System level carbon sequestration by riparian buffer systems as influenced by soil texture, vegetation type and age in southern Ontario

by

Sowthini Vijayakumar

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ABSTRACT

System level carbon sequestration by riparian buffer systems as influenced by soil texture, vegetation type and age in southern Ontario

Sowthini Vijayakumar
University of Guelph, 2019

The effect of soil texture, vegetation type and age on system level carbon (C) sequestration in eight riparian buffer systems (RBS) in southern Ontario was investigated. Biomass C sequestration was up to three times (247 Mg C ha\(^{-1}\)) greater in deciduous buffers than in coniferous buffers (100 Mg C ha\(^{-1}\)) regardless soil texture and age. Mature deciduous tree buffers in clay soils had the highest soil organic carbon (SOC - 177.62 ± 6.193 Mg C ha\(^{-1}\)) while the young coniferous buffers in loam soil had the lowest (94.71 ± 6.193 Mg C ha\(^{-1}\)). SOC sequestration (154 Mg C ha\(^{-1}\)) was significantly higher (p<0.05) in mature buffers compared to adjacent agricultural fields (88 Mg C ha\(^{-1}\)), irrespective of soil texture and vegetation type. All RBS had ~66% of SOC in the heavy fraction, indicating stable SOC. Soil NH\(_4\)^+-N was the predominant inorganic N source in all RBS.
ACKNOWLEDGEMENT

I express my sincerest gratitude to my advisor, Professor Naresh Thevathasan, for his financial and personal support, guidance and patience during the course of my study. His knowledge, encouragement, positive attitude and kindness all have helped me to complete this study on-time. For this, I say a ‘big thank you’!

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CHAPTER ONE
GENERAL INTRODUCTION

1.1 BACKGROUND

Climate change resulting from global warming is caused by increasing atmospheric concentrations of greenhouse gases (GHGs). The key GHGs are carbon dioxide (CO$_2$), nitrous oxide (N$_2$O), methane (CH$_4$), ozone (O$_3$) and water vapor (IPCC, 2007). Worldwide, CO$_2$, CH$_4$ and N$_2$O accounts for 74%, 16% and 9% of the GHGs emissions, respectively (EPA, 2008). The increased concentration of CO$_2$ in the atmosphere is being enhanced by anthropogenic activities such as burning of fossil fuel and deforestation (IPCC, 2007). During last century, conversion of land use from forest and grasslands to intensive agricultural cropping systems has also contributed to the increase in atmospheric CO$_2$ (Lal et al., 1998). Atmospheric CO$_2$ concentrations continue to rise at a rate of 2 ppm y$^{-1}$ or 4.4 Pg C y$^{-1}$ (IPCC, 2007) and it reached 411 ppm in the month of May 2018 (Scripps Institution of Oceanography, 2018).

Climate change mitigation refers to efforts reduce the level of GHG in the atmosphere by converting it into stable carbon (C) sinks such as forest biomass or establishing new C sinks through vegetation management (IPCC, 2007). This strategy for reducing CO$_2$ concentrations from in atmosphere is referred to as C sequestraiton. In this context, the role of trees has been recognized as a significant tool to capture and sequester atmospheric CO$_2$ in plants and soils (Griscom et al., 2017).

Further, improved soil management practices and soil C sequestration enhancement are also very helpful towards reducing the undesirable impacts of anthropogenic activities on global warming, soil degradation and desertification. This can be achieved by best management practices (BMPs) that include adoption of agroforestry. Agroforestry land-use systems are
considered as C accumulation systems particularly under the afforestation and/or reforestation activities promoted by the Kyoto Protocol (the international agreement made in 1997 to reduce emissions). Therefore, industrialized and developing countries have gained interest in agroforestry systems, which is accepted as having the most potential for C sequestration (Albrecht and Kandji, 2003; Sharrow and Ismail, 2004; Makundi et al., 2004; Haile et al., 2008; Schoeneberger et al., 2017). In support of the above and within the context of climate change, Schoeneberger et al. (2017) has released a policy paper entitled “Agroforestry: Enhancing Resiliency in U.S. Agricultural Landscapes Under Changing Conditions”, in order to emphasize the need for wider adoption of agroforestry land-use systems in the USA. However, wide adoption in Canada, in particularly in Ontario, is lacking. Studies of this nature will contribute to the knowledge gap and thereby assist conservation authorities, such as the Grand River Conservation Authority (GRCA) to implement riparian buffer systems [RBS] (agroforestry) in Ontario.

In this context, previous studies have stated that RBS, a form of agroforestry where strips of perennial plants such as meadows, shrubs and trees, are mainly used to control non-point source pollutants reaching the waterways, also have the potential to sequester atmospheric CO₂ both in biomass and in soil. RBS and their contributions to enhance water quality issues have been studied in detail over the previous two decades. However, there is a lack of literature related to system level (aboveground and belowground and in soil) C sequestration in RBS in Ontario, especially, as influenced by the factors such as, vegetation age, type and soil texture. Furthermore, an adequate quantification of C sequestration is required in order to assess the influence of various vegetation types, ages and soil types on system level C sequestration. Although riparian areas in southern Ontario only comprise a small geographical area, they
represent an extremely important component of the overall landscape. This research activity is therefore proposed to quantify riparian buffer C sequestration potential as influenced by buffer design (vegetation type, tree age-class, and soil textural class) within the GRCA watershed.

In addition, significant amounts of nutrients [readily available nitrogen source especially nitrate (NO$_3^-$) and ammonium (NH$_4^+$)] via surface and sub-surface flows enter the buffered area from adjacent agricultural fields. Based on the perennial vegetation, age and soil type, uptake of these nutrients by the riparian vegetation and the residue nutrients trapped in the buffered region could significantly influence GHG emissions, especially N$_2$O. However, the presence of microbial organisms responsible for N cycling coupled with soil C inputs in the buffered region via litterfall, fine root turnover, through fall and stemflow could also contribute to enhanced soil C sequestration within the RBS. Hence, this study will also focus on inorganic N concentrations (NO$_3^-$ and NH$_4^+$) within the different RBS in order assess residue inorganic N presence within the types of tested RBS.

Further, stabilization of SOC can be influenced by the soil texture, vegetation type and vegetation age, since these factors play major role in the decomposition of SOM. Therefore, this study also will focus on the SOM fractions, namely light fraction (LF) and heavy fraction (HF) determination. Hence, it can provide information on the effect of RBS on SOC stabilization.

1.2 RESEARCH CONTEXT

The research conducted for this study is a component of a larger AGGP project entitled “Riparian buffer plantings: An agroforestry land-use for greenhouse gas mitigation including multiple benefits to Canadian agricultural systems”. The ultimate goal of the AGGP project is to develop the strategies to mitigate global warming by reducing GHGs emissions through the adoption of riparian buffer systems. The overall AGGP project objectives are: a) to assess soil
nutrient dynamics (N and P) in soils within riparian buffer and understand their influence on GHG emissions, b) characterize soil microbial communities associated with N cycling and their influence on GHGs, c) characterize soil macro faunal diversity (earthworms) and its influence on CH$_4$ emission; d) assess RBS biodiversity and its effects on ecosystem processes and GHG emissions (CO$_2$, CH$_4$ and N$_2$O), and e) Quantify C sequestration in RBS perennial vegetation and soils and the develop C sequestration models for linear length (km) of RBS. Net annual C sequestration models for young and mature RBS will be created by undertaking system level assessments by taking into account both GHG emissions (negative C sequestration or C source) and annual C sequestration (positive C sequestration or C sink) in order to deliver verified and quantified results to demonstrate that RBS are ‘net’ C sinks and not C sources within the Grand River watershed as influenced by age, species and soil types. The research team of the project includes professors and graduate students from the University of Guelph, Waterloo, Toronto and Sherbrooke. Supporting organizations includes GRCA, Ontario Soil and Crop Improvement Association (OSCIA) and Biomass North Development Centre (NBDC) (Northern Ontario First Nation communities).

1.3 RESEARCH OBJECTIVES

The research conducted in this study has the following objectives:

1. To quantify system level C as influenced by soil textures (clay, loam), vegetation types (coniferous, deciduous) and age classes [young (<15 years), mature (≥15 years)].

2. To compare land use influence on SOC sequestration potential within RBS and in adjacent agricultural lands.

3. To compare the effect of soil depth (0-30 cm vs. 30-60 cm) on SOC in RBS.
4. To evaluate the stabilization of SOC by quantifying organic C in the light fraction (OC$_{LF}$) and heavy fraction (OC$_{HF}$) in RBS as influenced by vegetation type and age, and in comparison, to an adjacent agricultural field.

5. Quantification of soil inorganic N (NO$_3^-$ and exchangeable NH$_4^+$) concentrations as influenced by soil texture, vegetation type and age.

We hypothesize that

1. System level C sequestration will vary among the two different soil types, vegetation types and vegetation age in RBS.
2. SOC sequestration will be influenced by land use (RBS vs. adjacent agricultural land)
3. SOC concentration will vary with depth.
4. SOM fractions (LF and HF) and its stabilization in RBS will be influenced by vegetation type and age.
5. Soil type, vegetation type and age will influence the inorganic nitrogen (NO$_3^-$ and NH$_4^+$) concentration in soils of RBS.

1.4 RESEARCH DESIGN AND SITE DESCRIPTION

1.4.1 STUDY AREA

The study was conducted on selected riparian buffer systems within the Grand River watershed (GRW), southern Ontario, Canada. The GRW includes all the land drained by the Grand River and its tributaries and comprises 6,800 square kilometres (2,800 square miles). GRW is the largest watershed in southern Ontario and includes the cities of Brantford, Cambridge, Guelph, Kitchener and Waterloo. Cities, towns and villages make up about five per cent of the land. It is also an intensive agricultural area, with farms making up about 70 per cent
of the watershed. Four major rivers empty into the Grand River; the Conestogo, Nith, Speed and Eramosa and the combined length of all of the rivers and streams is about 11,000 kilometres.

Table 1: Sampling sites

<table>
<thead>
<tr>
<th>Vegetation Age</th>
<th>Vegetation Type</th>
<th>Soil Texture</th>
<th>Site No</th>
<th>Site Name</th>
<th>Soil Series</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature</td>
<td>Deciduous</td>
<td>Loam</td>
<td>2</td>
<td>Moorefield 1</td>
<td>Huron</td>
</tr>
<tr>
<td></td>
<td>Clay</td>
<td>8</td>
<td>Conostogo 2</td>
<td>Huron</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coniferous</td>
<td>Loam</td>
<td>5</td>
<td>Guelph Lake 1</td>
<td>Burford</td>
</tr>
<tr>
<td></td>
<td>Clay</td>
<td>7</td>
<td>Conostogo 2</td>
<td>Huron</td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>Deciduous</td>
<td>Loam</td>
<td>11a</td>
<td>Weber Property</td>
<td>Huron</td>
</tr>
<tr>
<td></td>
<td>Clay</td>
<td>13b</td>
<td>Alblas Property</td>
<td>Burford</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coniferous</td>
<td>Loam</td>
<td>11a</td>
<td>Weber Property</td>
<td>Huron</td>
</tr>
<tr>
<td></td>
<td>Clay</td>
<td>13b</td>
<td>Alblas Property</td>
<td>Brford</td>
<td></td>
</tr>
</tbody>
</table>

Site No 2: - RBS with deciduous trees, age of 37 years, is dominated by sugar maple (*Acer saccharum*), ash (*Fraxinus excelsior*), hawthorn (*Crataegus monogyna*), white elm (*Ulmus americana*), black cherry (*Prunus serotina*), dogwood (*Cornus florida*). Adjacent agricultural field was cultivated with soybean crop at the time of sampling. Loam textured soil.

