO<sub>2</sub>e/ha and the accumulation of 83.448 Mg CO<sub>2</sub>e/ha over the span of 9 years compared to the no cover control that accumulated 50.508 Mg CO<sub>2</sub>e/ha during this time (Chahal et al., 2020). This indicates a net gain of ~33 Mg CO<sub>2</sub>e/ha in 9 years or 3.66 Mg CO<sub>2</sub>e/ha on average annually for integrating cover crops. Their study confirmed SOC increases between 11 and 22% that can be attributed to above-ground biomass. Cereal rye and oilseed radish&rye mixtures had the greatest SOC increases, followed by singly-planted (sole) oilseed radishes and oats. However, Holos 4 did not allow for blended mixtures of cover crops, and single cover crops or winter crops need to be coupled with rotation crops.

Using cover crops alone (without residues) increases SOC by 8%, raising carbon stocks 7%, for cover-cropped sites compared to no cover controls (Peng et al., 2023). Additionally, according to them, using cover crops

augmented TN by 8% and water-stable aggregates by 15%. On average, in their study, cover crops sequestered 12.993 Mg CO<sub>2</sub>e/ha at a rate of 0.879 Mg CO<sub>2</sub>e/ha each year in the top 15 cm of soil. Nonleguminous cover crops were especially effective in raising SOC stocks, notably outperforming legumes and mixed cover crops (Peng et al., 2023).

#### 4.3.5. Crop Residues

Carbon:nitrogen (C:N) ratios are used to track the persistence of residues. Residues with low C:N ratio (e.g., soybeans; alfalfa = 13) rapidly decompose and do not greatly improve soil structure. Compared to higher C:N ratio wheat residues (wheat straw C:N = 80) and fresh tree leaves (C:N = 90) (Blanco-Canqui & Lal, 2004). According to them, a high C:N ratio increases the potential for the soil to function as a sink for atmospheric CO<sub>2</sub>.

Soil carbon and nitrogen increased early in the growing season when cover crop residues were incorporated, but concentrations were reduced with residue decomposition (Sainju et al., 2000). In their research, rye was associated with increased organic nitrogen and SOC after 3 years, whereas hairy vetch and crimson clover increased inorganic nitrogen with residue incorporation. The latter produced tomato yield and nitrogen uptake as produced by 90 and 180 kg N/ha of nitrogen fertilizer (Sainju et al., 2000). Using a split application of nitrogen fertilizer increased tomato yield and nitrogen uptake, but reduced SOC and nitrogen, compared to rye.

#### 4.4. Areas for Improvement

The Holos 4 software does not allow for mixtures of cover crops or intercropped systems that occur simultaneously. These need to be input as separate entries for specific fields (main crops). Since it is known that blends behave differently than singly planted (sole) crops (Chahal et al., 2020), this is a shortcoming of Holos 4. Additionally, the software does not allow for the use of composted manure and only quantities of manure can be entered, without elaboration on the concentration or quality of manure, although it does consider the application rate. A split nitrogen application cannot be specified, since only one application rate can be included with each run.

The results show that tillage has the most effect than nitrogen fertilization or cover crop use, which is supported by other research (e.g., Man et al., 2022). Perhaps the latter can be improved if measured biomass inputs are provided for specific cover crops – rather than automatic yields provided by the software based on rotation crops. Moreover, details about tilling are not input into the model even though it is known that chisel plowing and moldboard plowing affect soil mixing differently and vary in their influence on carbon sequestration (e.g., Yang & Kay, 2001) at different depths. The most room for improvement, however, is more cover crop options – including blended varieties as well as perhaps adding an application rate for cover crops.

