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Advances in Environmental and Engineering Research

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Communication

Quantification of the Carbon Sequestration Potential of a 31-year-old Tree-based Intercropping System in Southern Ontario, Canada

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Academic Editor: Paul Dargusch

Special Issue: Case Studies of Carbon Management in Practice

Adv Environ Eng Res
2022, volume 3, issue 4
doi:10.21926/aeer.2204043

Received: September 08, 2022
Accepted: October 18, 2022
Published: October 25, 2022

Abstract

Carbon (C) storage potential was quantified for four tree species which are commonly incorporated into tree-based intercropping (TBI) systems and compared with conventional agricultural systems in southern Ontario, Canada. In the 31-year-old TBI system at the University of Guelph's Agroforestry Research Station, Norway spruce (*Picea abies*), white cedar (*Thuja occidentalis*), black walnut (*Juglans nigra*) and red oak (*Quercus rubra*) were planted in tree rows, intercropped with soybean (*Glycine max*). In the conventional agricultural field, soybean was grown in a monocropping system. Above and belowground tree and crop C content, soil organic C (SOC) and system level C was quantified for each tree species as well as the conventional agricultural system. Red oak TBI systems had the highest SOC followed by black walnut, Norway spruce and white cedar with values of 93.2, 83.9, 78.1, and 72.2 t C ha^{-1}, respectively. Red oak TBI systems also had the highest mean tree C content, followed by black walnut, Norway spruce and white cedar, with values of 299.7, 285.8, 255.4 and 70.1 kg C tree^{-1}, respectively. Total system level C, which incorporated SOC, tree C content and tree planting densities was 134.8, 126.4, 115.7, 95.6 and 75.6 t C ha^{-1}

respectively for spruce, oak, walnut and cedar TBI systems and the agricultural field. TBI systems provide higher C sequestration potentials than conventional agricultural fields, and Norway spruce and red oak should be included into TBI systems in southern Ontario to maximize C sequestration benefits.

Keywords

C sequestration; agroforestry; intercropping; C pools and fluxes; Norway spruce, black walnut, white cedar, red oak

1. Introduction

To ratify the Paris Agreement signed in 2015, Canada has adopted a climate change plan to reduce greenhouse gas (GHG) emissions to 30% below their 2005 levels before 2030. The Intergovernmental Panel on Climate Change (IPCC) has recommended mitigation strategies for reducing atmospheric CO_2 emissions, including converting agricultural land into agroforestry systems [1]. Tree-based intercropping (TBI) is an agroforestry system in which cropping rows are established between rows of trees. TBI systems have been recommended as a climate change mitigation strategy through its ability to sequester carbon [1-3]. TBI systems have demonstrated a greater carbon sequestration potential relative to conventional monocropping systems, through two mechanisms: (1) higher carbon storage in tree biomass, and (2) a reduced decomposition rate of litter containing high volumes of lignin, resulting in further stabilization of soil organic carbon (SOC) [4]. Incorporating trees into agricultural systems in the form of TBI can offset atmospheric CO_2 emissions by sequestering carbon in both tree biomass and soils, while simultaneously providing many other environmental and economic benefits, including improved cycling of soil nutrients, protection from erosion, and creation of suitable habitat for wildlife [3, 5-7].

While no management system could ever replicate the amount of carbon stored by primary forests, TBI systems can sequester a substantial amount of carbon, while simultaneously providing food and other ecosystem services. Reforestation can remove massive amounts of carbon from the atmosphere, however, due to increasing population and food demands, this can be incredibly difficult to implement as the demand for land increases [6]. TBI systems can be very effective in this respect as they allow for reforestation which concurrently provides other human benefits, namely food production [3]. This can indirectly impact carbon sequestration by alleviating pressure on existing forested lands by providing a sustainable source of timber and food resources [4, 8]. The complete adoption of TBI systems in Canada would catalyze a multitude of environmental benefits including increased biodiversity, nutrient cycling, and soil fertility while simultaneously contributing towards the national goal of the Paris Agreement by enhancing C capture in the terrestrial ecosystem. The estimated total land in Canada that could be converted to a TBI system is estimated at above 45.5 million hectares, thus if intercropped, would result in a momentous effect on carbon sequestration [2, 3, 7].