Site No 5: - RBS with coniferous trees planted in 1983 (34 years), dominated by eastern white cedar (*Thuja occidentalis*) and buckthorn (*Rhamnus cathartica*). Some white pine (*Pinus strobus*) trees are present, and poplar (*Populus spp.*) trees towards downstream. Loam soil texture. Adjacent agriculture field had soybean at the sampling time.

Site No 7: - RBS having mature coniferous trees with clay textured soil. Currently dominated by white pine trees with understory shrubs namely, hawthorn, buckthorn and dogwood species.
Establishment year of the RBS is not known, however according to the diameter of the tree trunk and the height it was estimated to be about 60 years.

**Site No 8:** - RBS with deciduous trees which was dominated by sugar maple, black cherry, bur oak (*Quercus macrocarpa*), white elm, ash spp., and some invasive buckthorn. Trees were estimated to be close to 60 years. Clay textured soil. Adjacent cultivated land was wheat at the time of the study.

**Site No 11:** - This site has both deciduous and coniferous RBS in different sections of the buffer. Established in 2011 (6 years). Sampling was done in both deciduous (Named as 11a) and coniferous (Named as 11b) RBS. RBS with deciduous trees is dominated by silver maple (*Acer saccharinum*) trees whereas, RBS with coniferous trees is dominated with white spruce (*Picea glauca*) trees. Loam textured soil. Adjacent agricultural land was cultivated with wheat at the time of the study.

**Site No 13:** - This site also has both deciduous and coniferous RBS in different sections of the buffer, which was established in 2009 (8 years). Clay textured soil and sampling was done in both RBS. Deciduous RBS is dominated with Manitoba maple (*Acer negundo*), black walnut (*Juglans nigra*) and dogwood (Named as 13a) whereas, Coniferous RBS is dominated by white spruce and white pine trees. Adjacent agricultural land had clover cover crop at the time of sampling.
1.4.2 EXPERIMENTAL DESIGN AND SAMPLE COLLECTION

Soil samples were collected from 5th of July 2017 to 8th of August in 2017 and 21st of June in 2018. A network of replicated riparian buffer treatments (n = 3) comprising of a factorial array of 2 tree types (coniferous vs. deciduous) x 2 tree age classes (<15 years (young) vs. > 15 years (mature)) x 2 soil texture classes (clay vs. loam) were identified within the GRW, southern Ontario. In each replicated riparian buffer treatment, a research area of 150 m² (5m x 30m) along the stream was identified and was divided into ten equal plots (5m x 3m = 15m²). Five plots (pseudo replicates) were selected randomly from within the study area for perennial vegetation and soil data collection. With respect to all treatment combination, 8 sites were selected (4 mature sites and 4 young sites) within the GRW. Adjacent agricultural fields associated with each selected RBS were set for soil sample collection. Adjacent agricultural fields were treated...
as control treatment for soil C comparisons with RBS. Transect in the agricultural land was laid out perpendicular to the RBS due to the slope effect. RBS are established in the low land along the streams whereas the agricultural lands were located away from the stream in the upland. Therefore to reduce the slope effect on C analyses, several soil samples (0-30 cm, 3 samples; 30-60 cm, 3 samples) were collected along the transect in the agricultural land, and they were pooled to obtain 5 samples (for each soil depth 3 soil samples were pooled to obtain one sample per each location along the transect) per site for C analyses.

All riparian systems that were included in this study were established on previous agricultural lands. Therefore, the adjacent agricultural lands were considered as the control plots and representing the soils in the RBS prior to establishment. This allowed study of the carbon changes resulting from the conversion of agricultural lands RBS. The comparison with adjacent agricultural lands was done in order to quantify the impact of RBS on carbon sequestration in watersheds and their potential role towards climate change mitigation.
2.1 Riparian buffer systems (RBS)

2.1.1 Definition:

RBS, one of the temperate agroforestry systems, is defined as an area of trees and/or shrubs and other vegetation established on agricultural lands adjacent to a body of water and managed to maintain the integrity of streams and water quality from non-point source pollutants (Palone and Todd, 1997). RBS is known for its variety of ecosystem services it provides and it also can be used as one of the best management practices (BMPs) to sequester C in above and below ground vegetation and in the soil (Thevathasan et al., 2004).

2.1.2 Basic Structure of the RBS

Figure 2: Aerial view of the selected locations within the Grand River watershed (GRW), Ontario, Canada.
According to the guidelines developed by the U.S. Department of Agriculture (USDA) Forest Service (FS) and the USDA Natural Research Conservation Service (NRCS), streamside forest buffers have three distinct zones (Figure 2) (Welsch, 1991).

Zone 1: The recommended width of this zone is 5m wide (minimum width) with undisturbed matured trees starting from the edge of the streambank. This provides final filtering of materials moving through the buffer, shade, organic matter input, stream bank stability and stable ecosystem (Schultz et al., 2000; Swanson et al., 2017). This zone consists of native trees and shrub species, and tree removal is not acceptable in this zone to maintain the buffer strip effectiveness (Welsch, 1991).

Zone 2: Adjacent to zone 1, zone 2 has at least 18m width of managed trees to high infiltration of surface runoff, nutrient assimilation by plants while providing organic matter input for microbial processing of agrochemicals. This zone mainly provides the required space for the uptake and removal by plants, and microbial degradation of incoming non-point source pollutants like N, P and S. Growing of N fixing plant is not recommended since one of the major functions of this zone is to remove NO$_3^-$ (Schultz et al., 2000). Periodic removal of woody plant sinks is essential to encourage new plant growth, thereby increases the nutrient uptake in this zone (Welsch, 1991).

Zone 3: The minimum width of this zone is 6m consist of grazed or non-grazed grass to alter the concentrated upland flow to sheet flow. Hence, this zone filters sediments and enables water and agrichemicals to infiltrate into the rooting zone. This process helps the vegetation and microbes for their nutrient uptake and removal (Schultz et al., 2000). Controlled grazing and haying can be allowed when the structure of the zone is not disturbed (Welsch, 1991).
2.1.3 Functions of Riparian buffer systems

RBS carry out several functions in the environment while being between the terrestrial and aquatic ecosystem (Kozlowski, 2002). Even though they occupy an area as small as one percentage of the land area of a watershed (Alpert et al., 1999), these ecosystems are most protective in the watershed (Chaney et al., 1990). Some of their major functions are;

i. Filtering and retaining sediment: Large amount of suspended sediments and associated nutrients can be removed by RBS, which enter from the uplands through overland flow and from flooded waters (Correll, 1996; Mankin et al; 2007). Palone and Todd (1997) have reported 40 to 95% of sediment removal from 30m wide mature buffers.

ii. Immobilizing, storing and transforming chemical inputs from upland; several studies have shown the ability of the RBS to store, immobilize and transform residual chemical inputs from upland specially from fertilized adjacent agricultural fields. Mature buffers 30 m in width can remove nitrogen (N) and phosphorus (P), up to 68 to 92% and 70 to 81%, respectively, while the lowest level of N and P removal ranges from 15 to 54% and 24 to 50 %, respectively (Palone and Todd, 1997).

iii. Controlling stream environments; the light, temperature, size and shape of the channel can be controlled by riparian buffers. Gregory et al (1991), have suggested that riparian vegetation controls quantity and quality of solar radiation entering the water surface, thereby it impacts instream algae production, water temperature and O₂ content.

iv. Provides water storage and recharge of subsurface aquifers; riparian vegetation zones slowdown the flood flows by letting the water to spread and soak in to the soil thus recharging the ground water and outspreading the baseflow. Palone and Todd (1997)
indicated that forests can capture and absorb 40 times more rainfall than disturbed agricultural soils and 15 times more than turfgrass or pasture soils.

v. Provide terrestrial habitat; RBS provide habitat for both animals and plant in the both aquatic and terrestrial ecosystems since they are established in between both aquatic and terrestrial landscape (Gregory et al., 1991).

vi. Provide economic and social benefits; RBS can produce many products for marketing purposes like high quality wood and fiber, high quality forage for livestock and numerous recreational activities such as, fishing, hunting, boating, hiking, camping and picnicking can be enhanced by the communities. Besides, nature appreciation and relaxation can be enjoyed within the communities living in the watershed area (Palone and Todd, 1997).

vii. Carbon sequestration in both, soil and in biomass; diversified plant species can be established in the RBS, which can positively contribute to fix C in the biomass by absorbing atmospheric CO₂ and also in the soil through the decomposition of residue inputs. This process indirectly helps to mitigate climate change (Capon et al., 2013; Fortier et al., 2010; Oelbermann and Raimbault, 2015).

2.2 System level carbon sequestration (carbon storage in the aboveground biomass, belowground roots and in the soil) by riparian buffers:

Carbon sequestration implies CO₂ removal from the atmosphere and conversion into long-lasting pools such as in trees and soils (Lal et al., 2004). In RBS, system level C sequestration means; C fixed in the vegetation (i.e., aboveground biomass like leaves, trunks, branches etc. and below ground biomass like roots) and in the soil. Riparian zones have the potential to lessen climate change through C sequestration in vegetation biomass (Capon et al., 2013). As this study is being conducted within the Grand River watershed (GRW), any new
establishment of RBS by the GRCA should therefore, contribute to long-term C sinks. For example, it has been reported that RBS established on former agricultural lands with poplar hybrids (*Populus* spp.) and switchgrass (*Panicum virgatum* L.) sequester 3000 and 800 kg C ha\(^{-1}\) yr\(^{-1}\), respectively (Schultz et al., 2004).

Fortier et al. (2010) conducted research in RBS in Canada to assess their ability to sequester C in the aboveground biomass and they have suggested that hybrid poplar has the potential to produce more biomass in the RBS when compared with when they are grown in a plantation established in upland soils (Fortier et al., 2013). In addition, belowground biomass in RBS also has been quantified to be eight times higher than the belowground biomass in adjacent crop fields (Schultz et al., 2004). Research in belowground C sequestration in RBS in roots and soils (Fortier et al., 2013) have all provided excellent information on RBS’ ability to sequester C in selected sites in Canada. Further, studies conducted in the USA showed that the system level C sequestration potential of RBS is nearly 1.5 Mt C y\(^{-1}\) (Kimble et al., 2003), and they have stated that the maximum percentage of C is seen in the biomass in living trees. It is also assessed that soil C accumulation in buffer zones is about 10 times higher than agricultural lands.

**2.3 Effect of soil texture, vegetation type and age on carbon sequestration**

C sequestration depends on several factors including but not limited to, climate, environmental conditions, soil texture, vegetation type and age (Burke et al., 1989). C sequestration in trees is directly influenced by biomass production rate of trees, which depends on the morphology of trees, soil fertility, soil parameters, climate condition and water availability (Grote, 2002).
2.3.1 Vegetation type and age

Forest vegetation can be classified into two major types; deciduous and coniferous trees. Any tree that drops its leaves in the fall and goes dormant during cold weather referred to as deciduous. They produce new leaves when the weather warms. Process of shedding unneeded parts is known as abscission. The trees survive winter weather conditions conserving water through abscission. Coniferous trees are often referred to as evergreens, and the name is associated with trees that have needles instead of broad, flat leaves and they reproduce via cones.

Deciduous and coniferous trees have different morphology and growth habits, hence C sequestration by these tree species will also differ significantly. Hansen (1993) stated that hybrid poplar showed significant increase in C sequestration as they aged when compared to other deciduous trees. With respect to atmospheric C sequestration by deciduous and coniferous trees, Black et al. (2000) stated the C sequestration of a boreal deciduous forest in Canada in 1994, 1996, 1997 and 1998 were 144, 80, 116 and 290 g C m⁻² y⁻¹, respectively. Whereas Vetter et al. (2005) estimated 1.51 t C ha⁻¹ y⁻¹ of total biomass C sequestration in coniferous trees.

Several studies have stated that C sequestration potential by RBS in the aboveground biomass was 2.46 Mg C ha⁻¹ y⁻¹ (Boggs and Weaver 1994; Harner and Stanfoord 2003; Naiman et al. 2005). Furthermore, Hazlett et al. (2005) has reported 269 Mg ha⁻¹ C sequestration in Canada, while lower C sequestration was reported by Schroeder (1994) for another temperate riparian buffer (63 Mg C ha⁻¹).