Despite its limitations, Holos 4 can output annual per-hectare emissions as CO<sub>2</sub>e to facilitate GHG accounting as well as provide outputs for SOC modeling. The open-source software is based on regional climatic data from NASA, and its soil type information is also regionally based on locational polygons. Nevertheless, Holos 4

allows users to override the soil type to input specific soil data. Details about amendments can be added, as origin, type, handling system, application method, and manure amount. Residue inputs can also be added. The model also allows for information about grazing animals and infrastructure (e.g., anaerobic digestion). Although not considered in this paper, HoloS 4 allows users to input economic information, such as crop price and direct costs, for a socioeconomic outlook.

HoloS 4 appears to have high levels of uncertainty. For example, the most uncertainty is associated with indirect N<sub>2</sub>O emissions ( $\pm 60\%$ ), followed by direct N<sub>2</sub>O emissions ( $\pm 40\%$ ) and energy CO<sub>2</sub> ( $\pm 40\%$ ). The least uncertainty ( $\pm 20\%$ ) is for CH<sub>4</sub> (enteric, manure). It should be noted that since enteric or manure data were not input into the pilot study runs, there were no measured outputs for CH<sub>4</sub> release. The calculated error between the laboratory-tested data and modeled SOC conveys an error range of 25–48%, which is considerable. Nevertheless, the software can be used to make on-farm decisions about which BMPs to implement to promote regenerative agricultural practices.

Finally, it is noteworthy that the pilot study collects and tests soil samples twice each year from treatment and control fields. Its paired design allows for further validation of the modeled results. This study considered a single (sandy) field in Farm C, whereas only one field (out of the two sampled during the pilot) was examined here – the clayey field, but without modeling the results for the sandy field in Farm M.

## 5. Conclusions

HoloS 4 has been instrumental for this paper, which has focused on BMPs for regenerative agriculture in the context of carbon storage in soils and reduced GHG emissions from farming operations. Two farms in Brant and Norfolk Counties (Ontario, Canada) were sampled in southern Ontario and used as a basis for data entry and comparison in the pilot study. Their current (baseline) GHG emissions were compared to those after the implementation of BMP recommendations for enhanced carbon sequestration.

Developing sustainable agriculture means augmenting soil health while sequestering carbon. This paper has focused on ways to improve the latter and measure them in CO<sub>2</sub>e using HoloS 4 software. No-tillage appears to be a major contributor to both, since carbon is stored when soil structure is preserved. The tilling method is important because it affects the amount of disturbance (e.g., mixing depth); however, chisel plowing still disturbs the surface and, by doing so, releases carbon into the atmosphere. Ideally, no-tillage should be deployed as much as possible alongside reduced N<sub>2</sub>O sources (fertilizer application rate) and CH<sub>4</sub>, whose emissions can be reduced by producing manure on-farm and ideally composting it. Nevertheless, there are some factors that cannot presently be measured by HoloS 4 (discussed in this paper) that affect measurements of GHG emissions. This has affected cover crop measurements (including average yields) too.

## Author Contributions

M.T.: conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing—original draft preparation, visualization, supervision; M.Z.: software, resources, validation, writing—review and editing, project administration, funding acquisition; E.A.: software, resources, validation, writing—review and editing; C.M.: resources, writing—review and editing; E.K.: writing—review and editing; M.M.U.R.: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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## Data Availability Statement

Data are available by contacting the corresponding author.

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## Conflicts of Interest:

M.T. is Head of Carbon and Research and Development at CarbFarm, Inc.

## Use of AI and AI-assisted Technologies

No AI tools were utilized for this paper.

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## Abbreviations

The following abbreviations are used in this manuscript:

BMP	Best management practice
CH <sub>4</sub>	Methane
C:N	Carbon:nitrogen ratio
CO <sub>2</sub>	Carbon dioxide
CO <sub>2</sub> e	Carbon dioxide equivalent
GHG	Greenhouse gas
ICBM	Introductory Carbon Balance Model
IPCC	Intergovernmental Panel on Climate Change
N <sub>2</sub> O	Nitrous oxide
SOC	Soil organic carbon
SOM	Soil organic matter

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