In the past, studies have been conducted with the objective of determining C sequestration potentials in temperate zones [7, 9]. This study was completed in the University of Guelph's Agroforestry Research Station (ARS), North America's oldest TBI system. Previous studies have quantified carbon sequestration in the ARS's TBI system at earlier stages of maturity; [9] investigated C sequestration at 13 years (measurement year 2000), [10] at 21 years (measurement year 2008) and [5] at 25 years (measurement year 2012) after establishment. Understanding C storage potentials of temperate TBI systems and how they change as they mature in one of North America's most established agroforestry systems would allow for recommendations to be made regarding which tree species should be included in TBI systems to maximise C sequestration benefits. The overall goal of this manuscript is therefore to document this progressive gain of carbon sequestration in TBI systems in the temperate region.

The objectives of this study are, 1) to quantify the C sequestration potentials of four different tree species in a mature 31-year-old TBI system and 2) to compare the C sequestration potential of TBI systems to a conventional monocropping agricultural field. The results from this study will determine which tree species are suitable and should be included into TBI systems to maximize C sequestration potentials. Accurately quantifying C sequestration potentials for TBI systems, which are a nationally recommended natural climate solution [2], may allow for sequestration estimates to contribute to Canada's carbon budget while also aligning with emission goals in the province.

2. Methods

2.1 Site Description and Experimental Design

This study was conducted in 2018 at the University of Guelphs' Agroforestry Research Station (ARS), in southwestern Ontario (latitude 43°32'60" N, longitude 80°12'30" W). This site, established in 1987, encompasses approximately 30 ha of agricultural land and has a mean annual temperature of 7.2°C, as well as mean precipitation of 833 mm, with 344 mm falling within the growing season [10].

Prior to the establishment of the ARS, the land was being utilized for crop and hay production, however, this management system had experienced continuously declining crop yields as well as bedrock exposure as a consequence of intensive soil erosion. Beginning in 1987, twelve tree species were selected and planted in tree rows, with the between tree row spacing and within-tree row spacing being 12-15 m and 6 m, respectively, for hardwood trees and 3 m within row spacing for coniferous trees (Figure 1).

Figure 1 Tree-based intercropping system at the University of Guelph's Agroforestry Research Station. Tree rows are intercropped with soybean. Photo credit: Dr. Naresh Thevathasan.

There is a conventional agriculture system located adjacent to the ARS, which is planted in a monoculture with the same species that are planted each year in the TBI system. Data were collected from the conventional agricultural system in 2017, which will be referred to as the monocropped field from this point on, and was used to draw comparisons with data from the TBI system.

2.2 Soil Carbon

All soil samples from the TBI system were collected in November of 2018, and samples from the adjacent agricultural field were collected in 2017. A metal soil auger was used to collect a sample at a depth of 0-15 cm, and one at 15-30 cm. Both soil cores were placed in polyethylene bags and mixed to generate one sample for a 0-30 cm depth. Soil samples were taken at a distance of 2.0 m and 5.0 m from both the east and westward side of the tree. The east and westward samples were combined at their respective distance for each tree. Four tree species were selected for this experiment with 5 replications each. Therefore, a total of 40 soil samples were taken (2 samples per tree × 4 tree species × 5 replications per species). In the monocropped field, a total of 3 samples at 0-30 cm depth were collected. All soil samples were transported to the laboratory at the University of Guelph and stored frozen at -20°C. Once ready for analysis, all 40 soil samples were thawed and placed in aluminum trays to air dry for fourteen days. Once dry, samples were passed through a 2 mm sieve to remove any coarse roots or gravel. Any material unable to pass through the sieve was discarded. The remaining material was ground with a mortar and pestle and passed through a 0.149 mm sieve to ensure a homogenized sample for SOC analysis.

From each of the ground soil samples, two subsamples were taken. The first subsample was used to determine the soil inorganic carbon fraction (SIC). A 5 g sample was placed into a muffle furnace (Lindberg, Mi, USA) at 575°C for 24 hrs to remove the organic C fraction. A 0.2000-0. 2100 g sample was then taken from this subsample and analyzed for SIC using the LECO CR-412 dry combustion Carbon Analyzer with a lance flow of 1.2 L/min at a temperature of 1200°C (LECO Corporation, MI, USA). The second subsample (unheated) was used to determine the soil total carbon (STC) fraction and a 0.2000-0.2100 g sample was placed directly into the LECO Carbon CR412 analyzer.