2.3.2 Soil texture

The proportions of sand, silt and clay in the fine earth fraction is referred to as soil texture. A study conducted by Burke et al. (1989) in U.S grassland soils stated that SOC content increases with increasing clay content. However, Percival et al. (2000) mentioned that SOC
showed small variation with increasing clay content. It has also been stated that increased clay content can proportionally reduce the porosity resulting in low oxygen and high-water content in soils (Brady and Weil, 2010). These features seen in clay soils could possibly reduce the decomposition of organic matter and result in comparatively high SOC in comparison with other soil textures.

Clay textured soils have relatively more surface area than loamy soils this can lead to form higher organo-mineral complexes with the soil matrix. In addition, physicochemical protection is a major process which helps to retain SOC for long-term. Through which SOC stabilization is triggered by sorption into mineral surfaces, occlusion within aggregates and deposition in soil pores. Hence, clay soils have higher potential to protect the SOM from microbial degradation (Jastrow et al., 2006). Furthermore, aggregate formation is documented higher in clay soils which could also possibly delay SOM decay through the physical protection (Six et al., 2002).

2.4 Soil C depletion and release of GHGs

Globally, soil comprises of 2500 gigatons (Gt) of soil C including 1550 Gt of soil organic C (SOC) and 950 Gt of soil inorganic C (SIC) (Lal et al., 2004). Around 60% of the SOC pool tends to be depleted in the alteration of natural to agricultural ecosystems from the temperate region soils, and 75% in the tropics. However, due to continuous inputs to the SOC pool, the SOC pool is at an equilibrium considering the gains and losses. This equilibrium gets altered when the soil C outputs surpasses the inputs. Lal et al. (2004) have stated that some soils can lose up to 20 to 80 tons C ha\(^{-1}\) y\(^{-1}\), typically released into the atmosphere. This detrimental effect of soil degradation has exacerbated projected global warming potentials (Lal et al., 2004). Therefore, consideration of soil C sequestrations through judicious land use and BMPs are very
important. Mulch farming or crop residue inputs, conservation tillage, agroforestry and diverse cropping systems are some suggested BMPs for SOC sequestration (Lal et al., 2004).

Therefore, the RBS established within conservation authorities’ watershed areas can contribute to significant amount of landscape level C sequestration in Ontario’s watersheds. For example, GRCA is establishing close to 20 new riparian buffer plantings per year under the rural water quality program (Anne Loefler, personal communication. 2018). In addition, the length of degraded agricultural streams is \(~11,000\) km, and if we plant trees on both sides of the stream banks, potentially 22,000 km of RBS can be established just within the GRCA watershed area in order to derive landscape level impact.

2.5 Soil organic matter and its fractions

Soil organic matter (SOM) is a component of soil, consists of microbes and their by-products, plant and animal residues in various stages of decomposition, and humus (Paul and Clark, 1996; Brady and Weil, 2010). SOM undergoes a series of physical, chemical and biological transformation processes that cause complex interactions between soil minerals and SOM. Soil contains relatively smaller concentration of SOM specifically 1 – 10% of the total soil mass, of which only 58% is SOC (Brady and Weil, 2010).

Regardless of smaller concentration in the soil mass, SOM plays several beneficial functions in the soil. Soil organic matter is essential as it influences all other soil properties (physical, chemical and biological) except texture. Increased SOM amplifies many beneficial soil characteristics, such as improves bulk density, porosity, soil permeability, water holding capacity, aggregation and cation exchange capacity (Thevathasan et al., 2014). In addition, it is also important for supporting soil micro flora and fauna by providing nutrients and energy for their activities, enhances nutrient availability to crops not only as a source of nutrients but also
by chelating effects of micro nutrients there by also reducing metal toxicity to the plants (Doran and Parkin, 1994).

Further, SOM also plays a vital role in the global C cycle, especially with respect to climate change. The soil C pool is estimated to be two times larger than the C stored in the atmosphere and three times than in terrestrial vegetation (Lemus and Lal, 2005) therefore, management of SOC can be considered as one of the strategies to mitigate climate change (Lal, 2004). Depending on the inputs and the outputs of C, soil can act as sink or source of C. When inputs surpass the outputs, soil becomes a sink and vice versa. Reduced soil C level is a result of low SOM inputs and poor management practices (Lemus and Lal, 2005). With proper management strategies SOC level can be improved, which can lead to climate change mitigation. However, this depends on several factors including soil properties, crop types, cropping practices, tillage practices and climate (Lal, 2004; Gauder et al., 2016).

Decomposition of fresh crop residues by the soil microbial biomass results in organic C being oxidized to CO\textsubscript{2} or stabilized as humified organic matter. To better understand carbon stabilization in soil, scientists have developed multicompartment turnover models such as ROTHC (Jenkinson and Rayner, 1977) and CENTURY (Parton et al., 1987). They have classified the SOM into three compartments or pools in terms of C mineralization rates, stabilization and mean residence times (van Veen and Paul, 1981; Paustian et al., 1992). The CENTURY model shows SOC three kinetic pools with varying turnover times. There is an ‘active’ SOM pool with much lower turnover time (1 – 5 y) than other two pools containing mainly the soil microbial biomass, microbial products and root exudates. There is a ‘slow’ pool with an intermediate turnover time of 20–40 y consisting of physically protected or SOM more
resistant to microbial degradation. There is also a ‘passive’ pool consisting of chemically recalcitrant SOM with a turnover time of 200–1500 y (Parton et al., 1987).

Further, during the continuum of residue decomposition, a transitory intermediate pool known as “physically un-complexed organic matter” can be recovered, which consists of organic material not bound to soil minerals (Gregorich and Beare, 2008). Based on the method of separation, the “physically un-complexed organic matter” is either referred to as particulate organic matter (POM, particle-size fractionation using wet-sieving techniques) or the light fraction (LF, density separation). Density fractionation refers to the physical separation of soil organic matter using a liquid of known specific gravity, into a low- and high-density fraction, known as the light and heavy fraction (LF and HF), respectively (Sohi et al., 2001; Poirier et al., 2005). The LF embodies residues in various stages of decay, with high C and low ash concentrations (Golchin et al., 1994) and not experienced complete conversion (Gregorich and Janzen, 1996). The LF is considered as a labile SOM pool and highly influenced by management practices, and an chief indicator of soil quality (Gregorich et al., 1996; Leifeld and Kögel Knabner, 2005). It is included in the defined ‘active’ pool. The hydrolysable fraction of HF is illustrative of the ‘slow’ pool, and the non-hydrolysable (recalcitrant or resistant) fraction of HF is equivalent to the ‘passive’ pool (Collins et al., 2000).

2.6 Sources of nitrogen and its effects in the RBS

The important sources of nitrogen (N) in soil are organic matter, rainfall, animal manure and inorganic fertilizers. Nitrate (NO$_3^-$) and ammonium (NH$_4^+$) are available forms of N in the soil for the plant uptake, and, NO$_3^-$-N is extremely mobile in soil and water (Simmons et al., 1992).
Firstly, NH$_4^+$ and NO$_3^-$ forms occur in the RBS as part of the N cycle through the mineralization and nitrification process. Organic N, a component of SOM, accounts for most of the N in soil. It is converted to NH$_3$ by microorganisms in a process referred to as N mineralization or ammonification (Myrold, 1999). The NH$_4^+$ is transformed to NO$_3^-$ by the microbial activity mainly by bacteria called nitrifiers in a process termed nitrification. These two processes are important part of the N cycle since they produce the plant available form of N (Robertson et al., 1999). Secondly, N forms enter the RBS during surface runoff from the adjacent agricultural land. Dissolved nutrients mainly NO$_3^-$ and phosphorus and soil particles which holds cations in their negatively charged exchange sites, specially NH$_4^+$, enters the RBS. In this context, RBS slows down the surface flow and store the water within the RBS for a period of time and there by increases nutrient filtration by the riparian vegetation (Palone and Todd, 1997). It has been stated that presence of 30m buffer can filter 24 to 98 % of N efficiently when water flowing from cropped lands is passed through RBS. This process helps to maintain the water quality by reducing the concentration of NO$_3^-$ in the water below allowable concentration before entering in to the ground water. The World Health Organization (WHO) recommends a maximum concentration of 45 mg L$^{-1}$ NO$_3^-$ or its equivalent 10 mg L$^{-1}$ NO$_3^-$ -N for drinking water. Many other countries including Canada, India and Mexico have adopted the same standards for drinking water quality (Agarwal et al., 1999; Pacheco et al., 2001).

The general mechanism related to removal of chemicals in the RBS include plant and microbial uptake and immobilization, microbial transformations in surface and ground water, and adsorption to soil and organic matter particles. Lowrance (1992) suggested that effectiveness of these processes will depend on the age and the condition of the vegetation, soil characteristics
such as porosity, aeration and organic matter content, and the rate with which surface and subsurface waters move through buffers.

Significant amounts of nutrients via surface and sub-surface flows can enter the buffered area. Based on the perennial vegetation, age and soil type, uptake of these nutrients by the riparian vegetation and the residue nutrients trapped in the buffered region could significantly influence GHG emissions. The presence of microbial organisms responsible for N cycling coupled with soil C inputs in the buffered region via litterfall, fine root turnover, through fall and stemflow could also contribute to enhanced soil C sequestration within the RBS. Knowledge on the understanding of soil organic carbon fractions and available nutrients, mainly N forms, would yield appreciable information to improve the RBS management practices and thereby contributing to climate change mitigation.
CHAPTER THREE

EFFECT OF SOIL TEXTURE, VEGETATION TYPE AND AGE ON SYSTEM LEVEL CARBON SEQUESTRATION IN RIPARIAN BUFFER SYSTEMS AND COMPARISON OF SOIL ORGANIC CARBON WITH ADJACENT AGRICULTURAL LAND

3.1 INTRODUCTION

Agroforestry is recognized as an integrated land-use system promoting both, productivity and environmental integrity. This system has been widely recognized by global organizations, such as the Intergovernmental Panel on Climate Change (IPCC) and the Food and Agriculture Organization (FAO), as a land-use contributing to climate change mitigation and resilience (Jose and Bardhan, 2012). There is worldwide consent related to increasing greenhouse gases (GHG) in the atmosphere and their influence on global warming and climate change (IPCC, 2007). Carbon (C) sequestration process is considered as one of the main tools to mitigate the climate change via reducing the GHG concentration in the atmosphere. In this context, riparian buffer systems (RBS); a form of agroforestry where strips of perennial plants, shrubs and / or trees are planted along waterways mainly to control non-point source of pollutions reaching the water source. Therefore, RBS is considered as a best management practice (BMP) in order to enhance water quality. However, RBS also have the potential to sequester atmospheric CO$_2$ in the above ground biomass, and belowground in roots and in soil, referred in this thesis as system level C sequestration; capturing atmospheric C both above and belowground (roots and soil).

In relation to the above, RBS and their contributions to enhance water quality issues have been studied in detail over the previous two decades (Jose 2009; Zehetner et al., 2009; Vogt et al. 2015). However, the effect of the perennial component, their age class and type and soil texture on system level C sequestration (above ground and below ground) by RBS is not well
understood. There is a research gap related to the quantification of C sequestration potentials in RBS by assessing both above and below ground biomass C as well as soil organic C (SOC) content as influenced by soil textures (clay and loam), vegetation types (deciduous and coniferous) and vegetation age (mature and young). This study therefore addressed this research gap by quantitatively assessing C sequestration potential of RBS as influenced by the factors indicated above. Eight RBS sites were selected within the Grand River Watershed (GRW) to quantify C sequestration. The Grand River Conservation Authority (GRCA) has digitized 11,000 km of linear length of degraded agricultural streams within the 7,000 km² of their watershed. This study, therefore, should provide the needed information to all conservation authorities in Canada to potentially plant RBS along the degraded agricultural streams and thereby optimize C sequestration in order to bring about landscape level impact within the watersheds in Canada.

