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The effect of land-use conversion from agriculture to perennial biomass crops and nitrogen fertilizer on soil organic carbon stock in southern Ontario, Canada

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ABSTRACT

Switchgrass [*Panicum virgatum*, (SG)] and miscanthus [*Miscanthus* spp., (Mis)] are perennial biomass crops (PBCs) commonly grown in Ontario, Canada. By the integration of PBCs on marginal/degraded agricultural (Ag) fields, a positive gain in soil organic carbon (SOC) sequestration has been reported in the scientific literature. Therefore, using PBCs to enhance SOC sequestration can potentially contribute to Canada's goal to reach net-zero greenhouse gas emissions by 2050. However, long-term field research (>10 years) to clearly demonstrate this trend in Canada is limited. In the above context, this study compared the current SOC stock (Mg C ha^{-1}) in SG and Mis to that of baseline Ag SOC obtained from three different locations, namely Elora (2008–2019), Guelph (2009–2020) and Burlington (2016–2020), Ontario, Canada. SOC stock at the time of land-use conversion from Ag to SG and Mis was considered as baseline. Woodlots (WLs), undisturbed natural forests, were used as reference SOC (potential maximum SOC stock) to predict the potential SOC sequestration in the future by PBCs at the respective locations. Results showed that SOC stocks in all SG fields, Mis fields and WLs were higher compared to respective baseline Ag fields. SOC stocks (Mg C ha^{-1}) in PBCs were significantly higher in Elora (SG: 96.3 ± 3.44, Mis: 100.0 ± 1.48) and Guelph (SG: 88.5 ± 5.72, Mis: 87.9 ± 6.43) over 11 years, whereas SOC stock in Burlington (SG: 87.5 ± 5.04, Mis: 94.7 ± 4.86), showed no significant difference over 4 years compared to their respective baseline Ag fields (77.6 ± 3.09, 59.3 ± 1.17 and 84.1 ± 3.39). All WLs had significantly higher SOC stock compared to their respective baseline Ag fields, showing the potential for future SOC stock gain by PBCs. SOC stock values in SG, with different nitrogen (N) fertilizer application rates (0, 40, 80 and 160 kg N ha^{-1}), were not significantly different. Conversely, SOC stock at all N rates were significantly higher in SG than baseline Ag SOC stock values. For Mis, only N fertilizer rates 40, 80, 160 kg N ha^{-1} significantly increased SOC stock. However, within the four N fertilizer rates, SOC significantly increased only at 80 kg N ha^{-1}. Increases in SOC stock suggest that converting marginal lands to PBCs over the long-term (>10 years) could create additional terrestrial C sinks. In Canada, there are close to 10 million ha of degraded Ag lands that can potentially be repurposed for PBCs, therefore the conversion of these unproductive Ag lands to PBCs could significantly contribute to Canada's climate mitigation strategies.

1. Introduction

Rising levels of greenhouse gasses (GHGs) in the atmosphere have resulted in negative climatic changes experienced by several terrestrial ecosystems (Anderson et al., 2016; Wuebbles and Jain, 2001). In particular, carbon dioxide (CO_2) released by global land-use changes has been cited as the primary cause for the increased atmospheric concentration (Strassmann et al., 2008). There is a growing global interest to develop strategies that mitigate GHG emissions and to enhance soil organic carbon (SOC) sequestration in terrestrial ecosystems (Follett

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et al., 2005; Kim et al., 2021). Hudiburg et al. (2015) have reported that perennial biomass crops (PBCs) have the potential to produce high biomass yields that can be used for bioenergy conversion, but at the same time can also reduce GHG emissions via SOC sequestration (Feng et al., 2015; Eichelmann et al., 2016). Awareness of PBC production as a renewable biomass source for the creation of advanced biofuels, direct combustion for heating and electricity, the manufacturing of bioproducts and as a material for animal bedding is increasing (Kludze et al., 2013). In addition, land-use systems with PBCs in temperate regions such as Canada are also considered as temperate agroforestry systems (Graham et al., 2019). Perennial biomass land-use systems embrace agroforestry principles such as the safety net hypothesis in relation to nutrient cycling, and promote ecosystem services such as soil erosion control, C sequestration, bird diversity enhancement and provide habitats for bird nesting. All temperate agroforestry systems contribute to climate mitigation strategies (Thevathasan et al., 2012) that are comparable to perennial biomass cropping systems in the same region.

The most commonly grown PBCs in Ontario, Canada are switchgrass (*Panicum virgatum,* SG) and miscanthus (*Miscanthus* spp., Mis). These PBCs can be readily grown on degraded agricultural (Ag) lands to enhance SOC sequestration (Lemus and Lal, 2005; Sanscartier et al., 2014; Agostini et al., 2015; Deen, 2017), thereby improving the quality of the soil (Graham et al., 2019). This is due to the ability of PBCs to sequester C through their high biomass production, deep roots and perennial nature (Lemus and Lal, 2005). In addition, a recent UN report on climate change also encourages integrating perennial vegetation to enhance C sinks in terrestrial soils (UN Report, 2019). In Canada, there are close to 10 million ha of degraded Ag lands that can be repurposed for PBCs (Liu et al., 2017). In Ontario, current statistics show that there are >1200 ha of SG and Mis PBC fields (Liu et al., 2017). However, this land area in Ontario is expected to increase to 15,000 ha in the next 5 to 10 years, given the Canadian government's commitment towards building green energy technologies. These lands, with sound management practices, could potentially increase SOC and support ecosystem services (Liu et al., 2017; Bazrgar et al., 2020).

In a recent study in southern Ontario, Graham et al. (2019) reported that the mean SOC stock was highest in woodlots (WLs; undisturbed natural forests), followed by both SG and Mis fields and lastly Ag fields, suggesting that SG or Mis crops could enhance SOC sequestration over Ag fields. Additional research studies have indicated that PBCs may be able to increase SOC levels and provide long-term C storage in lands where they have been introduced (Agostini et al., 2015; Qin et al., 2016). With respect to lower productivity Ag lands, the accumulation of SOC might be enhanced with the introduction of PBCs, since C emissions associated with land-use change to PBCs are low (Gelfand et al., 2013). Overall, long-term trends indicate net gains of SOC in both SG and Mis grown across North America. However, annual SOC sequestration rates by PBCs and how long it takes to achieve SOC gain since conversion to PBCs are not completely understood.

In the above context, this study was conducted in three different sites, namely Burlington, Guelph and Elora in Southern Ontario, Canada and was designed to investigate the following questions: firstly, is there any potential gain in SOC stock on a long/short-term basis when land is converted to SG and Mis from Ag production in class 1 (Elora) and class 3/4 (Guelph and Burlington) lands as classified under Canada Land Inventory (CLI)? Secondly, what are the annual SOC sequestration rates of SG, Mis and Ag at the three sites? Lastly, is SOC sequestration by both SG and Mis affected by variable annual nitrogen (N) fertilizer rate applications of 0, 40, 80 and 160 kg N ha^{-1} in Elora over 11 years? The results from this study can therefore help provide projections for climate change mitigation strategies brought about by land-use change from Ag fields to PBCs.