The LECO Carbon CR412 analyzer determines the percent carbon of a sample in relation to its total mass. Consequently, a moisture correction factor (MC_F) was also calculated to account for any moisture present in the soil subsamples. The first subsample, which was used to determine the percentage of SIC present, was placed into the LECO Carbon CR412 analyzer immediately after being removed from the muffle furnace, thus the MC_F was not applied to the SIC values. However, for the second subsample, the MC_F was applied as the STC samples had been only air dried, as shown in Equation (1). The MC_F was determined by taking a third subsample (15 g) from the original ground soil samples. These subsamples were then placed in tins and their weight was recorded before and immediately after being placed into an oven at a temperature of 105°C for 48 hrs. Equation (2) was then used to calculate the MC_F, in which M_{tin} is the mass of the tin (g), M_{OD} is the mass of the oven-dried soil subsample (g) and M_{AD} is the mass of the air-dried soil subsample (g).

$$\mathrm{SOC}\ (\%) = [\mathrm{STC}\ (\%) \times \mathrm{MCF}] - \mathrm{SIC}\ (\%) \tag{1}$$

$$\mathrm{MCF} = [\mathrm{M_{AD}}\ (\mathrm{g}) - \mathrm{M_{tin}}\ (\mathrm{g})]/[\mathrm{M_{OD}}\ (\mathrm{g}) - \mathrm{M_{tin}}\ (\mathrm{g})] \tag{2}$$

For each sample, SOC concentrations were used to determine SOC stocks in t C ha^{-1}, by considering a bulk density of 1.34 [11]. Consequently, the mean SOC stocks (t C ha^{-1}) at distances of 2.0 m and 5.0 m for each species were determined by averaging the values of five samples taken from each distance.

2.3 Tree Carbon Content

In November of 2018 diameter at breast height (DBH) and tree height were measured for all twenty tree samples. A tree diameter tape was used to determine the diameter of the tree at 1.3 m from ground level. Tree height was measured using a Haga altimeter at a distance of 15 m from each tree. The aboveground biomass (AGB) could then be determined using allometric equations with regression coefficients specific for each species, as shown in Equation (3). Regression coefficients *a* and *b* were determined by Ter-Mikaelian and Korzukhin [12] (Table S3).

$$\text{Aboveground biomass (kg/tree)} = a \times \mathrm{DBH}\ (\mathrm{cm})^{b} \tag{3}$$

Belowground biomass (BGB) was subsequently determined by multiplying ABG by a root: shoot (RS) ratio. This ratio was calculated using AGB and BGB values determined by Wotherspoon *et al.* [5] on the same TBI system, 6 years prior. Percent BGB was divided by percent AGB to determine the RS ratio and is displayed in Table 1. Total biomass (TB) (kg tree^{-1}) was then determined by combining AGB and BGB values.

Table 1 Root:shoot ratio determined by utilizing aboveground biomass (AGB) and belowground biomass (BGB) data derived on the same experimental site 6 years prior, in the Agroforestry Research Station, Guelph, Ontario, Canada (Wotherspoon *et al*. [5]).

Tree species	AGB (%)	BGB (%)	Root:shoot ratio
Norway spruce	73	27	0.37
Black walnut	82	18	0.22
White cedar	93	7	0.08
Red oak	74	26	0.35

Tree C content was determined by multiplying TB by carbon fractions of 48.8% for red oak and black walnut, and 50.8% for Norway spruce and white cedar. Carbon fraction values were provided by Thomas and Martin [13]. System-level carbon content (t C ha^{-1}) for each species was then determined by multiplying the average biomass C for each tree species by its relative planting density. Red oak and black walnut were planted at a density of 111 trees ha^{-1}, while Norway spruce and white cedar were planted at densities of 222 and 333 trees ha^{-1}, respectively.

2.4 SOC Calculation on a Per Hectare Basis

SOC stock (t C ha^{-1}) for each TBI system and the monocropped agricultural field was determined by incorporating bulk density and carbon percentages, and was calculated using Equation (4).

$$\mathrm{SOC\ (t\ C\ ha^{-1})} = [(10{,}000 \times 10{,}000\ (\mathrm{m^2})) \times \mathrm{Depth\ (m)}] \times [\mathrm{Bulk\ density\ (Mg\ m^{-3})}] \times [\mathrm{SOC\ (\%)}] \quad (4)$$

Average bulk density values (0-30 cm depth) for the measurement year 2018 were determined by Bazrgar *et al.* [11] on the same experimental site. Bulk density determination methods were reported in detail by Bazrgar *et al*. [11].