**Significance of the study**

It is anticipated that when the current federal government is seeking ways to enhance atmospheric CO₂ capture in terrestrial ecosystems, results from this study could contribute valuable data to inform policy. Further, 11,000 km of liner length of degraded agricultural streams within the GRW are flowing through various soil textural classes. Results derived from this study with respect to suitability of vegetation type in different soil textural classes could also help GRCA officials to recommend tree species based on soil texture in order to optimize tree growth and C sequestration at the landscape level. The differences in SOC within the riparian buffer and in the adjacent control agricultural field will provide information on soil C sequestration enhancement by adopting / planting RBS along degraded streams within the agricultural landscapes. Due to the presence of perennial vegetation, SOC build-up over time is expected to be high within the buffered areas. As the riparian plantings that are being
investigated in this study were established in previous agricultural lands, any difference in SOC can be attributed to the influence of perennial vegetation. These initiatives coupled with climate change mitigation efforts that are being promoted by both federal and provincial governments thereby could financially benefit landowners within the GRW by adopting RBS in their respective lands: (1) Federal government’s C pricing policy, and also (2) They can get revenue from any understory plantings such as, berries, ginseng, log mushrooms etc. that can be grown within the buffered areas under shaded conditions.

In addition to C sequestration and climate change mitigation potential of RBS within the GRW, it can also provide several environmental services such as soil erosion control, reduce flood damage, nutrient filtration and thereby reduce eutrophication in Lake Erie.

### 3.2 MATERIALS AND METHOD

#### 3.2.1 Study area

Detailed description of the study area is given in chapter one (section 1.4.1).

System level C sequestration in the RBS = \( \text{Biomass C sequestration} + \text{SOC sequestration} \)

Here, Biomass C sequestration = \( \text{[Aboveground + Belowground (roots)]} \) C sequestration

#### 3.2.2 Biomass C sequestration:

3.2.2a Vegetation Sampling and Data Collection:

All trees and shrub species were identified and the diameter at breast height (DBH) was recorded for all trees > 2.5cm DBH in each randomly selected plot as described in the chapter one (section 1.4.2). Counts of individuals for each species were made for smaller trees (< 2.5cm DBH and > 1m height). For shrub species, i.e. those with multiple stems or low growth habitat, the tallest and widest dimensions of the shrub were recorded.
3.2.2b. Calculation: Biomass C sequestration

Published allometric/regression equations (Ter-Mikaelian and Korzukhin, 1997) were used to determine species-specific biomass quantification for trees (> 2.5cm DBH) and shrubs. Belowground living biomass, mostly the large root system of trees, were not sampled since it can cause an undesirable level of soil disturbance on private lands close to the streams. However, biomass stored in root systems were estimated as a percentage of aboveground biomass based on the literature for various species and age classes (e.g. Kurz et al., 1996; Cairns et al., 1997; Rheinhardt et al., 2012a; Addo-Danso et al., 2016).

Further, biomass C in both above and below ground vegetation was determined by using the % C in various vegetation types from previously published paper (Thomas and Martin, 2012). This % C in various biomass samples were used to convert the dry biomass quantitative estimates to quantitative biomass C.

Equation 1: Aboveground biomass (Ter-Mikaelian and Korzukhin, 1997)

\[ M = aD^b \]

Where, \( M \) - oven-dry weight of the biomass component of a tree (kg)

\( D \) – Diameter at breast height (DBH) (cm)

\( a, b \) – Parameters

Equation 2: Aboveground biomass carbon of a tree (Thomas and Martin, 2012)

Average C % in the aboveground biomass = 47.7%

\[ AGBC = \text{Equation 1} \times 47.7\% \]

\[ = aD^b \times 47.7\% \]

Where, \( AGBC \) – Aboveground biomass carbon (kg C)
Equation 3: Belowground biomass carbon of a tree (Thomas and Martin, 2012)

Average C % in the aboveground biomass = 30% of the AGBC

\[ BGBC = \text{Equation 2} \times 30\% \]
\[ = aD^b \times 47.7\% \times 30\% \]

Where, BGBC – Belowground biomass carbon (kg C)

Equation 4: Total biomass carbon (TBC) of a tree

\[ TBC = \text{Equation 2} + \text{Equation 3} \]
\[ = \text{AGBC} + \text{BGBC} \]
\[ = [(aD^b \times 47.7\%) + (aD^b \times 47.7\% \times 30\%)] \text{ kg C} \]

TBC in the sampled plot in RBS (5m width x 15m length plot) was estimated by the summation of TBC of all trees (TBC\text{plot}). Finally, TBC\text{plot} in the linear length of RBS (3m width x 1000m linear length) was calculated as follows;

Equation 5: TBC\text{plot} in the linear length of RBS (TBC\text{LL})

\[ TBC_{\text{LL}} \text{ (Mg C)} = \left(\frac{TBC\text{plot kg C}}{(5m \times 15m)}\right) \times \left(\frac{(3m \text{ width} \times 1000m)}{1000}\right) \]

Further, annual biomass C sequestration (ATBC\text{LL}) was calculated by dividing the TBC\text{LL} by the age of the RBS and CO₂ equivalent for the biomass C sequestration was calculated by multiplying the ATBC\text{LL} by 3.67 [ conversion of C to CO₂ equivalent, see below].

Equation 6: Annual biomass carbon sequestration in the linear length of RBS

\[ ATBC_{\text{LL}} \text{ (Mg C y}^{-1} / m^2) = \frac{\text{Equation 5}}{\text{Buffer age}} \]
\[ = \left(\frac{TBC\text{plot} \text{ / (5m x 15m)}}{(3m \text{ width} \times 1000m)}/ \text{buffer age}\right) \]

Equation 7: Determination of CO₂ fixation through biomass C sequestration

Annual CO₂ fixed in biomass C (Mg CO₂ y\text{-1} / m²) = Equation 6 x CO₂ equivalent
\[ = \left(\frac{TBC\text{plot} \text{ / (5m x 15m)}}{(3m \text{ width} \times 1000m)}/ \text{buffer age} \times 3.67\right) \]
Where, CO$_2$ equivalent = (molecular weight of CO$_2$ / molecular weight of C)

= (44/12)

= 3.67

3.2.3 Soil C sequestration

3.2.3a Soil Sampling:

In each selected plot (pseudo replicates) within the strip transect, representative soil samples were collected from three randomly selected locations per plot (sub samples) in both riparian buffer zones and in the adjacent agricultural fields. Soil samples were collected from two depths (0-30 cm and 30-60cm) as trees can store C in deeper soil layers due to their extensive root systems. Altogether 480 soil samples were collected from all sites [5 plots x 3 sub samples x 2 depths x (8 RBS + 8 adjacent agriculture fields)]. Soil bulk density was also collected at all sampling sites and at each soil depths to determine the total SOC on a mass basis. Soil samples stored at -20°C until analyzed.

3.2.3b SOC sample preparation:

Soil sub samples were taken from the freezer and air dried for 10-14 d. After air drying, samples were passed through a 2 mm sieve. Small rocks and root material that were collected in the 2 mm sieve was discarded. The soil was passed through a hammermill (Custom Laboratory Equipment, FL, US) to grind the soil in order to homogenize the soil sample. The soil from the hammermill was again passed through the 2 mm sieve. The remaining material above the sieve was then discarded. In preparation for subsequent SOC analysis 15-20 g of soil was ground ≤0.250 mm using a mortar and pestle (Graham et al., 2018).
3.2.3c SOC determination:

SOC was determined using direct combustion methods adapted from Wang and Anderson (1988) and further outlined by Wotherspoon et al. (2015) and Graham et al. (2018). Each ground soil sample was divided up into two subsamples. The first subsample was placed in a muffle furnace and heated to 575°C for 24 h to burn off SOC so that only the soil inorganic carbon remained (SIC). After SOC removal, the subsample was analyzed for its remaining SIC content by combusting 0.2000 - 0.3000 g of soil in a Leco CR-412 carbon analyzer (LECO Corporation, MI, USA) at 1300°C using a lance flow of 1.2 L min⁻¹ as outlined in the Leco operational manual. A second subsample was analyzed directly using the same method to determine soil total carbon (STC). STC and SIC from the two subsamples were then used in Equation 8 to calculate SOC (Tabatabi and Bremmer, 1970).

The Leco CR-412 determines C content by analyzing the CO₂ evolved from the soil sample and displays %C on a total mass basis. Therefore, correction factor for moisture content (CFM) was applied to account for the air-dry soil moisture in the air-dried STC subsample. Since the SIC subsample was analyzed directly after being treated in the muffle furnace (575 °C), these samples did not contain any moisture and no correction for soil moisture was applied. To determine the CFM, soil was placed in tins and weighed before and after oven-drying for 48 h at 105°C. Equation 9 was then used to calculate the CFM. Soils used in the CFM determination were not used for C analysis.

Equation 8: SOC determination

\[
SOC (\%) = [STC (\%) \times CFM] - SIC (\%)
\]

Equation 9: CFM determination

\[
CFM = \frac{[M_{ADS} (g) - M_{tin} (g)]}{[M_{ODS} (g) - M_{tin} (g)]}
\]
Where, $M_{\text{tin}}$ - mass of the tin (g),

$M_{\text{ADS}}$ - mass of air-dry soil (g)

$M_{\text{ODS}}$ - mass of oven-dry soil (g)

3.2.3d Bulk density determination

The Leco CR-412 displays SOC on a percent basis. To calculate the total mass of SOC associated with each field, soil bulk density was used. For each field (RBS and Ag sites), bulk density samples were collected from two depths (0-30 cm and 30-60 cm) by digging a 1m x 1m x 1m pit and three samples were taken from each depth. Bulk density samples were first oven dried for 48 h at 105°C and weighed to determine their oven dried mass ($M_{\text{ODS}}$). Since root debris and rocks were present in the samples, bulk density was corrected to capture only the bulk density of the soil material (Hao et al., 2008). After oven-drying, soil samples were passed through a 2 mm sieve. The mass ($M_{\text{debris}}$) and volume ($V_{\text{debris}}$) of the root and rock material that were retained on the 2 mm sieve was recorded and Equation 10 was used to calculate bulk density. $V_{\text{debris}}$ was obtained through water displacement (Hao et al., 2008). The total soil volume ($V_{\text{total}}$) was fixed at 250 cm$^3$ as this was the volume of the UMS soil sampling ring used. Finally, the mean bulk density and mean SOC (%) of each field was used in Equation 11 to calculate the total Mg of organic C per hectare in each field found within the both depths.

Equation 10: Bulk density determination

$$\text{Bulk density (g cm}^{-3}) = \frac{[M_{\text{ODS}} (g) - M_{\text{debris}} (g)]}{[V_{\text{total}} (cm^3) - V_{\text{debris}} (cm^3)]}$$

Equation 11: SOC determination on mass basis

$$\text{SOC (Mg C ha}^{-1}) = [10,000m^2 \times 0.3m] \times [\text{bulk density (Mg m}^{-3})] \times [\text{SOC(%) \}}]$$
3.2.3e Soil texture determination

Soil texture was determined on 2017 and 2018 using the hydrometer method (Kroetsch and Wang 2007).