2. Abbreviations

To enhance readability, abbreviations are used throughout this paper. These abbreviations are summarized in Table 1.

Table 1
Abbreviations with proper definitions used in the study.

Abbreviation	Definition
Ag	Agriculture
ACS	Agricultural Climate Solutions
BMP	Best management practice
C	Carbon
CLI	Canada Land Inventory
CO_2	Carbon dioxide
ERS	Elora Research Station
GHG	Greenhouse gas
GTI	Guelph Turfgrass Institute
Mis	Miscanthus (*Miscanthus* spp.)
N	Nitrogen
PBC	Perennial biomass crop
SG	Switchgrass (*Panicum virgatum*)
SIC	Soil inorganic carbon
SOC	Soil organic carbon
STC	Soil total Carbon
WL	Woodlot

3. Materials and method

3.1. Study sites

Three different sites were identified in Southern Ontario, Canada for the study. Two sites were research stations established and operated by the University of Guelph [Elora Research Station in Elora (ERS) and Guelph Turfgrass Institute in Guelph (GTI)] and one site was a commercial farm in Burlington. Soil samples were collected from four different land-uses: SG, Mis, Ag and adjacent WLs from the identified sites (Fig. 1).

The SOC stocks in the Ag fields before PBCs were grown were used as baseline SOC stocks (initial SOC stock). Immediately after baseline soil sample collection from the Ag fields, part of each field was converted to SG and Mis cultivation, while the remaining field was left as Ag field. Annually, SG and Mis crops were harvested in all three sites.

SOC in the Ag field prior to land conversion to PBC was used as baseline SOC in each specific location to calculate SOC change after 11 years of PBC cultivation. And adjacent WLs that have been undisturbed for >200 years were used as a point of comparison for land-use change impact on SOC loss or gain. This research work also assumed that the SOC value measured in WLs, located adjacent to the PBC fields (SG and Mis), would be higher and will not change significantly over time. Therefore, we used the SOC in adjacent WLs as a reference SOC for maximum SOC sequestration and compared the difference in SOC between PBC fields and WLs as potential SOC gain in the future that can be attained by these PBCs in their specific locations. The difference in SOC between the Ag field and adjacent WLs was attributed as loss of SOC resulting from past agricultural management practices.

It should be noted that adoption of PBCs will likely increase SOC stock more than Ag crops but may not sequester SOC up to the values recorded in the WLs in this study. The PBC and WL systems will have different SOC equilibrium contents, since PBC systems are associated with biomass removal, differences in vegetation properties and the soil environment (temperature/moisture). However, it is not currently known when and at which SOC equilibrium content the PBCs will plateau. Therefore, in this study, to explain the above concept, the maximum SOC sequestration potential of PBCs was calculated by using SOC in the adjacent WLs in the respective sites.

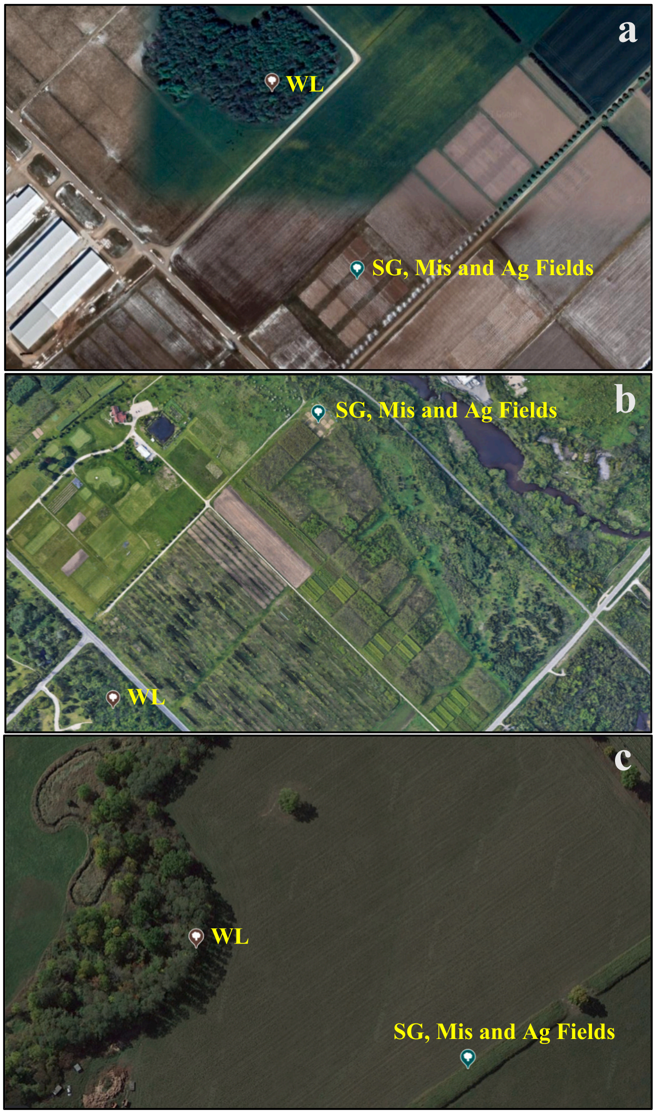

Fig. 1. Aerial view of the study sites; a) Elora (Elora research station), b) Guelph (Guelph Turfgrass Institute) and c) Burlington (Commercial farm) in Southern Ontario, Canada created in Google Earth Pro. [SG- Switchgrass, Mis- Miscanthus, Ag- Agriculture and WL- Woodlot].

3.2. Site description

Elora – University of Guelph [(ERS, Latitude 43°38'42" N, longitude 80°24'16" W) (Fig. 1a)]: SG and Mis were established in 2008 and the land was converted from Ag production to PBCs. Prior to initiation of the trial, the field was predominantly corn (*Zea mays L.*)- soybeans (*Glycine max L.*)- winter wheat (*Triticum avestivum L.*)] rotation. During the study, the Ag field was under corn-soy rotation with minimum tillage and received 112 kg N ha^{-1} annually, whereas PBCs were maintained with no tillage and received 60 kg N ha^{-1} annually. Average monthly temperature ranges from −11 °C in February (coldest) to 25 °C in July (warmest) and the mean annual precipitation is approximately 979 mm. The soils at the site belong to the London series (Hoffman et al., 1963) and are classified as a Gray Brown Luvisol in the Canadian System of Soil Classification (Soil Classification Working Group, 1998). The soil type is silt loam (sand-27%, silt-56%, clay-17%) and the land is classified as Class 1 land based on the CLI classification (Canada Land Inventory, National Soil Database, Agriculture and Agri-Food Canada. 1998). Class 1 lands have no limiting features to restrict crop production. They are well drained with good water-holding capacity, easily maintained in good fertility and soil erosion is low (Ashiq et al., 2017; Bock et al., 2018;).