2.5 System-level Carbon Sequestration Calculation

Total system level C was calculated by combining the total C captured in both crop and tree biomass in addition to SOC. Historical crop biomass data was used to estimate the C content of soybeans planted on the monocropped field. The aboveground biomass data (minus grain yield) for soybeans was estimated at 1.71 t ha^{-1}, with the belowground biomass estimated to be 1.55 t ha^{-1} [14]. C concentration in the soybean leaf and stalk was assumed to be 43%, and therefore the monocropping system would have a C input of 1.40 t C ha^{-1} from soybean biomass. The tree component of the TBI system occupies 13% of the total agricultural land area, leaving 87% of the field to be planted with a crop component. Consequently, the potential soybean C input would be 1.22 t C ha^{-1} in established TBI systems [5].

2.6 Statistical Analyses

Treatments were analyzed for statistical parameters using R (2022.02.3 Version "Prairie Trillium"). Data was assessed for normality, equal variance and independence and an analysis of variance (ANOVA) was used to compare interactions between SOC influenced by tree species, and to compare TBI systems with the monocropped field. A Kruskal-Wallis test was used to assess statistical significance between SOC and distance in the TBI system as data was non-normal. Tree carbon content was log transformed to fit normality, as the dataset failed normal distribution. The level of statistical significance was assessed at $P < 0.05$. Post-hoc t tests, specifically Fisher's least significant difference (LSD), were used to compare different SOC rates and total system C between species. A tukey test was used to compare SOC between TBI systems and to a monocropped agricultural field.

3. Results

3.1 Soil Carbon

A total of 40 samples were used in the SOC analyses for the four tree species. The mean values of SOC for each species were calculated for distances of 2.0 m and 5.0 m. The total mean SOC was found to be approximately 77.3 (±9.8), 86.9 (±3.5), 71.83 (±3.3) and 97.9 (±9.2) t C ha^{-1} at 2.0 m and 78.9 (±2.3), 80.9 (±3.3), 72.6 (±2.5) and 88.4 (±3.4) t C ha^{-1} at 5.0 m from the tree row for spruce, walnut, cedar and oak, respectively. A Kruskal-Wallis test indicated that the interaction between SOC and tree species was significant (H value = 16.4415, $P < 0.001$). A LSD test indicated that there was a significant difference when comparing red oak SOC to Norway spruce and white cedar (Figure 2). The interaction between distance and plant species was not significant. There was no significant difference when comparing SOC in black walnut TBI systems to red oak, Norway spruce or white cedar systems.