3.3 STATISTICAL ANALYSIS

A three factor factorial in completely randomized design (CRD) with two levels in each factor was used to conduct this study. The SOC data were analyzed using PROC GLIM in SAS 9.4 package. The assumptions for the variance analysis was confirmed using scatterplots of studentized residuals against the various independent variables and their predicted values. Outliers were identified and removed based on Lund’s critical value. To confirm the assumption of normality Shapiro-Wilkes Test was used. Test for significance was employed by F-test in order to test the above hypothesis, and a Tukey’s test was employed to determine the significance of treatment means. To find the significant differences in sample means in each treatment combinations, least squares means (LSM) were computed and, compared pairwise using a Tukey’s multiple range test (p≤0.05). It is also important to observe that pseudo replicates were used in the experimental design. This was necessary because it was not possible to find five replicates of each treatment combination in the study area (could not find so many RBS sites). However, many watershed studies have used pseudo replicated designs (e.g. Bormann and Likens 1979) and have generated much useful information.
3.4 RESULTS

3.4.1 The physical characteristics of soils

3.4.1a Soil bulk density

The bulk density and soil texture of the soils collected from both RBS and adjacent agricultural fields at 0-30 cm and 30-60 cm are given in Tables 2, 4 and 4, respectively.

Table 2: Soil bulk density in riparian buffer systems and adjacent agricultural fields at 0-30 and 30-60 cm depth

<table>
<thead>
<tr>
<th>Land use</th>
<th>Bulk Density (g cm(^{-3}))</th>
<th>Land use</th>
<th>Bulk Density (g cm(^{-3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-30 cm</td>
<td>30-60 cm</td>
<td>0-30 cm</td>
</tr>
<tr>
<td>Buffer systems</td>
<td></td>
<td></td>
<td>Agricultural field</td>
</tr>
<tr>
<td>MDL</td>
<td>1.13 (±0.014)</td>
<td>1.22 (±0.026)</td>
<td>Soybean</td>
</tr>
<tr>
<td>MCL</td>
<td>1.11 (±0.089)</td>
<td>1.21 (±0.045)</td>
<td>Soybean</td>
</tr>
<tr>
<td>MDC</td>
<td>1.00 (±0.087)</td>
<td>1.29 (±0.026)</td>
<td>Wheat</td>
</tr>
<tr>
<td>MCC</td>
<td>1.23 (±0.033)</td>
<td>1.18 (±0.035)</td>
<td>Wheat</td>
</tr>
<tr>
<td>YDL</td>
<td>1.16 (±0.053)</td>
<td>1.25 (±0.021)</td>
<td>Wheat</td>
</tr>
<tr>
<td>YCL</td>
<td>1.10 (±0.046)</td>
<td>1.31 (±0.019)</td>
<td>Wheat</td>
</tr>
<tr>
<td>YDC</td>
<td>1.27 (±0.060)</td>
<td>1.34 (±0.062)</td>
<td>Clover</td>
</tr>
<tr>
<td>YCC</td>
<td>1.03 (±0.018)</td>
<td>1.20 (±0.024)</td>
<td>Clover</td>
</tr>
</tbody>
</table>

This study was conducted in the Grand River Watershed, southern Ontario in 2017-2018. (MDL- Mature deciduous loam, MCL- Mature coniferous loam, MDC- Mature deciduous clay, MCC- Mature coniferous clay, YDL- Young deciduous loam, YCL- Young coniferous loam, YDC- Young deciduous clay and YCC- Young coniferous clay). Numbers in the parentheses are standard error of the mean (n=3).

In the RBS, soil bulk density varied between 1.00 to 1.27 g cm\(^{-3}\) and 1.20 to 1.34 g cm\(^{-3}\) at 0-30 cm and 30– 60 cm depth, respectively. Bulk density in the agricultural fields ranged between 1.14 to 1.50 g cm\(^{-3}\) and 1.14 to 1.58 g cm\(^{-3}\) at 0-30 cm and 30-60 cm, respectively. All RBS had lower bulk density at the surface layer (0-30 cm) compared to the lower layer (30-60 cm) except MCC. Most of the adjacent agricultural fields had higher bulk density level at the lower layer than upper layer. However, results showed that all RBS had lower bulk density than in agricultural fields at both soil depths, but it was significantly lower at 0-30 cm soil depth.
(P=0.0017, according to pooled t-test) and no significant different was observed at 30-60 cm soil depth (P=0.1560) (Table 2).

3.4.1b Soil texture

Soil texture, sand %, silt % and clay % are given in Tables 3 and 4 for RBS and adjacent agricultural lands respectively. RBS and adjacent agricultural land had the same soil texture as shown in the tables below.

Table 3: Soil texture in riparian buffer systems

<table>
<thead>
<tr>
<th>Site No</th>
<th>Riparian buffer systems</th>
<th>Texture</th>
<th>Sand %</th>
<th>Silt %</th>
<th>Clay %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Mature deciduous loam (MDL)</td>
<td>Loam</td>
<td>33</td>
<td>46</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>Mature coniferous loam (MCL)</td>
<td>Loam</td>
<td>42</td>
<td>46</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>Mature deciduous clay (MDC)</td>
<td>Clay</td>
<td>22</td>
<td>38</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>Mature coniferous clay (MCC)</td>
<td>Clay</td>
<td>28</td>
<td>30</td>
<td>42</td>
</tr>
<tr>
<td>11a</td>
<td>Young deciduous loam (YDL)</td>
<td>Loam</td>
<td>44</td>
<td>46</td>
<td>10</td>
</tr>
<tr>
<td>11b</td>
<td>Young coniferous loam (YCL)</td>
<td>Loam</td>
<td>42</td>
<td>47</td>
<td>11</td>
</tr>
<tr>
<td>13a</td>
<td>Young deciduous clay (YDC)</td>
<td>Clay</td>
<td>20</td>
<td>35</td>
<td>45</td>
</tr>
<tr>
<td>13b</td>
<td>Young coniferous clay (YCC)</td>
<td>Clay</td>
<td>23</td>
<td>34</td>
<td>43</td>
</tr>
</tbody>
</table>

"This study was conducted in the Grand River Watershed (GRW), southern Ontario in 2017-2018.

Table 4: Soil texture in adjacent agricultural fields

<table>
<thead>
<tr>
<th>Site No</th>
<th>Agricultural land</th>
<th>Texture</th>
<th>Sand %</th>
<th>Silt %</th>
<th>Clay %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Soybean</td>
<td>Loam</td>
<td>28</td>
<td>48</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>Soybean</td>
<td>Loam</td>
<td>44</td>
<td>48</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>Wheat</td>
<td>Clay</td>
<td>19</td>
<td>30</td>
<td>51</td>
</tr>
<tr>
<td>7</td>
<td>Wheat</td>
<td>Clay</td>
<td>31</td>
<td>28</td>
<td>41</td>
</tr>
<tr>
<td>11a</td>
<td>Wheat</td>
<td>Loam</td>
<td>38</td>
<td>48</td>
<td>14</td>
</tr>
<tr>
<td>11b</td>
<td>Wheat</td>
<td>Loam</td>
<td>41</td>
<td>47</td>
<td>12</td>
</tr>
<tr>
<td>13a</td>
<td>Clover</td>
<td>Clay</td>
<td>22</td>
<td>36</td>
<td>42</td>
</tr>
<tr>
<td>13b</td>
<td>Clover</td>
<td>Clay</td>
<td>23</td>
<td>35</td>
<td>42</td>
</tr>
</tbody>
</table>

"This study was conducted in the Grand River Watershed (GRW), southern Ontario in 2017-2018.
3.4.2 System level carbon sequestration in the RBS

3.4.2a Biomass carbon sequestration

Total biomass C, annual biomass C sequestration and CO₂ equivalent for the annual C sequestration in 1000m linear length (1 km) x 3m (landowner preferred buffer width) width of the buffer systems and total biomass C in Mg C per hectare are given in the Table 5.

Table 5: Biomass carbon sequestration in riparian buffer systems

<table>
<thead>
<tr>
<th>Riparian Buffer</th>
<th>Buffer Age</th>
<th>Total biomass C Mg C (3m width x 1000m linear length)</th>
<th>Annual biomass C sequestration Mg C y⁻¹ (3m width x 1000m linear length)</th>
<th>CO₂ equivalent Mg C y⁻¹ (3m width x 1000m linear length)</th>
<th>Total biomass C Mg C ha⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDL</td>
<td>37</td>
<td>64.71</td>
<td>1.75</td>
<td>6.42</td>
<td>215.70</td>
</tr>
<tr>
<td>MCL</td>
<td>34</td>
<td>31.75</td>
<td>0.93</td>
<td>3.43</td>
<td>105.83</td>
</tr>
<tr>
<td>MDC</td>
<td>60</td>
<td>82.54</td>
<td>1.38</td>
<td>5.05</td>
<td>275.13</td>
</tr>
<tr>
<td>MCC</td>
<td>60</td>
<td>27.26</td>
<td>0.45</td>
<td>1.67</td>
<td>90.87</td>
</tr>
<tr>
<td>YDL</td>
<td>6</td>
<td>1.40</td>
<td>0.23</td>
<td>0.86</td>
<td>4.67</td>
</tr>
<tr>
<td>YCL</td>
<td>6</td>
<td>0.65</td>
<td>0.11</td>
<td>0.40</td>
<td>2.17</td>
</tr>
<tr>
<td>YDC</td>
<td>8</td>
<td>3.82</td>
<td>0.48</td>
<td>1.75</td>
<td>12.74</td>
</tr>
<tr>
<td>YCC</td>
<td>8</td>
<td>1.43</td>
<td>0.18</td>
<td>0.65</td>
<td>4.77</td>
</tr>
</tbody>
</table>

This study was conducted in the Grand River Watershed, southern Ontario in 2017-2018. (MDL- Mature deciduous loam, MCL- Mature coniferous loam, MDC- Mature deciduous clay, MCC- Mature coniferous clay, YDL- Young deciduous loam, YCL- Young coniferous loam, YDC- Young deciduous clay and YCC- Young coniferous clay).

Among the mature trees, MDC (82.54 Mg C) sequestrated the highest biomass C compared to the other buffer sites followed by MDL (64.71 Mg C), MCC (27.26 Mg C) and MCL (31.75 Mg C). Results indicate that the CO₂(g) sequestration rate was higher in deciduous trees irrespective of age and soil types compared to coniferous trees. According to the data, MDL, MCL, MDC, MCC, YDL, YCL, YDC and YCC have sequestered 6.42, 3.43, 5.05, 1.67, 0.86, 0.40, 1.75 and 0.65 Mg CO₂ y⁻¹ per linear kilometer of buffer strip with 3m width, respectively (Table 5).
3.4.2b Soil carbon sequestration

3.4.2b.1 Soil organic carbon percentage and stock at 0-30 cm depth in the RBS

Figure 3 and 4 show mean soil organic carbon percentage (%) and mean soil organic carbon content (Mg C ha\(^{-1}\)) respectively in all the sites sampled based on treatment combination (each site represents one treatment combination) in the RBS. Though mean SOC percentage showed significant difference (p<0.05), mean SOC content showed no significance difference (p≥0.05) among the sites (Three-factor treatment combination). Both mean SOC % and mean SOC content followed similar numerical order [MDC > MDL > MCL > YDC > MCC > YCC > YDL > YCL] (Figures 3 and 4).

![Graph showing mean soil organic carbon percentage (%) in the sites of riparian buffer systems sampled at 0 – 30 cm depth in Grand River Watershed, southern Ontario in 2017-2018.](image-url)

Figure 3: Mean soil organic carbon (%) in the sites of riparian buffer systems sampled at 0 – 30 cm depth in Grand River Watershed, southern Ontario in 2017-2018. (MDL- Mature deciduous loam, MCL- Mature coniferous loam, MDC- Mature deciduous clay, MCC- Mature coniferous clay, YDL- Young deciduous loam, YCL- Young coniferous loam, YDC- Young deciduous clay and YCC- Young coniferous clay). Means followed by the same letter are not significantly different according to a Tukey’s multiple range test (P≥0.05). Error bars indicate the standard error of the mean (n=5).
Figure 4: Mean soil organic carbon content (Mg C ha\(^{-1}\)) in the sites of riparian buffer systems sampled at 0 – 30 cm depth in Grand River Watershed, southern Ontario in 2017-2018. (MDL- Mature deciduous loam, MCL- Mature coniferous loam, MDC- Mature deciduous clay, MCC- Mature coniferous clay, YDL- Young deciduous loam, YCL- Young coniferous loam, YDC- Young deciduous clay and YCC- Young coniferous clay). Error bars indicate the standard error of the mean (n=5).