Guelph – University of Guelph [(GTI, latitude 43°32'59" N, longitude 80°12'28" W) (Fig. 1b)]: SG and Mis were established in 2009 after >35 years of Ag production with crop rotation (corn-soybeans-winter wheat). During the study, the Ag field was under corn-soy rotation with minimum tillage and received average annual N fertilizer application of 112 kg N ha^{-1}. 60 kg N ha^{-1} was applied annually to PBCs and they were maintained with no tillage. Average monthly temperature ranges from -11 °C in January/February (coldest) to 26 °C in July (warmest) and the mean annual precipitation is approximately 958 mm. This land is classified as Class 3 to 4 land based on CLI classification for Ag production. "Class 3 lands have moderate limitations that reduce the choice of crops or require special conservation practices, and Class 4 lands have severe limitations that restrict the choice of crops or require special conservation practices and require very careful management, or both" (Ashiq et al., 2017; Bock et al., 2018). The soils at GTI are Gray Brown Luvisol with fine sandy loam texture (sand-56%, silt-34%, and clay-10%).

Burlington: Commercial SG and Mis farm [(43°27'03 N, 79°53'28" W) (Fig. 1c)], established in 2016. The Ag field was corn-soybeans-winter wheat rotation, which received average annual fertilizer application of 112 kg N ha^{-1} with minimum tillage. PBCs were maintained with no tillage and annual N fertilizer application of 60 kg N ha^{-1}. Average monthly temperature ranges from -7 °C in January (coldest) to 26 °C in July (warmest) and the mean annual precipitation is approximately 979 mm. This farmland is classified as Class 3 to 4 land under CLI classification. Soils at this site belong to the Chinguacousy series and are classified as Gleyed Gray Brown Luvisol in the Canadian System of Soil Classification (Soil Classification Working Group, 1998). The soil type is silt loam (sand-45%, silt-39%, clay-16%).

3.3. Experimental design

To monitor SOC sequestration and quantify SOC change in PBC production, 11-year-old [GTI (Guelph) - 2009 to 2020 and (ERS (Elora) - 2008 to 2019] and 4-year-old (Burlington - 2016 to 2020) sites were used. The experimental design was set up as a randomized complete block design with PBCs and Ag (land-uses) as a factor at three levels (SG, Mis and Ag) with three replicates at each site. Research plots of SG and Mis in Guelph and Elora were smaller than the commercial farm (Burlington) fields surveyed. The Guelph plots were 10 m by 10 m and the Elora plots were 3.05 m by 6.1 m. In Burlington, as the PBCs were grown in an open field (commercial production), the sampling positions were located at evenly spaced intervals (10 m) along the transect within the strip of PBCs. Baseline SOC stocks were obtained from the Ag fields prior to PBC establishment (in 2009 - Guelph, 2008 - Elora and 2016 - Burlington). At the time of baseline soil sampling, GPS coordinates (Garmin GPS unit accurate to within 2 m) were recorded to find exact locations to do sampling in the future (current SOC stock). Current SOC stock values were obtained from the SG, Mis and Ag fields using the previously established GPS coordinates. Similarly, GPS coordinates were established in the adjacent WLs, and the current SOC stock values were derived from those GPS coordinates.

To investigate the effect of variable N fertilizer rates on SOC sequestration by PBC (SG and Mis) fields at the Elora site only (other sites did not have N fertilizer treatments), with three replicates, four different N fertilizer [Ammonium nitrate (34–0-0)] rates (0, 40, 80 and 160 kg N ha^{-1}) were applied annually by surface broadcasting (early to mid-May) over a period of 11 years. For this experiment, twenty-four strip plots [(SG; 12 plots = 4 N fertilizer rates x 3 replicates) + (Mis; 12 plots = 4 N fertilizer rates x 3 replicates)] were selected randomly. The 160 kg N ha^{-1} fertilizer rate was chosen to ensure that N was not a limiting factor on biomass growth and did not negatively impact the amount of biomass inputs entering the soil. SOC stock in Ag fields (2008) with annual N fertilizer application of 80 kg N ha^{-1} were used as the control treatment (average N fertilizer application for corn-soybean-wheat annual crop rotation is 80 kg N ha^{-1} at the Elora research site). After an 11-year period (in 2019), SOC stocks in SG and Mis fields with variable N fertilizer rates were quantified to determine the SOC change and compare with the Ag control plots.

3.4. Soil sample collection

At the Guelph and Elora sites, (SG, Mis, Ag and WL), soil samples from 0 to 30 cm were collected randomly using a spade shovel. However, at the Burlington site and in all WLs, three transects were used to collect the topsoil. Three sub-samples were collected randomly from each replicated plot and along a transect and subsamples were mixed well to get one homogenous sample, which yielded three soil samples per plot/transect. Altogether, nine homogenous samples (3 homogenous samples x 3 replicates/transects) were collected from each field (SG, Mis, Ag and WL) and each N treatment (SG and Mis) for SOC analysis. In addition, two samples were collected for bulk density using a 250 cm^3 UMS soil sampling ring/core (Meter Group AG, Munich, DE) (V$_{\text{total}}$) from all fields. The soil sampling rings had a height of 5 cm and an inner diameter of 8 cm. This allowed us to calculate the SOC stock on a mass basis.

Soil samples were collected from Ag crop fields (representing baseline SOC stock) prior to PBC (SG and Mis) establishment at each experimental field (sampling year at each experimental field: Elora - 2008, Guelph - 2009, and Burlington - 2016). For current SOC stock derivation, soil samples were collected from PBC and Ag fields and adjacent WLs to calculate SOC change at all sites (current sampling year at each experimental field: Elora - 2019, Guelph and Burlington - 2020). To investigate any potential SOC stock change in WLs, samples were collected for two different time periods (Elora and Burlington: 2016 and 2020 and Guelph: 2009 and 2020).

$$Soil\ bulk\ density\ \left(Mg\ m^{-3}\right) = \left[M_{OD}\ (Mg) – M_{debris}\ (Mg)\right] / \left[V_{total}\ \left(m^3\right) - V_{debris}\ \left(m^3\right)\right] \tag{1}$$

3.5. Soil sample preparation

Immediately upon returning from the field, samples were air dried for 10–14 days, then rocks and roots were manually discarded while sieving through a 2 mm sieve. To improve the homogenization, large soil aggregates were ground by using a hammermill (Custom Laboratory Equipment, FL, US) and the resulting soil samples were again passed through the 2 mm sieve. Material filtered out by the sieve was discarded. Subsequently, it was ground using mortar and pestle to pass through a 0.250 mm sieve for SOC analysis (Graham et al., 2019; Vijayakumar et al., 2020).

$$SOC\ Stock\ \left(Mg\ C\ ha^{-1}\right) = \left[\left(Bulk\ density\ \left(Mg\ m^{-3}\right)\ x\ 0.3\ m\ x 10,000\ m^2\right)/ha\right]\ x\ [SOC\ (\%)] \tag{2}$$

3.6. SOC determination

Direct combustion method was used to determine SOC. Ground soil samples were divided into two subsamples (a pair). From one subsample, organic C was removed by muffle furnace treatment (samples were burned at 575 ^{o} C for 24 h). The other untreated subsample and the muffle furnace treated soil subsample (paired subsamples) were analyzed by using a LECO CR-412 Carbon Analyzer to get soil total C (STC) and soil inorganic C (SIC) percentages, respectively (Wang and Anderson, 1998; Wotherspoon et al., 2014; Graham et al., 2019; Bazrgar et al., 2020; Vijayakumar et al., 2020;). Finally, SOC percentage (%) was calculated by subtracting SIC from STC.