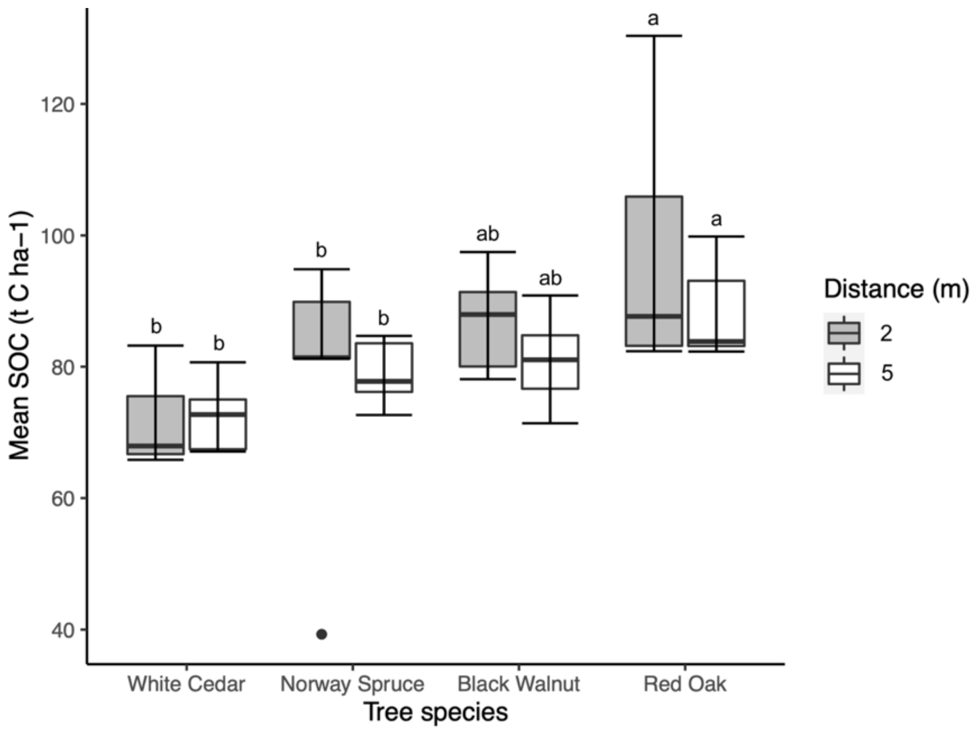

Figure 2 Mean soil organic carbon (0-30cm soil depth) (t C ha^{-1}) ± SE for four tree species in a 31-year-old tree-based intercropping system in southern Ontario, Canada at a distance of 2.0 m and 5.0 m from each tree (*n = 5*). [a, b] means with different superscripts between tree species are significantly different at $P < 0.05$.

Total SOC stocks (t C ha^{-1}) were calculated using soil bulk density obtained the same year on the same site [11] and by combining all SOC measurements across all distances for each tree species. This allowed for a comparison between total SOC for each tree species to be compared with total SOC from the neighboring monocropped agricultural field. Total SOC stock was 93.2 (±15.4), 83.9 (±7.9), 78.1 (±15.1), and 72.2 (±6.2) t C ha^{-1} for red oak, black walnut, Norway spruce and white cedar TBI systems, respectively. Total SOC stock for the monocropped agricultural field was 74.2 (±2.3) t C ha^{-1}. There was a significant difference between the monocropped field and red oak TBI system (P = 0.0037) and the black walnut TBI system (P = 0.0038), where oak and walnut TBI systems had significantly higher SOC (Figure 3).

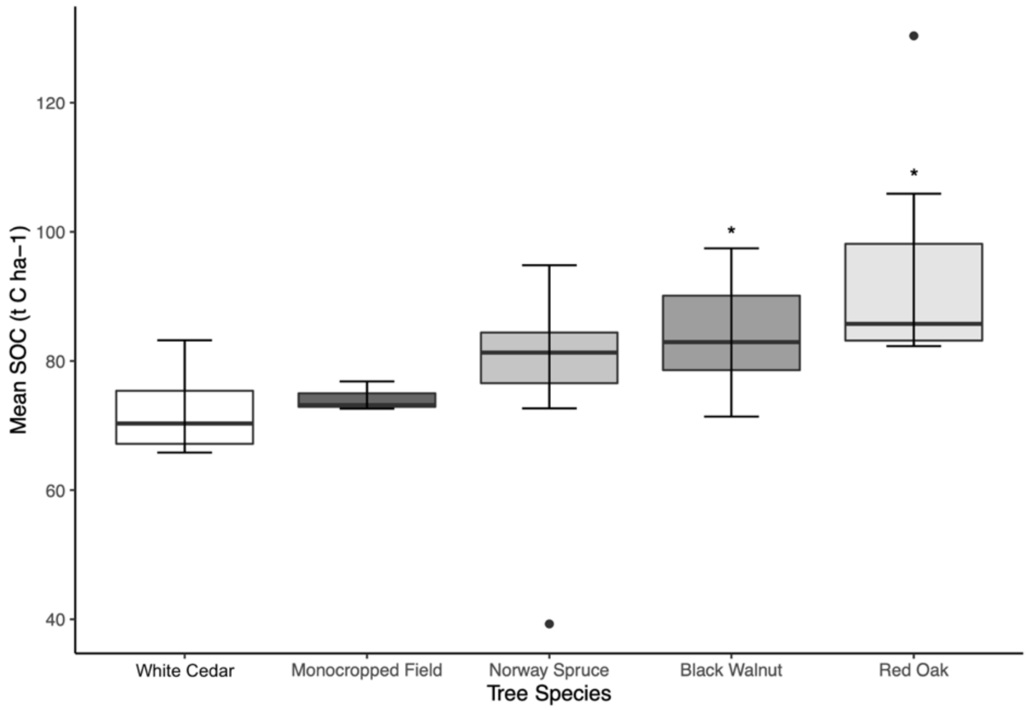

Figure 3 Mean soil organic carbon stocks (0-30cm soil depth) (t C ha^{-1}) ± SE associated with four different tree species in a 31-year-old tree based intercropping system and a soybean monocropping system in southern Ontario, Canada. * means with asterisks are significantly different from the mean SOC stock in the monocropped field at P < 0.05.