Riparian buffer systems with mature deciduous trees in clay soils had numerically higher mean SOC content (177.62 ± 6.193 Mg C ha\(^{-1}\)), while riparian buffers with young coniferous trees in loam soils had the lowest mean SOC content (94.71 ± 6.193 Mg C ha\(^{-1}\)). Mean SOC content was also numerically higher in RBS having mature deciduous trees in both clay (177.62 ± 6.193 Mg C ha\(^{-1}\)) and loam soils (159.21 ± 6.193 Mg C ha\(^{-1}\)) compared to mature coniferous trees in both clay and loam soils. Higher mean SOC content was observed in YDC (143.62 ± 6.906 Mg C ha\(^{-1}\)) compared to MCC (134.37 ± 6.193 Mg C ha\(^{-1}\)), and other RBS with young trees in both clay and loam soils (YCC =114.22 ± 6.193 Mg C ha\(^{-1}\), YDL=107.02± 6.1934 Mg C ha\(^{-1}\) and YCL = 94.71 ± 6.193 Mg C ha\(^{-1}\)) (Figures 4).

Further, deciduous trees in clay soils (DC = 160.62 ± 4.531 Mg C ha\(^{-1}\)) showed statistically higher (p<0.05) mean SOC over deciduous trees in loam soils (DL= 133.11± 4.266...
Mg C ha$^{-1}$) and coniferous trees in both clay (CC =124.3 ± 4.266 Mg C ha$^{-1}$) and in loam soils (CL=119.11 ± 4.266 Mg C ha$^{-1}$). Mean SOC did not show significant difference (p≥0.05) among DL, CC and CL, however, SOC results indicate that there is a numerical trend in the following order for the sites that were not significantly different from each other; DL > CC > CL (Figure 5a).

According to the vegetation age and soil type interaction effect, mature trees in both clay (MC=155.99 ± 4.266 Mg C ha$^{-1}$) and loam soils (ML=151.36 ± 4.266 Mg C ha$^{-1}$) had significantly higher (p<0.05) mean SOC compared to younger trees in both soils (YC and YL). The lowest (p<0.05) mean SOC was observed in (YL=100.86 ± 4.266 Mg C ha$^{-1}$) (Figure 5b). However, riparian sites on YC had significantly higher SOC when compared to sites in YL.

Although vegetation age and vegetation type showed no significant difference (p≥0.05) on mean SOC content, mature deciduous trees (MD= 168.41 ± 4.266 Mg C ha$^{-1}$) had numerically higher mean SOC and followed by mature coniferous (MC= 138.94 ± 4.266 Mg C ha$^{-1}$), young deciduous (YD= 125.32± 4.531 Mg C ha$^{-1}$) and young coniferous (YC= 104.46 ± 4.266 Mg C ha$^{-1}$) (Figure 5c).
Figure 5: Two-factor interaction effects on mean soil organic carbon content (Mg C ha\(^{-1}\)) among the riparian buffer systems sampled at 0 – 30 cm depth in Grand River Watershed, southern Ontario in 2017-2018. Means followed by the same letter are not significantly different according to a Tukey’s multiple range test (P≥0.05). Error bars indicate the standard error of the mean (n=10).
Among the two soil types, results showed significantly higher (p<0.05) mean SOC content in clay soils than in loam soils. Mean SOC content in clay and loam soils were 142.46 ± 2.950 Mg C ha\(^{-1}\) and 126.11 ± 2.849 Mg C ha\(^{-1}\), respectively (Figure 6a). Further, deciduous trees resulted in statistically greater (p<0.05) mean SOC sequestration (146.87 ± 2.950 Mg C ha\(^{-1}\)) compared to coniferous trees (121.7 ± 2.85 Mg C ha\(^{-1}\)) in RBS (Figure 6b) while, mature trees had significantly higher (p<0.05) mean SOC sequestration (153.68 ± 2.850 Mg C ha\(^{-1}\)) than young trees (114.89 ± 2.950 Mg C ha\(^{-1}\)) in the RBS (Figure 6c).

Figure 6: Main factor effects on mean soil organic carbon content (Mg C ha\(^{-1}\)) RBS sampled at 0 – 30 cm depth in Grand River Watershed, southern Ontario in 2017-2018. Means followed by the same letter are not significantly different according to a Tukey’s multiple range test (P≥0.05) Error bars indicate the standard error of the mean (n=20).
3.4.2b.2 Soil organic carbon stock at 30-60 cm depth

Figure 7 and 3.6 show mean soil organic carbon percentage (%) and mean soil organic carbon content (Mg C ha$^{-1}$), respectively, in all the RBS sites sampled at 30 – 60 cm soil depth.

Figure 7: Mean soil organic carbon (%) in riparian buffer systems sampled at 30 – 60 cm soil depth within the Grand River Watershed, southern Ontario in 2017-2018. (MDL- Mature deciduous loam, MCL- Mature coniferous loam, MDC- Mature deciduous clay, MCC- Mature coniferous clay, YDL- Young deciduous loam, YCL- Young coniferous loam, YDC- Young deciduous clay and YCC- Young coniferous clay). Means followed by the same low case letters are not significantly different according to Tukey’s multiple range test (P≥0.05). Error bars indicate the standard error of the mean (n=5).

Figure 8: Mean soil organic carbon content (Mg C ha$^{-1}$) in the sites of riparian buffer systems sampled at 30 – 60 cm depth in Grand River Watershed, southern Ontario in 2017-2018. (MDL- Mature deciduous loam, MCL- Mature coniferous loam, MDC- Mature deciduous clay, MCC- Mature coniferous clay, YDL- Young deciduous loam, YCL- Young coniferous loam, YDC- Young deciduous clay and YCC- Young coniferous clay). Error bars indicate the standard error of the mean (n=5).
SOC (%) and SOC content results were very similar to the results obtained for 0-30cm soil depth. Mean SOC percentage showed significant difference (p<0.05) and mean SOC content showed no significant difference (p≥0.05) among the sampled sites. Unlike the results observed for 0-30cm soil depth, for 30-60 cm soil depth, both mean SOC % and Mean SOC content did not follow same C sequestration numerical trend.

Mean SOC % for MDC, MCC, YDC, YCC, MDL, MCL, YDL and YCL were 3.17 ± 0.144 %, 3.00 ± 0.144 %, 2.72 ± 0.131 %, 3.04 ± 0.144 %, 3.54 ± 0.131 %, 3.52 ± 0.144 %, 2.99 ± 0.144 % and 2.57 ± 0.144 %, respectively. Mean SOC content for MDC, MCC, YDC, YCC, MDL, MCL, YDL and YCL were 122.53 ± 5.013 Mg C ha⁻¹, 106.13 ± 5.013 Mg C ha⁻¹, 109.48 ± 4.488 Mg C ha⁻¹, 103.26 ± 5.013 Mg C ha⁻¹, 129.96 ± 4.488 Mg C ha⁻¹, 128.37 ± 5.013 Mg C ha⁻¹, 111.91 ± 5.013 Mg C ha⁻¹ and 99.93 ± 5.012 Mg C ha⁻¹, respectively (Figures 3.5 and 3.6). SOC % was significantly higher for MDL and MCL. However, there were no statistically significant differences in SOC content at 30 - 60 cm among the study sites. The SOC % was significantly lowest in the YCL and YCC sites (Figures 7 and 8).

Among the two-factor interaction, vegetation age and soil type interaction showed significant difference (p<0.05) and other two two-factor interactions (vegetation type x soil type and vegetation age x vegetation type) did not show statistical difference (p≥0.05) Figure 9).

Though no significant difference were observed in the interaction of vegetation type and soil type, deciduous trees grown on both clay (DC = 116.01 ± 3.430 Mg C ha⁻¹) and loam (DL= 120.93 ± 3.431 Mg C ha⁻¹) soils have numerically more mean SOC content (0 - 30 cm) compared to coniferous trees in both soil types (CC= 104.70 ± 3.605 Mg C ha⁻¹ and CL= 114.15 ± 3.604 Mg C ha⁻¹). Besides, both coniferous and deciduous trees have contributed to more SOC in the loam soils compared to clay soils at 30 – 60 cm depth (Figure 9a).
Further, loam soils with mature trees (ML = 129.17 ± 3.431 Mg C ha\(^{-1}\)), had significantly higher mean SOC content than others. Even though no statistical difference was observed among MC=114.33 ± 3.604 Mg C ha\(^{-1}\), YC = 106.37 ± 3.431 Mg C ha\(^{-1}\), and YL= 105.91 ± 3.604 Mg C ha\(^{-1}\), numerically higher mean SOC content was observed in MC, followed by YL and YC. Mature trees in both soils had higher mean SOC than young trees (Figure 9b).
In the case of vegetation type and vegetation age interaction, \( \text{MD}=126.25 \pm 3.430 \text{ Mg C ha}^{-1} \) had higher mean SOC and followed by \( \text{MC}=117.25 \pm 3.620 \text{ Mg C ha}^{-1}, \text{YD}=110.65 \pm 3.431 \text{ Mg C ha}^{-1} \) and \( \text{YC}=101.59 \pm 3.604 \text{ Mg C ha}^{-1} \). Among the mature trees, soils under deciduous trees had more SOC than those under coniferous trees. Like mature trees, young deciduous trees sequestrated more SOC than young coniferous trees in the RBS at 30 – 60 cm depth (Figure 9c).

Among the two soil types and unlike at 0-30 cm depth, results showed significantly higher (p<0.05) mean SOC content in loam soils than in clay soils at the depth of 30 – 60 cm. Mean SOC contents in loam and clay soils were 117.54 ± 2.575 Mg C ha\(^{-1}\) and 110.35 ± 2.575 Mg C ha\(^{-1}\), respectively (Figure 10a). As observed at 0-30 cm depth, deciduous trees had statistically higher (p<0.05) mean SOC sequestration (118.47 ± 2.516 Mg C ha\(^{-1}\)) compared to coniferous trees (109.42 ± 2.637 Mg C ha\(^{-1}\)) at 30-60 cm soil depth too (Figure 10b). Mature trees had significantly higher (p<0.05) mean SOC sequestration (121.75 ± 2.580 Mg C ha\(^{-1}\)) than young trees (106.14 ± 2.580 Mg C ha\(^{-1}\)) (Figure 10c) in these RBS systems.

![Figure 10: Main factor effects on mean soil organic carbon content (Mg C ha\(^{-1}\)) in the sites of riparian buffer systems sampled at 30 – 60 cm depth in Grand River watershed, southern Ontario in 2017-2018. Means followed by the same letter are not significantly different according to a Tukey’s multiple range test (P≥0.05) Error bars indicate the standard error of the mean (n=20)]](image=content)
3.4.2b.3 Comparison of mean SOC content between riparian buffer systems and adjacent agricultural field

Effects of land use (RBS vs. adjacent agriculture fields) on mean SOC content were analyzed in the sampled sites at both depths. Tukey’s multiple range test was conducted to compare the mean SOC between RBS and adjacent agricultural field (Table 6).

At 0-30 cm soil depth, a significant difference (p<0.05) was observed in the mean SOC for all the sites with mature trees compared to respective cropped lands. MDL (159.21 ± 3.391 Mg C ha$^{-1}$), MCL (143.51 ± 4.556 Mg C ha$^{-1}$), MDC (177.62 ± 5.480 Mg C ha$^{-1}$) and MCC (134.37 ± 5.104 Mg C ha$^{-1}$) had significantly higher mean SOC compared to their respective adjacent agricultural fields; soybean (85.10 ± 3.391 Mg C ha$^{-1}$), soybean (98.42 ± 4.556 Mg C ha$^{-1}$), wheat (109.43 ± 5.480 Mg C ha$^{-1}$) and wheat (59.67 ± 5.104 Mg C ha$^{-1}$), respectively.