3.7. Bulk density determination

Soil bulk density was used to compute total soil mass at a depth of 0–30 cm to calculate SOC stock in Mg C ha^{-1} from SOC %, which was derived from the direct combustion C analysis. Bulk density core soil sample mass was weighed after oven drying (M$_{\text{OD}}$) at 105 °C for 14 h. Then the roots and rock material (debris) were obtained by passing the samples through a 2 mm sieve. The debris mass (M$_{\text{debris}}$) and volume (V$_{\text{debris}}$) were obtained. V$_{\text{debris}}$ was obtained through the water displacement method described by Hao et al. (2008). Soil bulk density was calculated by using Eq. (1).

3.8. SOC stock (equivalent soil mass basis) and equivalent CO$_2$ sequestration calculation

The mean bulk density and mean SOC % of each field was used in Eq. (2) to calculate SOC stock in mass basis (Mg C ha^{-1}) (Graham et al., 2019; Vijayakumar et al., 2020). SOC stock was calculated based on equivalent soil mass basis. Equivalent soil mass was calculated by multiplying soil volume from 0 to 30 cm soil depth and soil bulk density from Ag fields (baseline soil mass at 0–30 cm soil depth) obtained 11 years ago at the respective sites. Soil masses from respective Ag fields were used to calculate SOC stock in Ag fields and in all other land uses (SG, Mis and WLs) (Ellert and Bettany, 1995; Georgiadis et al., 2017; Vijayakumar et al., 2020).

The SOC stock change (gain/loss), mean annual SOC sequestration rate and potential SOC sequestration in the future by PBCs were calculated by using Eqs. (3), (4) and (5), respectively.

$$SOC\ stock\ change\ \left(Mg\ C\ ha^{-1}\right) = \left[Current\ SOC\ stock\ (SG\ or\ Mis\ or\ Ag)\ \left(Mg\ C\ ha^{-1}\right) – Baseline\ SOC\ stock\ (Ag)\ \left(Mg\ C\ ha^{-1}\right)\right] \tag{3}$$

$$Mean\ annual\ SOC\ sequestration\ rate\ \left(Mg\ C\ ha^{-1}\ y^{-1}\right) = \left[SOC\ stock\ change\ \left(Mg\ C\ ha^{-1}\right)/years\ of\ PBCs\ cultivation\ (y)\ or\ Ag\ crop\ cultivation\right] \tag{4}$$

$$Potential\ SOC\ sequestration\ \left(Mg\ C\ ha^{-1}\right) = \left[SOC\ stock\ in\ WL\ \left(Mg\ C\ ha^{-1}\right) - Current\ SOC\ stock\ in\ PBCs\ fields\ (SG\ or\ Mis)\ \left(Mg\ C\ ha^{-1}\right)\right] \tag{5}$$

Finally, the quantity of CO_2 sequestered (during the study period) and the potential future CO_2 mobilization from the atmosphere into the soil by PBCs in each location were calculated as shown in Eqs. (6) and (7), respectively. In these equations, sequestered SOC (SOC stock change by PBCs during the study period) and potential SOC sequestration were multiplied by 3.67 [44 g CO_2/ 12 g C] to convert the SOC stock value (Mg C ha^{-1}) to equivalent CO_2 (Mg CO_2 ha^{-1}). This is the appropriate conversion method used in the scientific literature to obtain CO_2 equivalents of SOC (Environmental Protction Agency USA, 2022).

$$Sequestered\ CO_2\ \left(Mg\ CO_2\ ha^{-1}\right) = \left[SOC\ stock\ change\ \left(Mg\ C\ ha^{-1}\right)\ x\ 3.67\ CO_2/C\right] \tag{6}$$

$$\begin{aligned} &Potential\ CO_2\ sequestration\ \left(Mg\ CO_2\ ha^{-1}\right) \\ &\quad = \left[Potential\ SOC\ stock\ change\ \left(Mg\ C\ ha^{-1}\right)\ x\ 3.67\ CO_2/C\right] \end{aligned} \tag{7}$$

3.9. Statistical analysis

All treatments were tested for statistical parameters using SAS software, Version 9.4, (SAS Institute, Inc. Cary NC). PROC GLIMMIX (Generalized linear mixed model) and replications were considered as random effects. For comparing current SOC stocks in SG, Mis, Ag and adjacent WLs with baseline SOC stock (Ag fields) and for comparing baseline SOC stock and current SOC stock in different N rates in SG and Mis fields, the paired "*t*-test" ($p < 0.05$) was used. To find the significant differences among the sample means of each N-treatment, least squares means were computed and compared pairwise using Tukey's multiple range test ($p < 0.05$). The Shapiro-Wilkes Test was used to test normality.

4. Results

4.1. Effect of land-use change on SOC sequestration in soil by PBCs

Comparisons of mean SOC stock (Mg C ha^{-1}) between current SOC stock in SG, Mis, Ag and adjacent WL with baseline SOC stock (Ag), at the respective sites, are shown in Fig. 2. SOC stock changes in WLs in two different time periods, at the respective sites, are also shown in Fig. 2.

In Elora (Class 1 land), mean SOC stock (Mg C ha^{-1}) for Ag was 77.6 (± 3.09) in 2008. After the land conversion to PBCs in 2008 (SG, Mis) mean SOC stocks quantified in 2019 in SG and Mis fields were 96.3 (± 3.44) and 100.0 (± 1.48), respectively and were significantly higher (p