3.2 Tree Carbon Content

Overall, red oak had the highest mean C content (kg C tree^{-1}) followed by walnut, spruce, and cedar, with values of 299.7 (±17.7), 285.8 (±43.9), 255.4 (±10.8) and 70.1 (±12.8) kg C tree^{-1}, respectively. The ANOVA indicated that there was a significant difference between plant species (P < 0.0001). A t-test (LSD) determined that mean C content in white cedar was significantly different from Norway spruce, black walnut and red oak species. When C content was estimated in relation to planting densities (333, 222 111 and 111 trees ha^{-1} for cedar, spruce, oak and walnut, respectively), Norway spruce had the highest C sequestration potential on an area basis (per ha) followed by oak, walnut, and cedar with values of 56.7, 33.3, 31.7 and 23.4 t C ha^{-1}, respectively.

3.3 System-level Carbon Sequestration

Total system level C was 136.0 (±2.4), 127.6 (±2.0), 117.0(±4.9), 96.8 (±4.3) and 75.6 (±2.3) t C ha^{-1} for spruce, oak, walnut, cedar TBI systems and the soybean monocropped field, respectively. An ANOVA indicated that there was a significant interaction between system level C and the different cropping systems, ($P < 0.0001$). A t-test (LSD) determined that total system level C was significantly different between each individual cropping system (four TBI systems and one monocrop system) (Figure 4).

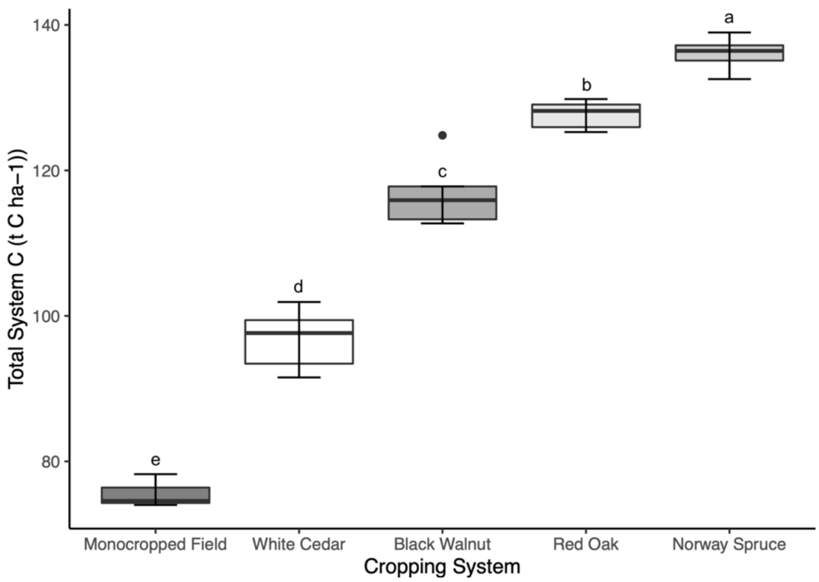

Figure 4 Mean total system C (t C ha^{-1}) ± SE for four tree species in a 31-year-old tree-based intercropping system in southern Ontario, Canada and a monocropped soybean field (*n = 5*). Total system C includes SOC stock and tree/soybean C content. [a, b, c, d, e] means with different superscripts between cropping systems are significantly different at $P < 0.05$.

4. Discussion

4.1 Soil Carbon

In red oak and black walnut TBI systems, SOC concentrations were numerically higher closer to the tree row (at 2.0 m) and reduced in the crop row (at 5.0 m), however, these values were not significantly different. The spatial variation of SOC for tree species oak, walnut, spruce, and cedar are not significant between a 2.0 m and 5.0 m distance from the tree row, which is in agreement with Wotherspoon *et al.* [5]. However, this contrasts with previous findings on the same site, where SOC was higher in locations closer to tree rows when compared to the crop interface in a 21-year old TBI system [10]. These findings can be explained by litterfall distribution patterns. In younger TBI systems, higher SOC concentrations in close proximity to the tree row are accredited to high organic matter inputs from tree leaf litter, which are rich in carbon and augment the SOC stock [10, 15, 16]. As trees mature and grow in height, litterfall is spread in an increasingly homogenous pattern [5], which may explain the spatial homogeneity found within the first 5 meters of the tree-crop interface in a mature 31-year-old TBI system.