Unlike sites with mature vegetation and associated adjacent agricultural fields, all the sites with young vegetation (YDL = 107.02 ± 5.447 Mg C ha$^{-1}$, YCL= 94.71 ± 4.636 Mg C ha$^{-1}$, YDC = 144.32 ± 9.285 Mg C ha$^{-1}$ and YCC = 114.22 ± 5.633 Mg C ha$^{-1}$) did not show significant difference (p≥0.05) in mean SOC when compared with the respective adjacent agricultural fields. However, all the RBS had numerically higher mean SOC contents compared to adjacent agricultural fields. When considering adjacent agricultural fields as a control treatment to the buffer, difference in mean SOC were 74.11, 45.09, 68.19, 74.70, 1.39, 1.60, 25.99 and 3.61 Mg C ha$^{-1}$ for MDL, MCL, MDC, MCC, YDL, YCL, YDC and YCC, respectively. YDC showed much higher SOC change compared to other young sites (i.e., almost seven times higher), though the mean SOC content in all agricultural fields was approximately similar across all tested sites. Results associated with annual mean SOC change in MDL, MCL, MDC, MCC, YDL, YCL, YDC and YCC were 2.00, 1.33, 1.14, 1.25, 0.23, 0.27, 3.25 and 0.45 Mg C ha$^{-1}$ yr$^{-1}$. In the RBS
with mature coniferous (MCC) and mature deciduous trees (MDC); even though the MDC had 43.25 Mg C ha\(^{-1}\) of more SOC than MCC, annual sequestration rate of SOC is almost similar for both systems (Table 6). These differences in SOC clearly shows that RBS have positive impact on C sequestration over cultivated fields.

At 30 – 60 cm soil depth, similar to what was observed at 0 – 30 cm depth, mean SOC in all mature sites showed statistically higher SOC when compared to respective adjacent agricultural lands; MDL (129.96 ± 3.912 Mg C ha\(^{-1}\)), MCL (129.35 ± 5.070 Mg C ha\(^{-1}\)), MDC (122.00 ± 6.095 Mg C ha\(^{-1}\)) and MCC (108.00 ± 5.648 Mg C ha\(^{-1}\)). However, some agricultural fields had higher SOC compared to YCL (99.765 ± 5.180 Mg C ha\(^{-1}\)), YDC (109.48 ± 8.469 Mg C ha\(^{-1}\)) and YCC (108.56 ± 6.289 Mg C ha\(^{-1}\)) among the young sites but a significant difference was observed only in YCL (Wheat = 119.29 ± 4.636 Mg C ha\(^{-1}\)). Even though YDL did not show a significant difference, SOC was higher compared to its adjacent agriculture field among the young sites. Annual change in mean SOC was approximately similar in MDL and MCL, however, MDC showed the lowest change compared to MCC in same soils when considering the adjacent agricultural field as control treatment. This was due to the mean SOC content in agricultural fields near to MDC and MCC (i.e., wheat= 116.43 ± 5.480 Mg C ha\(^{-1}\) and wheat= 58.152 ± 5.152 Mg C ha\(^{-1}\)) and it was two times higher in cultivated land adjacent to the MDC than MCC (Table 6).
Table 6: Effect of land use (RBS vs. adjacent agricultural field) on mean SOC content at 0-30 cm and 30-60 cm depth

<table>
<thead>
<tr>
<th>Depth</th>
<th>Landuse</th>
<th>Site 02</th>
<th>Site 05</th>
<th>Site 08</th>
<th>Site 07</th>
<th>Site 11</th>
<th>Site 11b</th>
<th>Site 13</th>
<th>Site 13b</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-30 cm</td>
<td><strong>Buffer</strong></td>
<td>MDL</td>
<td>MCL</td>
<td>MDC</td>
<td>MCC</td>
<td>YDL</td>
<td>YCL</td>
<td>YDC</td>
<td>YCC</td>
</tr>
<tr>
<td></td>
<td>(Mg C ha(^{-1}))</td>
<td>159.21</td>
<td>143.51</td>
<td>177.62</td>
<td>134.37</td>
<td>107.02</td>
<td>94.71</td>
<td>144.32</td>
<td>114.22</td>
</tr>
<tr>
<td></td>
<td>(±3.391)a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Adjacent</strong> agriculture</td>
<td>Soybean</td>
<td>Soybean</td>
<td>Wheat</td>
<td>Wheat</td>
<td>Wheat</td>
<td>Clover</td>
<td>Clover</td>
<td>Clover</td>
</tr>
<tr>
<td></td>
<td>(Mg C ha(^{-1}))</td>
<td>85.10</td>
<td>98.42</td>
<td>109.43</td>
<td>59.67</td>
<td>105.63</td>
<td>93.11</td>
<td>118.33</td>
<td>110.61</td>
</tr>
<tr>
<td></td>
<td>(±3.912)b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Difference in SOC</strong></td>
<td>74.11</td>
<td>45.09</td>
<td>68.19</td>
<td>74.7</td>
<td>1.39</td>
<td>1.6</td>
<td>25.99</td>
<td>3.61</td>
</tr>
<tr>
<td></td>
<td>(Mg C ha(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Buffer age (years)</strong></td>
<td>37</td>
<td>34</td>
<td>60</td>
<td>60</td>
<td>6</td>
<td>6</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td><strong>Annual change in SOC</strong></td>
<td>2.00</td>
<td>1.33</td>
<td>1.14</td>
<td>1.25</td>
<td>0.23</td>
<td>0.27</td>
<td>3.25</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>(Mg C ha(^{-1}) yr(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-60 cm</td>
<td><strong>Buffer</strong></td>
<td>MDL</td>
<td>MCL</td>
<td>MDC</td>
<td>MCC</td>
<td>YDL</td>
<td>YCL</td>
<td>YDC</td>
<td>YCC</td>
</tr>
<tr>
<td></td>
<td>(Mg C ha(^{-1}))</td>
<td>129.96</td>
<td>129.35</td>
<td>122.00</td>
<td>108.00</td>
<td>113.94</td>
<td>99.77</td>
<td>109.48</td>
<td>108.56</td>
</tr>
<tr>
<td></td>
<td>(±3.912)x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Adjacent</strong> agriculture</td>
<td>Soybean</td>
<td>Soybean</td>
<td>Wheat</td>
<td>Wheat</td>
<td>Wheat</td>
<td>Clover</td>
<td>Clover</td>
<td>Clover</td>
</tr>
<tr>
<td></td>
<td>(Mg C ha(^{-1}))</td>
<td>103.54</td>
<td>105.08</td>
<td>116.43</td>
<td>58.15</td>
<td>105.81</td>
<td>119.29</td>
<td>128.16</td>
<td>121.92</td>
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<td>(±3.912)y</td>
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<td>(Mg C ha(^{-1}))</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Buffer age (years)</strong></td>
<td>37</td>
<td>34</td>
<td>60</td>
<td>60</td>
<td>6</td>
<td>6</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td><strong>Annual change in SOC</strong></td>
<td>0.71</td>
<td>0.71</td>
<td>0.09</td>
<td>0.83</td>
<td>1.36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Mg C ha(^{-1}) yr(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aThis study was conducted in the Grand River Watershed, southern Ontario in 2017-2018.

a-b Means followed by the same letter along the column at 0-30 cm are not significantly different according to a Tukey's multiple range test (P≥0.05) and numbers in the parentheses are standard error of the mean (n=5).

x-y Means followed by the same letter along the column at 30-60 cm are not significantly different according to a Tukey's multiple range test (P≥0.05) and numbers in the parentheses are standard error of the mean (n=5).
3.4.2b.4 RBS effects on the mean SOC stocks in surface and subsurface soils

Table 7: SOC content in the riparian buffer systems

<table>
<thead>
<tr>
<th>Treatment Combination</th>
<th>0 - 30 cm</th>
<th>30 - 60 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature Deciduous Clay (MDC)</td>
<td>177.6 (± 5.48)a</td>
<td>122.0 (± 6.10)b</td>
</tr>
<tr>
<td>Mature Coniferous Clay (MCC)</td>
<td>134.4 (± 5.10)a</td>
<td>106.1 (± 5.65)b</td>
</tr>
<tr>
<td>Young Deciduous Clay (YDC)</td>
<td>143.3 (± 9.29)a</td>
<td>109.5 (± 8.47)b</td>
</tr>
<tr>
<td>Young Coniferous Clay (YCC)</td>
<td>114.2 (± 5.63)a</td>
<td>108.6 (± 6.29)b</td>
</tr>
<tr>
<td>Mature Deciduous Loam (MDL)</td>
<td>159.2 (± 3.39)a</td>
<td>130.0 (± 3.91)b</td>
</tr>
<tr>
<td>Mature Coniferous Loam (MCL)</td>
<td>143.5 (± 4.56)a</td>
<td>129.4 (± 5.07)b</td>
</tr>
<tr>
<td>Young Deciduous Loam (YDL)</td>
<td>107.0 (± 5.45)a</td>
<td>113.9 (± 5.90)a</td>
</tr>
<tr>
<td>Young Coniferous Loam (YCL)</td>
<td>94.7 (± 4.64)a</td>
<td>99.8 (± 5.18)a</td>
</tr>
</tbody>
</table>

*a This study was conducted in the Grand River Watershed, southern Ontario in 2017-2018. a-b Means followed by the same letter along the row are not significantly different according to a Tukey’s multiple range test (P≥0.05) and numbers in the parentheses are standard error of the mean (n=15).

Table 7 shows the RBS effects on the mean SOC in surface and subsurface soils. To test for statistical significance Tukey’s multiple range test was conducted at 95% confidence level. All the RBS with clay soils (MDC, MCC, YDC and YCC) and MDL have significantly higher (p<0.05) mean SOC content at the surface (0-30 cm) layer compared to the deeper layer (30-60 cm). In loam soils, mature trees had higher SOC in the top layer whereas young trees had higher SOC in the lower layer.
3.4.2c System Level C sequestration in the riparian buffer systems per linear kilometer length with 3m width.

Figure 11: System level carbon sequestration (Mg C/ (3m x 1000m linear length) in riparian buffer systems sampled within the Grand River Watershed, southern Ontario in 2017-2018. (MDL- Mature deciduous loam, MCL- Mature coniferous loam, MDC- Mature deciduous clay, MCC- Mature coniferous clay, YDL- Young deciduous loam, YCL- Young coniferous loam, YDC- Young deciduous clay and YCC- Young coniferous clay).