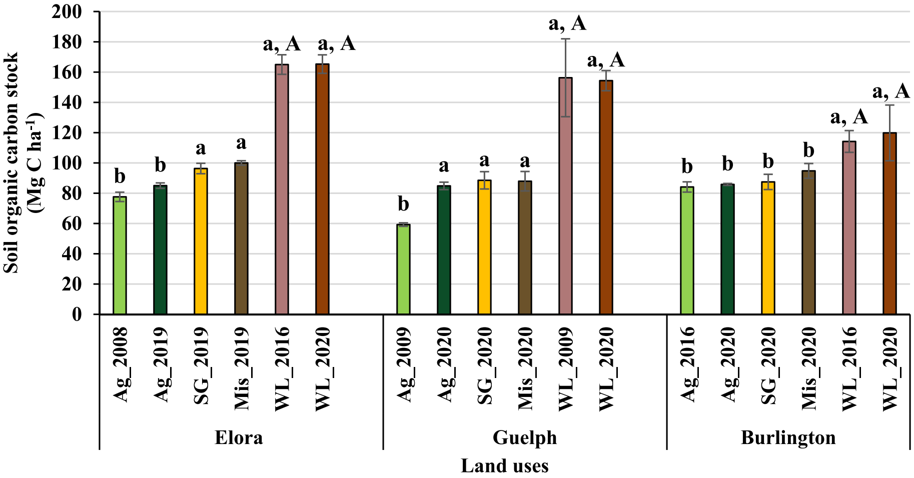

Fig. 2. Comparison of mean SOC stock (Mg C ha^{-1}) between baseline Ag [agriculture: corn (*Zea mays* L.)- soybeans (*Glycine* max L.)- winter wheat (*Triticum avestivum* L.) annual rotation] (Sampled year: Elora-2008, Guelph – 2009 and Burlington – 2016)] and other land uses [Current SOC in Ag, SG-Switchgrass, Mis-Miscanthus and WL-Woodlot (both baseline and current) (Sampled year: Elora – 2019, Guelph – 2020 and Burlington – 2020)] at the respective sites; Elora, Guelph and Burlington, southern Ontario, Canada. Means followed by the same letter in each site are not significantly different according to a paired "*t*-test" ($P \geq 0.05$). Error bars indicate the standard error of the mean ($n = 3$). **Lowercase letters** (a,b) represent the comparison between baseline Ag SOC stock values with current SOC stock values in other land uses at the respective sites and **uppercase letters** (A) represent comparison between baseline and current SOC stock values in WL at the respective sites [Elora- 2016 vs 2020, Guelph- 2009 vs 2020 and Burlington- 2016 vs 2020].

Table 2

SOC stock gain, mean annual SOC sequestration rate, potential SOC gain, sequestered CO_2 equivalent (during the study period) and potential equivalent of CO_2 sequestration by biomass crops and agriculture crops into the soil in the future in Elora, Guelph and Burlington, Ontario, Canada.[a]

Locations	SOC Stock Gain (Mg C ha^{-1})			Years of Crop Cultivation (y)	Mean Annual SOC Sequestration Rate (Mg C ha^{-1} y^{-1})			Sequestered CO_2 Equivalent (Mg CO_2 ha^{-1})			Potential SOC Stock Gain (Mg C ha^{-1})			Potential CO_2 Sequestration (Mg CO_2 ha^{-1})		
	Ag	SG	Mis	Ag/SG/Mis	Ag	SG	Mis	Ag	SG	Mis	Ag	SG	Mis	Ag	SG	Mis
Elora	7.4	18.7	22.4	11	0.67	1.70	2.04	27.6	68.6	82.2	80.3	69.0	65.3	294.7	253.2	239.7
Guelph	25.5	29.2	28.6	11	2.32	2.65	2.6	93.6	107.2	105.0	69.5	65.9	66.5	255.1	241.9	244.1
Burlington	1.7	3.4	10.6	4	0.46	0.85	2.65	6.2	12.5	39.0	34.0	32.3	25.1	124.8	118.5	92.1

[a] This study was conducted in Elora (2008–2019), Guelph (2009–2020) and Burlington (2016–2020), southern Ontario, Canada. {SG- Switchgrass, Mis- Miscanthus and Ag- Agriculture [corn (*Zea mays* L.)- soybeans (*Glycine* max L.)- winter wheat (*Triticum avestivum* L.)] annual rotation}.

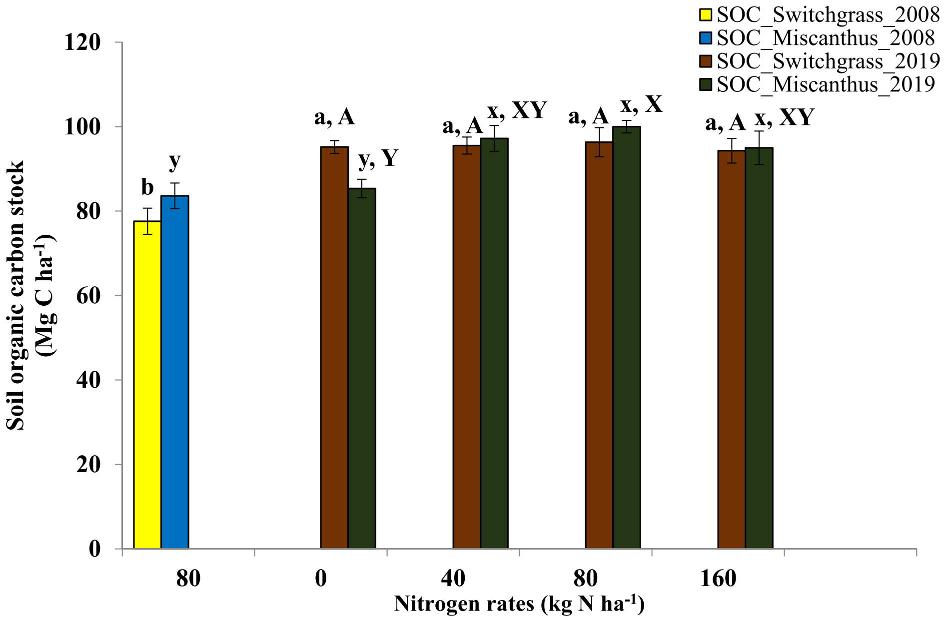

Fig. 3. Comparison of SOC stock (Mg C ha^{-1}) at four different rates of nitrogen fertilizer application in switchgrass (SG) and Miscanthus (Mis) crop fields with baseline SOC stock (Ag) and comparison of SOC stock among the N-treatment in SG and Mis in Elora, southern Ontario Canada (2008–2019). SOC_Switchgrass_2008 represents the baseline SOC stock value at the time of land-use change to SG from Ag and SOC_Miscanthus_2008 represents the baseline SOC stock value at the time of land-use change to Mis from Ag. Means followed by the **same lowercase letters** in each biomass crop [SG-(a,b), Mis-(x,y)] are not significantly different according to a paired "t-test" ($P \geq 0.05$). Means followed by the **same uppercase letters** in each N-treatment in SG and Mis [SG- A, Mis- (X-Y) are not significantly different according to Tukey's multiple range test ($P \geq 0.05$). Error bars indicate the standard error of the mean ($n = 3$).

< 0.05) compared to baseline Ag field SOC stock over 11 years (2008–2019). However, current (2019) SOC stock in the Ag field was 85.0 (± 1.80) Mg C ha^{-1} and did not show a significant difference ($p \geq 0.05$) with baseline SOC stock. In Guelph (Class 3/4 land), baseline SOC stock was obtained in 2009 and was 59.3 (± 1.17) Mg C ha^{-1}. Current (2020) SOC stocks in SG, Mis and Ag fields were 88.5 (± 5.72), 87.9 (± 6.43) and 84.8 Mg C ha^{-1} respectively and were significantly higher (p < 0.05) compared to baseline SOC stock over 11 years of cultivation (2009–2020). Conversely, at the Burlington site, current (2020) SOC stocks obtained in SG (87.5 ± 5.04 Mg C ha^{-1}), Mis (94.7 ± 4.86 Mg C ha^{-1}) and Ag (85.8 ± 0.65 Mg C ha^{-1}) were not significantly greater (p ≥ 0.05) after 4 years (2016–2020) of cultivation when compared with the baseline SOC stock in the Ag field (84.1 ± 3.39 Mg C ha^{-1}) (Fig. 2).