Red oak, Norway spruce and black walnut TBI systems had higher SOC concentrations when compared to the monocropped agricultural field. These results are in agreement with previous studies done at the same site 13, 21 and 25 years after establishment [5, 9, 10]. Peichl *et al.* [9] reported that a monocropped agricultural system had 16.0 and 2.7 t C ha^{-1} less than hybrid polar (*Populus deltoides × Populus nigra* clone DN-177) and Norway spruce TBI systems, respectively, 13 years after establishment. Bambrick *et al.* [10] reported a monocropped agricultural system to have 6.3 t C ha^{-1} less than the hybrid poplar TBI system, 21 years after establishment. Lastly, Wotherspoon *et al.* [5] reported the monocropped system had 15.8, 12.7, 12.1, 7.2 and 5.8 t C ha^{-1} less than poplar, oak, cedar, spruce and walnut TBI systems, respectively, 25 years after establishment. SOC stocks continued to grow with red oak and black walnut sequestering 19.0 and 9.7 t C ha^{-1} more than the monocropped system, 31 years after establishment. Given the well-documented relationship between soil fertility and SOC [4, 7], increasingly large SOC stocks found within TBI systems demonstrate the potential of restoring crop productivity and sustaining soil fertility, contrary to monoculture systems.

4.2 Tree Carbon Content

Both above and belowground tree biomass are responsible for greater C inputs in TBI systems relative to conventional monoculture systems and thus have a higher capability to sequester atmospheric CO_2. In previous studies on the same site, fast-growing hybrid poplar species sequestered almost twice as much C relative to slow growing coniferous species Norway spruce and white cedar 13 years after establishment [9]. However, high assimilation rates associated with fast-growing species began to plateau when these species reached maturity and experienced significant die back [5]. Now, 31-years after establishment, the slow growing coniferous species, Norway spruce, had the highest tree C content relative to oak, cedar and walnut systems with a total of 56.7 t C ha^{-1} when taking into account planting density. This was unexpected as not only is spruce a slow-growing coniferous species, it was also measured to have the lowest sequestered C in both previous studies [5, 9]. In the TBI system, spruce had the lowest recorded C sequestered in above and belowground tree components at 6.4 t C ha^{-1} 13 years (year 2000) and 13 t C ha^{-1} 25 years (year 2012) after establishment when planted at a density of 222 trees ha^{-1}. However, over time, it is interesting to note higher C sequestration in Norway spruce. While other species such as poplar may begin to experience a plateau in assimilation rates as they become senescent, Norway spruce has yet to mature and is continuously sequestering higher amounts of C.

Another noteworthy trend observed in this study was the C content accumulation in cedar trees. In the TBI system, cedar had very minimal mean biomass growth from the last time measured by Wotherspoon *et al.* [5] (measurement year 2012), increasing from 93.9 to just 138 kg $tree^{-1}$ (measurement year 2018), but still had a significant increase in the amount of C sequestered, increasing from 16.0 to 23.4 t C ha^{-1}. A similar trend can be observed with red oak TBI system, which has continued to sequester higher amounts of tree C content likely due to its slow-growing and long-lived nature [17]. Therefore, long-lived species such as spruce, red oak, and cedar will continue to sequester atmospheric CO_2 until harvested (at around 70 years or longer), however short-lived species should be harvested before experiencing die back, with a second cycle of trees planted around 10 years after the initial planting to ensure continuous C sequestration [5]. This information can be utilized to construct effective TBI management systems by selecting appropriate tree species depending on the objective of a particular system. Management strategies such as relay or staggered planting could be utilized, which would combine the benefits of both short-term and long-term C storage.

4.3 System-level Carbon Sequestration

To determine total system level C storage in the TBI system SOC, above and belowground tree and soybean C content were combined. Tree density was maintained at their established planting densities (333, 222, 111 and 111 tree ha^{-1} for cedar, spruce, oak and walnut, respectively) rather than standardizing density because species like Norway spruce and white cedar have an inherent advantage as they can be planted at higher densities since their vertical branching structure does not compete with the crops for sunlight. Results from this study reported a higher C pool in all TBI systems compared with the monocropped system, 31 years after establishment. Spruce, oak, walnut and cedar TBI systems have sequestered 80, 69, 55 and 28% more C at the system level than the soybean monocropped system, respectively.