SOC stock change observed in SG, Mis and Ag fields were respectively, 18.7 Mg C ha^{-1}, 22.4 Mg C ha^{-1} and 7.4 Mg C ha^{-1} in Elora and 29.2 Mg C ha^{-1}, 28.6 Mg C ha^{-1} and 25.5 Mg C ha^{-1} in Guelph over 11 years of cultivation. Whereas in Burlington, SOC stock values were increased numerically by 3.4 Mg C ha^{-1}, 10.6 Mg C ha^{-1} and 1.7 Mg C ha^{-1}, in SG, Mis and Ag fields, respectively over 4 years of cultivation. Mean annual SOC sequestration rates observed in SG, Mis and Ag were respectively, 1.70 Mg C ha^{-1} y^{-1}, 2.04 Mg C ha^{-1} y^{-1} and 0.67 Mg C ha^{-1} y^{-1} in Elora, 2.65 Mg C ha^{-1} y^{-1}, 2.60 Mg C ha^{-1} y^{-1} and 2.32 Mg C ha^{-1} y^{-1} in Guelph and 0.85 Mg C ha^{-1} y^{-1}, 2.65 Mg C ha^{-1} y^{-1} and 0.46 Mg C ha^{-1} y^{-1} in Burlington (Table 2). Additionally, 68.6, 82.2 and 27.6 Mg CO_2 ha^{-1} equivalent of atmospheric CO_2 sequestered into soil in SG, Mis and Ag fields, respectively in Elora during the study period (11 years). In Guelph, observed mobilized atmospheric CO_2 equivalent into soil in SG, Mis and Ag were 107.2, 105.0 and 93.6 Mg CO_2 ha^{-1}, respectively over 11 years of cultivation. Whereas over 4 years of PBC cultivation in Burlington, mobilized atmospheric CO_2 into the soil were 12.5 Mg CO_2 ha^{-1} in SG, 39.0 Mg CO_2 ha^{-1} in Mis and 6.2 Mg CO_2 ha^{-1} in Ag fields (Table 2).

Current SOC stocks obtained (in 2020) from adjacent WLs in Elora, Guelph and Burlington were significantly higher ($p < 0.05$) than their respective Ag fields (Fig. 2). However, the SOC stocks obtained in WLs in two different periods did not show significant differences ($p \geq 0.05$) in all sites [Elora; 165.3 (± 6.10) Mg C ha^{-1} in 2020 and 165.0 (± 6.46) Mg C ha^{-1} in 2016, Guelph; 154.4 (± 6.66) Mg C ha^{-1} in 2020 and 156.3 (± 25.72) in 2009 and Burlington; 119.8 (± 18.40) Mg C ha^{-1} in 2020 and 114.17 (± 7.18) Mg C ha^{-1} in 2016].

In addition, potential SOC stock change and associated CO_2 sequestration by SG, Mis and Ag were predicted assuming WL SOC stock as a reference for maximum SOC sequestration, in all three locations studied as the WLs SOC did not change over the 11-year period (Table 2).

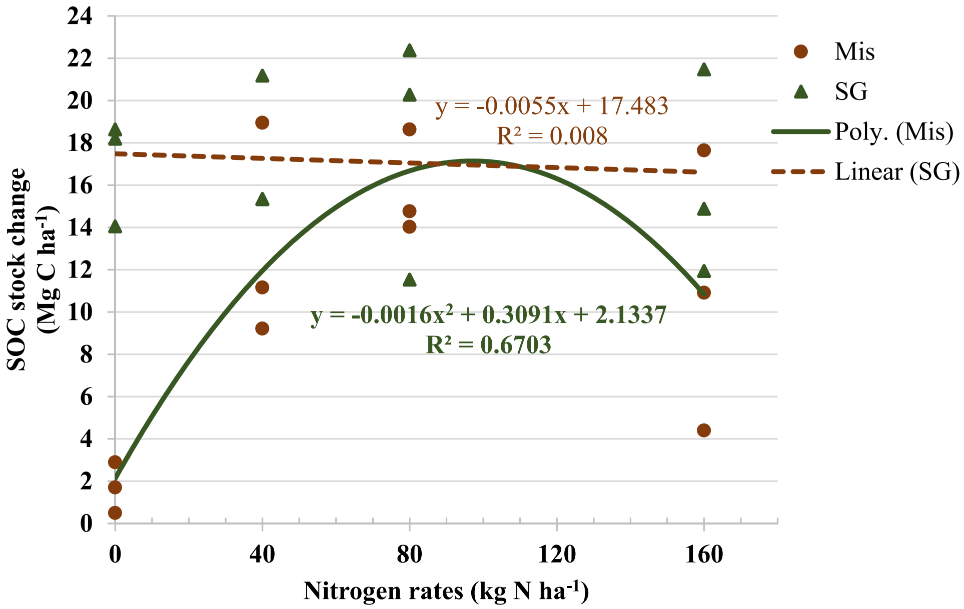

Fig. 4. SOC stock change (Mg C ha^{-1}) with four different rates of nitrogen fertilizer application in switchgrass (SG) and miscanthus (Mis) fields in Elora, southern Ontario Canada (2008–2019).

Potential SOC stock change in SG, Mis and Ag, respectively were 69.0, 65.3 and 80.3 Mg C ha^{-1} in Elora, 65.9, 66.5 and 69.5 Mg C ha^{-1} in Guelph and 32.3, 25.1 and 34.0 Mg C ha^{-1} in Burlington. The predicted equivalent of atmospheric CO_2 mobilization into the soil in the future in Elora, Guelph and Burlington were 253.2, 241.9 and 118.5 Mg CO_2 ha^{-1}, respectively in SG fields, 239.7, 244.1 and 92.1 Mg CO_2 ha^{-1}, respectively in Mis fields and 294.7, 255.1, 124.8 Mg CO_2 ha^{-1}, respectively in Ag fields.

4.2. *Effect of different rates of N fertilizer on SOC sequestration in soil by PBCs*

Comparison of SOC stock (in 2019) in SG and Mis crop fields at four different N fertilizer rates with baseline SOC stock (Ag) (obtained in 2008, with 80 kg N ha^{-1} fertilizer application) after 11 years of cultivation is shown in Fig. 3. In addition, comparison of SOC stock among all N fertilizer treatments (four different N fertilizer rates) in both SG and Mis separately, is shown in Fig. 3.

In the case of SG, there were no significant differences ($p \geq 0.05$) observed in SOC stock among the N fertilizer treatments. However, the SOC stock in SG was significantly increased ($p < 0.05$) in all N fertilizer rates compared to the baseline SOC stock (Ag) value (77.6 ± 3.09 Mg C ha^{-1}) (Fig. 3).

In contrast to SG, the SOC stock in Mis showed significant differences ($p < 0.05$) among N fertilizer treatments. SOC stock at fertilizer rate 80 kg N ha^{-1} [100.0 (±1.48) Mg C ha^{-1}] increased significantly ($p < 0.05$) compared to SOC stock at fertilizer rate 0 kg N ha^{-1} [84.4 (±2.19) Mg C ha^{-1}]. However, there were no significant differences observed in SOC stock among fertilizer rates 0, 40 and 160 kg N ha^{-1}. Additionally, SOC stock at fertilizer rate 0 kg N ha^{-1} did not significantly differ ($p \geq 0.05$) compared to baseline SOC stock [Ag, 83.6 (±3.05) Mg C ha^{-1}], whereas SOC stock at fertilizer rates 40, 80 and 160 kg N ha^{-1} were significantly increased compared to baseline SOC stock in 11 years (Fig. 3).

In response to increasing N fertilizer rates in Mis, SOC stock change increased with increasing fertilizer rates and started to decline between 80 and 160 kg N ha^{-1}. Hence, the optimum rate of N fertilizer application lies between 80 and 160 kg N ha^{-1}. Therefore, the predicted N fertilizer rate for optimum/higher SOC sequestration for Mis was 97 kg N ha^{-1} according to the polynomial regression equation ($y = -0.0016 \times {}^{2} + 0.3091\times + 2.1337$, $R^2 = 0.6703$) (Fig. 4).

SOC stock gains observed in SG fields were 17.6, 17.9, 18.7 and 16.7 Mg C ha^{-1} at fertilizer rates 0, 40, 80 and 160 kg N ha^{-1}, respectively. In Mis fields, SOC stock gains were 1.8, 13.6, 16.4 and 11.4 Mg C ha^{-1} at fertilizer rates 0, 40, 80 and 160 kg N ha^{-1}, respectively (Fig. 4).

5. Discussion

5.1. *Effect of land-use change on SOC sequestration in soil by PBCs*

Results from this study show a positive relationship between the integration of PBCs (SG and Mis) and mean SOC stock change. There was a statistical significance between land uses (baseline Ag vs Mis/Sg) in Elora and Guelph, where Ag lands were converted to PBCs at the beginning of the 11-year study. About 24% and 29% of SOC stock increases occurred with annual SOC sequestration rates of 1.70 Mg C ha^{-1} y^{-1} and 2.04 Mg C ha^{-1} y^{-1} in SG and Mis fields, respectively in Elora. Whereas 49% and 48% of SOC stock increases were observed with annual SOC sequestration rates of 2.64 Mg C ha^{-1} y^{-1} and 2.60 Mg C ha^{-1} y^{-1} in SG and Mis fields, respectively in Guelph. Similar results were reported for the potential SOC sequestration rates in Mis fields when converted from Ag fields; rates were between 2 and 3 Mg C ha^{-1} y^{-1} with variations based on initial SOC stock and crop yield in Dondini et al. (2009). In a meta-analysis, SG and Mis SOC sequestration rates have been reported to be 1.59 Mg C ha^{-1} y^{-1} and 1.21 Mg C ha^{-1} y^{-1}, respectively (Agostini et al., 2015). The rate of change in SOC sequestration does not remain constant over time, and the rate of change will eventually stabilize as soil reaches a new equilibrium (Agostini et al., 2015). The values vary across the scientific literature as SOC sequestration may be influenced by many factors (Graham et al., 2019). Factors such as previous land-use and crop management and duration may lead to different values (Lemus and Lal, 2005; Qin et al., 2016). It is interesting to note in this study that higher percentage increases in SOC stock occurred when the initial SOC stock was low (Fig. 2). For example, the Elora site, consisting of class 1 land, had a higher initial SOC stock value of 77.6 Mg C ha^{-1}, whereas at the Guelph site, consisting of class 3/4 land, the initial SOC stock value was only 59.3 Mg C ha^{-1}. A similar trend has also been reported by Dondini et al. (2009), where the highest SOC stock gain was observed in soil having low initial SOC stock.

The significant SOC stock gains shown in Fig. 2 clearly depict a relationship between the number of years under PBCs and SOC sequestration. At the Elora and Guelph sites, significant increases in SOC stocks were observed after 11 years, whereas such a significant gain was not observed ($p \geq 0.05$) at the Burlington site, where PBCs were only grown for 4 years. Gain in SOC at this site can be expected in the years to come, however such gain would be relatively low compared to future gains in the other two sites. This is due to lower differences in SOC currently observed between WLs and PBCs at the Burlington site (Table 2). However, it is important to note that the numerical values do show an increase in SOC stock in PBC plots at the Burlington site when compared with Ag land-use (baseline). Other studies have also shown that having PBCs for a short period of time did not significantly influence the SOC. In a study by Ma et al. (2000), it has been reported that 3 years of continuous production of SG did not significantly change SOC stock, whereas 10 years of production yielded a 45% increase in SOC stock, compared to an adjacent fallow crop. A report on "A comprehensive guide to switchgrass management" in Ontario by Samson et al. (2018) states that SG typically will not reach full yield response in the first two years and will depend on soil type, crop variety and crop establishment. Results from a modelling exercise (DayCent Model) showed SOC benefits derived from incorporating SG or Mis into marginal agricultural lands using a hypothetical cultivation duration of the crop; 50–60 years (Jarecki et al., 2020). However, in practice, no actual field study has demonstrated the benefits of PBCs on SOC for the above indicated hypothetical period.

It is reported that the extensive root systems associated with SG and Mis (below ground biomass) and leaflitter are the main source of C input to soil, since the above ground biomass is harvested, and this C input can last for many years (Lemus and Lal, 2005; Agostini et al., 2015). Therefore, the accumulation of SOC in the soil is expected to increase, in theory, over 50–60 years for SG or Mis (Lemus and Lal, 2005; Jarecki et al., 2020). In this context, the results and trends found in this study indicate that the SOC stock measured at all sites will likely continue to increase over the coming years and, especially at the Burlington site, statistically higher SOC stocks could be recorded in the future. However, as indicated above, at what level SOC equilibrium or saturation will occur at 30 cm soil depth as influenced by PBCs is yet to be seen.

This study also shows significant increases in Ag field SOC stock over the 11-year sampling period in Guelph. This increase can be linked to the adoption of best management practices (BMPs) such as minimum tillage and the change in crop rotation from corn-soybean-wheat to corn-soybean during the study. It is likely that these BMPs have contributed to the gain in SOC, especially in the low productivity Class 3 and 4 lands. It could be argued that such gain in SOC in Ag field by BMPs may not justify conversion of Ag field to biomass crops. However, agricultural crops are more susceptible to climate extremes than PBCs. In this context, Ontario agriculture growers are converting a part of their Ag fields to biomass crops as a climate resilient strategy. However, in the highly productive Class 1 land (i.e., Elora, Ontario) there was no significant increase in SOC stock represented over the 11-year study period irrespective of adopted BMPs. The initial SOC stock was relatively high in Elora compared to Guelph, which likely resulted in lower mean annual SOC increases (or C sequestration rates).