Results from this study align with studies done on the same site in previous years. Peichl *et al*. [9] found that the poplar TBI system had 16.0 t C ha^{-1} more than a barley monocropped system, 13 years after establishment. Wotherspoon *et al.* [5] found that, at a tree density of 111 trees ha^{-1}, hybrid poplar, white cedar, red oak, black walnut sequestered 42.3, 28.3, 28.1, 20.4 and 20.2 t C ha^{-1} more than a soybean monocrop, 25 years after establishment. Although total C was highest in the poplar TBI system, which is in agreement with previous studies [7, 9], an abatement in net assimilation rates was observed, possibly due to tree age. However, slow-growing species such as Norway spruce were found to add continuously larger amounts of carbon as they continued to mature [5].

While earlier studies provide evidence toward high initial C sequestration from fast-growing species such as hybrid poplar, this study highlights the importance of incorporating slow-growing spruce and oak species for long-term C storage. This may suggest that management strategies such as relay planting, where there is a secondary planting around 10 years after the initial TBI establishment, may provide continuously high C sequestration rates as the TBI system matures. This would allow for each species to be contributing maximum system C sequestration benefits to the TBI system.

4.4 Further CO_2 Reduction through TBI Systems

When assessing the potential atmospheric CO_2 reduction through TBI systems, it is important to note that this study only provides insight to direct benefits associated with C storage. There are a multitude of indirect benefits that intercropping systems provide as well, including; reducing demand for clear-cutting and fertilizer consumption, lessening CO_2 emissions by replacing wood for fossil fuels and intensifying long term C storage in wood products [4, 8]. In fact, it has been estimated that these indirect benefits can increase C storage by 2-15 times in TBI systems [18].

There is a growing interest in the utilization of biomass for bioenergy, specifically with willow (*Salix* spp.) as an intercrop. The perennial nature of bioenergy crop production is associated with lower greenhouse gas emissions, as it counterbalances CO_2 from fossil fuel emissions by using bioenergy to generate an equivalent amount of energy. Biomass crops could represent a sustainable long-term alternative to fossil fuels [19], and have been found to grow successfully as an intercrop in TBI systems [11, 20].

5. Conclusions

This study was the first to investigate how C pools in SOC and tree C content at the system-level change between a 13, 25 and 31-year-old TBI system compared to a conventional agricultural system. Reported C pools should inform future TBI systems regarding which tree species to incorporate to maximize both short-term and long-term C sequestration. The integration of trees into conventional agricultural lands provides carbon storage in both above and belowground biomass, as well as SOC, which increases carbon storage relative to monoculture systems. While earlier studies on the same site suggested that fast growing species had the highest rates of C sequestration, results from this study suggest that the longer-lived but slow growing species should be incorporated into TBI systems for long term C storage while incorporating fast-growing species which can provide high initial C sequestration and potential revenue sources for landowners. However, the fate of trees and their associated harvested wood products will be important in determining the true C sequestration potential of a TBI system, with long-lived products ensuring continual C storage. Results from this study may help landowners choose different tree species depending on site-specific goals. Accurately quantifying the sequestration potential of TBI systems is critical in their adoption in temperate regions and for determining effective natural climate solutions. Implementing TBI systems across Canada's agricultural lands would not only aid in remediating degraded lands and alleviating pressure on forested regions, but would also contribute to Canada's Climate Change mitigation strategy.

Author Contributions

Made contributions to data analysis, formal analysis, methodology and writing (original draft and review and editing): Ijzerman, M.M., Bazrgar, A.B., Gordon, A.M., Thevathasan, N.V. All authors have read and agreed to the published version of the manuscript.

Funding

This project was funded by the Government of Canada's Agricultural Greenhouse Gas Program (AGGP - 2).

Competing Interests

The authors have declared that no competing interests exist.

Additional Materials

The following additional materials are uploaded at the page of this paper.

1. Table S1: Soil carbon raw data for tree species Norway spruce (NS), black walnut (BW), white cedar (WC) and red oak (RO) collected in 2018 in the Agroforestry Research Station, Guelph, Ontario, Canada.
2. Table S2: Tree carbon content data for tree species Norway spruce (NS), black walnut (BW), white cedar (WC) and red oak (RO) collected in 2018 in the Agroforestry Research Station, Guelph, Ontario, Canada.
3. Table S3: Allometric coefficients from Ter-Mikaelian and Korzukhin (1997).

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