The data collected in the WL locations (Fig. 2) was utilized to provide a reference value for maximum SOC sequestration potential. There was no statistically significant change in SOC stock in WLs between the two sampling periods (4 years in Elora and Burlington, 11 years in Guelph). As the soils in these WLs are undisturbed, it is expected that the amount of SOC present is representative of the maximum saturation level for that site, as the WLs are located in close proximity to the test sites (Fig. 1) (Coleman et al., 2018). These values are important to keep in mind when comparing the levels of SOC stock in each sampling location. To minimize confounding variables due to various environmental factors present (i.e. weather, precipitation, soil properties etc.), the sampling locations for SG/Mis/Ag were located within 1 km of their corresponding WLs. Table 2 presents an overview of the SOC stock gain present in the soil after the introduction of PBCs and the potential for SOC stock gains in the future (maximum SOC saturation levels based on SOC stock values in the undisturbed WL locations).

In this context, as there are 10 million ha of degraded Ag lands in Canada, and if this land extent is brought under PBCs, a significant amount of atmospheric CO_2 could be captured in these soils as SOC stock. In Ontario, Canada, degraded or low productive (marginal) lands are being converted to PBCs by the Ontario Biomass Producers and there are currently about 1200 ha of biomass crops either under SG or Mis (OBPC (Ontario Biomass Producers Co-operative), 2023). The land extent is expected to increase to 15,000 ha in the next 10 years, therefore, this land-use change can significantly enhance atmospheric C capture via SOC sequestration in the province of Ontario. It is becoming increasingly well-known in the scientific literature and governmental sources that the integration of PBCs is economically and environmentally beneficial (Government of Canada, 2022a). In Canada, 672 Mt. CO_2 equivalent of GHGs were released into the atmosphere in 2020 (Government of Canada, 2022b). Therefore, to ensure that Canada reaches its goal outlined in the Paris Agreement related to GHG emission reduction, the government adopted the Pan-Canadian Framework on Clean Growth and Climate Change in 2016 (Government of Canada, 2022b). This initiative further allocated resources to enhance the development of effective mitigation strategies to address GHG emissions (i.e., CO_2). One aspect of this framework was for the federal, provincial, and territorial governments to collaborate and ensure that C sinks are being increased and protected (Government of Canada, 2022b). Emphasis is given in the framework to enhance C sinks in Ag fields. It is therefore evident from this study that potential CO_2 mobilization from the atmosphere into the soil can be realized by the adoption of PBCs in low productive Ag fields (Table 2).

5.2. *Effect of different rates of N fertilizer on SOC sequestration in soil by PBCs in Elora*

Among the two PBCs, SG and Mis, only Mis had a positive SOC response to N fertilizer application among the N fertilizer treatments. However, among N application rates, there was no significant difference in SG fields (Fig. 3). This means that in fertile soils (class 1 soils), it appears that additional N application is not needed for SG. Thomason et al. (2005) have reported that no trend was observed on SOC increases with various N applications in their study. However, it was evident that maintaining SG production increased SOC over the long-term (Thomason et al., 2005). It is important to note that fertilization may increase SG yields over time, but it does not have a significant effect on SOC stocks, potentially due to removal of above ground biomass (Thomason et al., 2005). It is becoming more apparent that N fertilization affects plant C partitioning by increasing aboveground biomass (Leyshon, 1991; Russell et al., 2009; Sprunger et al., 2018), but not belowground biomass, which is critical in the formation of more stable SOC (Russell et al., 2009; Sokol et al., 2019; Xu et al., 2021).

Regarding Mis, however, there was no significant increase in SOC when varying N rates until 80 kg N ha^{-1} was applied. According to the polynomial equation, the calculated optimum N rate is 96 kg N ha^{-1} (Fig. 4) to attain higher SOC stock sequestration. Lee et al. (2017) reported that the average annual yield in Mis was higher with 60 and 120 kg N ha^{-1} (20.0 dry Mg ha^{-1}) compared to unfertilized fields (11.8 dry Mg ha^{-1}). It was also reported that total dry root biomass in Mis was 13.9 Mg ha^{-1} (Neukirchen et al., 1999) and about 50% of the roots are found in the top 0–30 cm soil depth (Agostini et al., 2015). It should be noted that the N application experiment was only conducted at the Elora site (fertile soil) and was not conducted at the Guelph or Burlington sites (marginal or degraded soil). Hence, further investigation is needed in relation to studying the effect of N fertilization on SOC sequestration by PBCs. In addition, the N response was seen in Mis not in SG. This aspect also needs further research.

Recommendations for future research include the following:

1. Continue to investigate the long-term accumulation of SOC by PBCs and establish carbon credit quantification metrics so that PBC landowners can trade carbon credits and financially benefit. However, increase in the acreage of biomass crops in the province of Ontario in particular and in Canada will depend on demand for biomass crops by green technologies, market prices and landowner perception.
2. Conduct research to find the minimum required time period to enhance SOC levels and other soil health indicators in marginal Ag lands. Once the above indicators have been achieved, can the land be converted back to Ag production with BMPs, and still maintain or enhance SOC and other beneficial soil health indicators? A modeled (DayCent) study by Jarecki et al. (2020) has indicated that when PBCs are converted back to Ag production, the SOC will decline. This aspect should be investigated further.
3. N fertilization effect on SOC sequestration in SG and Mis fields should be investigated on more sites in southern Ontario.

6. Conclusion

The results found in this study show that integrating Mis or SG into degraded Ag fields could increase SOC sequestration. Statistically significant increases in SOC stocks resulted in 11 years, whereas increasing numerical values were observed in 4 years in the fields sampled in this study. This enhancement of SOC by PBCs may contribute to Canada's efforts on climate change mitigation. The Agricultural Climate Solutions (ACS) 10-year plan initiative, that was recently implemented in April 2021, acknowledges the importance of increasing terrestrial C sinks in non-forested lands (Government of Canada, 2021). This program's investment of CAD 185 million focuses on current climate change mitigation practices that aim to enhance C sinks in terrestrial ecosystems, more specifically in the Ag sector (Government of Canada, 2021). In this context, introducing PBCs in low productive agricultural lands can positively contribute to ACS objectives. It is estimated that about 10 million ha in Canada are suitable for PBC (herbaceous PBC and perennial woody crops) production. Therefore, if SG and/or Mis were planted at a widespread scale, about 80 Mt. CO_2 y^{-1} and 85 Mt. CO_2 y^{-1}, respectively (average SOC sequestration rate SG: 2.18 Mg C ha^{-1} y^{-1}, Mis: 2.32 Mg C ha^{-1} y^{-1}) would be mobilized from the atmosphere into the soil annually. According to the Government of Canada's most recent dataset, GHG emissions were 672 Mt. CO_2 eq in 2020. Therefore, planting SG and/or Mis throughout the ~10 million ha in Canada would cause an annual C emission reduction of 7.9 to 8.4%. Studies of this nature are not only beneficial for Canada, but similar research can be conducted in other regions of the world too.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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