Figure 11 shows system level C sequestration per linear kilometer length with 3m buffer width (Mg C/ (3m x 1000m linear length). Overall, MDC (172.59) showed highest C sequestration and followed by MDL (151.46), MCC (99.41) and MCL (83.51) among mature RBS. Same trend was observed in young RBS as well. System level C sequestration for YDC, YDL, YCC and YCL were 79.75, 67.08, 66.67 and 59.04, respectively.
3.5 DISCUSSION

3.5.1 Soil physical properties

3.5.1a Soil bulk density

Soil bulk density was recorded to be higher in the lower layer (30 – 60 cm) than in the upper layer (30- 60 cm) in most of the sampled RBS and in the adjacent agricultural fields. Low bulk density in the upper layer of soil can be a result of continuous addition of crop residues and tree inputs, litter and fine roots, together can increase the SOM content in the top soil and can contribute to low bulk density values in the upper layer. Further, at the time of soil samples were taken, all agricultural lands adjacent to RBS were subject to minimum tillage. This tillage practice is mainly recommended as a Best Management Practice in Ontario in order to avoid soil compaction resulting in enhanced porosity in the upper layer of soil. Therefore, increased organic matter inputs coupled with minimum tillage practice in combination could have contributed to lower bulk density values as recorded in this study. Similar to bulk density range in agriculture fields in our study (1.14 to 1.50 g cm$^{-3}$ at 0-30 cm depth, Table 2), Khorami et al. (2018) also have postulated the bulk density as 1.36 g cm$^{-3}$ in minimum tillage agricultural soil in Iran. However, all the RBS has lower bulk density than respective adjacent cropped fields. Similar bulk density range was reported in other agroforestry systems like in the RBS in our study (1.12 to 1.24 g cm$^{-3}$ at 0-30 cm depth). For an example, Wotherspoon et al. (2014) have reported 1.15 to 1.21 g cm$^{-3}$ in tree based intercropping systems at 0- 40 cm soil depth in Canada, while in Costa Rica, Oelbermann. et al (2006) have stated the bulk density of 1.2 g cm$^{-3}$ at 0-20 cm soil depth in alley cropping systems. It has also been mentioned in the literature that in general, RBS have lower bulk density than its adjacent agricultural field. Nair et al., (2010) have stated that RBS typically exhibit reduced levels of soil disturbance, resulting in reduced bulk
density and greater soil macro porosity as riparian buffer systems age. No/minimum soil
disturbance and accumulation of SOC might be enhanced by residue retention through increasing
biomass yields and concurrently by slowdown SOC loss (Lal et al., 1999; Alam et al., 2018)
which result in low bulk density in RBS. In addition, soil porosity increases by soil macro
organisms like earthworms (Rizhiya et al., 2007) and pores created while tree root establishment
will also reduce the bulk density in RBS than the average bulk density of agricultural soil (1.1 to
1.5 g cm$^{-3}$) in southern Ontario.

3.5.1b Soil texture

Difference in soil texture (percentages of sand, silt and clay) between RBS and its
adjacent cultivated land was observed. It is mainly due to the slope of the land; RBS being at the
lower elevation compared to adjacent agricultural lands. Therefore, during spring thaw and high
rainfall events finer soil particles, silt and clay, can be transported via surface runoff to riparian
areas and get deposited within the riparian systems (Robinson et al., 1996; Dillaha et al., 1989;
Dabney et al., 1996; Haghnazari et al., 2015). Overall, silt and clay percentages in soils sampled
from RBS were higher compared to adjacent agricultural fields (Table 3a and 4b).

3.5.2 System level carbon sequestration

3.5.2a. Biomass carbon sequestration

Total biomass C and annual biomass C sequestration were 2 to 3 times higher in the
riparian buffers planted to deciduous trees compared to coniferous trees irrespective of
vegetation age and soil types (Table 5). This result supports the fact that the net-photosynthetic
rate of a canopy is a complex function of different tree morphology including leaf area, specific
leaf area (SLA), Leaf area index (LAI), solar radiation, canopy architecture, canopy
microclimate, photosynthetic capacity and stomatal conductance, all of which may vary
diurnally, daily, seasonally and annually (Baldocchi et al, 2001; Gratani, 1997; Legner et al., 2014). These parameters differ in both deciduous and coniferous trees (Chabot and Hicks, 1982; Coley, 1988; Reich et al., 1997). Though the photosynthetic rate and SLA for deciduous trees is greater than photosynthetic rate and SLA reported for coniferous trees (Kloeppel, 1998; Gower, 1993; Dang et al., 1991), coniferous trees can hold their needles throughout the years and thus capable of producing dry matter even during late fall and winter months. However, results from this study suggest that irrespective of coniferous trees’ ability to accumulate dry matter throughout the year in the temperate region, biomass accumulation was much higher in deciduous trees. Photosynthetic C gain is directly proportional to biomass yields, suggesting that the increased photosynthesis will result in more C or biomass in plants (Wittig et al, 2005; Rasineni et al., 2011). Peichl et al (2006) also have stated that fast growing poplar (Populus spp.) trees are able to sequester more than twice as much C than slow growing coniferous trees like Norway spruce (Picea abies).

Results from this study indicate that mature deciduous trees grown in loamy soil (MDL; 37 years) had higher annual biomass C accumulation compared to mature deciduous trees grown in clayey soil (MDC; 60 year, whereas young deciduous trees grown in clayey soil (YDC; 8 years) had higher C accumulation in the biomass than young deciduous trees grown in loamy soil (YDL; 6 years) (Table 5). These observed differences in biomass accumulation rates could be explained by the sigmoid growth curve pattern commonly seen in trees. When trees age, growth rate reduces compared to the initial growth rate when trees are young (Weiskittel et al., 2011; Kassier, 2011). In the case of coniferous trees grown in loamy soils, results from this study indicate that the biomass carbon (C) accumulation declined with tree age (MCL = 0.06 and YCL = 0.65 Mg C y⁻¹). However, biomass C accumulation in coniferous trees grown on clayey soils, it
appears that even at age 60 trees are able to fix biomass C compared to young trees that are of 8 years old. This implies that soil and vegetation types can influence tree growth rates and thereby affect biomass C accumulation in trees. Either way, as a thumb rule, biomass C accumulation in trees can increase with age until the tree species reaches its full growth stage; a condition often seen in old growth forest ecosystems (Ryan et al., 1997; Long and Smith, 1992; Kassier, 2011).

3.5.2b. Soil carbon sequestration

3.5.2b.1 Soil C stock at 0 – 30 cm depth in RBS

Results from this study suggest that SOC sequestration cannot be only relied on percentage SOC (Figure 3 and Figure 3). When bulk density is factored in, at both depths, on a mass basis SOC sequestration amounts differed significantly between various RBS studied.

SOC was higher in the buffers having deciduous trees in both clay and loam soils, which indicates that the deciduous trees have more potential to sequester atmospheric CO$_2$ in the soil than coniferous trees (Figure 3). It is also interesting to note that the above observation was not influenced by the maturity stage of the deciduous trees (Table 5). The biomass C sequestration too was found to be higher for deciduous than coniferous trees, which could have resulted in higher SOC sequestration in the soil from the decomposing litter. This makes sense to the fact that the deciduous trees shed their leaves during fall compared to coniferous trees. However, even though coniferous trees shed needles throughout the year, the amount of the needles is low, and the fact that the needles are highly lignified the coniferous litter may not contribute to SOC accumulation in the soil at a higher rate compared to deciduous trees (Enoch Ofosu, 2019 pers. Comm). Decomposition of the litter is influenced by several factors including but not limited to, residue quality (chemical composition, type and size) (Vigil and Sparks, 2004), biodiversity of soil organisms and communities, which can be influenced by individual tree species (Kasprowicz
et al., 2011; Mueller et al., 2012; Knight et al., 2008). Reich et al., (2005) have also stated that species diversity, and biomass of soil organisms inhabited beneath the crowns of tree stands, influenced by the tree types, and various composition of litters coming from the needles and leaf fall can all influence the decomposition process and SOC addition to the soil. Skorupski et al., (2012) have postulated that chemical composition of leaf litter (most importantly nitrogen, carbon, phenolics and non-structural carbohydrates) is influenced by tree species of both deciduous and coniferous trees. This indicates a range of potential species differences in the leaf litter decomposition by micro- and macro organisms.

Among the young tree buffers, YDC had higher level of SOC compared to other young buffers studied (YDL, YCC and YCL) (Figure 3). Besides, YDC also had higher level of SOC than MCC. These results also suggest that when deciduous trees are grown on clay soils, they have more potential to accumulate / sequester SOC. In addition, results also suggest that growth rate or biomass production rate in deciduous trees was higher than what was recorded for the coniferous trees when both tree types were grown on clayey soils (Table 5). Further, the results on two-factor interactions showed that the significant variation was in the interaction between vegetation type x soil type and vegetation age x soil type. From these interaction effects, it can be seen that soil type is determining or influencing the overall results on SOC sequestration as there was significant differences in SOC sequestration between the tested soil types irrespective of the interactions with both vegetation type and vegetation age.

When considering soil type, results showed higher SOC accumulation in clay soils compared to loam soils. Clay soil possesses more surface area (both internal and external surface areas) than loam soils, and therefore, more organic carbon can be adsorbed in the surface area and also get trapped between the layers of clay particles (Feng et al., 2013; Ding et al., 2014). In
addition, mineral also adsorb soil organic matter to their surface thereby protecting them from microbial degradation contributing to physical stabilization of organic matter, which is less seen in loam soils (Tisdale and Oades, 1982). In the decomposition process, earthworms (soil macroorganisms) are also equally important as soil microorganisms. Earthworm population is said to be higher in clay soils than other coarse-textured soils like sandy and loamy soils since they prefer moist and cool environment. Results from another parallel study in RBS, during the same time period as this study, on earthworm population also indicated that earthworm population was higher in clayey soils than loamy soils. The same study, Ashley Cameron (personal Communication, 2019) has indicated that earthworm numbers were significantly lower in the adjacent agricultural soils (mean earthworm abundance in RBS and adjacent agriculture fields were 122.66 ±10.885 m⁻² and 82.72 ±8.871 m⁻², respectively). Earthworms ingest organic matter and mix it with inorganic soil material in their gut and are excreted as a cast, which contributes to soil aggregation. Casts generally have more organic matter than the surrounding soil and they occur mostly in the upper 0–20 cm of the soil (Lee and Foster, 1991; Bossuyt et al., 2004; Lavelle and Spain, 2001). Microbial polysaccharides and other organic products in the casts may strengthen bonds between organic and mineral components, resulting in less microbial degradation. Martin (1991) also has reported that a decrease in SOM decomposition in the long term was observed when earthworms were present, possibly due to the physical protection of SOM by the formation of aggregates in clay soils (Shipitalo et al., 2004; Six et al., 2004). In this study too, as discussed above, in the RBS there were more litter inputs not only from the trees but also from the understory vegetation (e.g; the parallel study reports averagely 8-12 Mg ha⁻¹ of litter input from different RBS, Enoch Ofosu, 2019 pers. Comm.), coupled with more presence
of earthworms (Ashley Cameron, 2019 pers. Comm.) in soils with increasing clay content may have contributed to significantly higher SOC in the RBS situated in clayey soils.

3.5.2b.2 Soil C stock at 30 - 60 cm depth in RBS

Very similar to the results derived for the surface layer, irrespective of vegetation and soil types, SOC content was numerically higher in the 30 - 60 cm soil layer for sites with matured trees compared to young trees. However, in the MCC RBS, SOC in the 30-60 cm soil layer was lower than that was recorded for YDC in the same depth. As explained in the above section 3.5.2b.1 this observed results is mainly due to the influence by the presence of deciduous trees. If early growth stages are compared (first 20 years) deciduous trees can grow faster than coniferous trees and also result in higher amounts of litter (above and belowground) inputs contributing to higher SOC in the 30-60 cm soil layer (Kloeppel, 1998; Gower, 1993; Dang et al., 1991; Wittig et al, 2005; Rasineni et al., 2011; Peichl et al; 2006). A drawback in this study is that baseline SOC values were not available. Therefore, initial SOC value for the YDC site at 30 to 60 cm depth could have been much higher to begin with. However, across all sites studied in this project, there was a clear trend to indicate that mature deciduous trees contributed to higher amounts of SOC compared to mature coniferous trees.

Results also indicate that SOC% and SOC content at 30 to 60 cm soil layer were higher for MDL and MCL than MDC and MCC among mature tree sites and, YDL than YDC among young tree sites. Surprisingly, the above results were contrast to the results obtained for the surface layer, where SOC was numerically higher in RBS sites with clayey soils, irrespective of tree types. As mentioned in the above section 3.5.2b.1, clayey soils have the potential to hold more SOC than loamy soils mainly due to the physical protection of the organic carbon (Lehmann and Kleber, 2015; Schmidt et al., 2011; Luo et al., 2017). There can be many reasons
as to why loamy soils have more SOC in the lower layer. Firstly, this might be due to, when excess rainfall and flooding occurs water will be stored for a while in the RBS. Surface flow water not only carries dissolved nutrients, but also has the potential to carry small soil particles with them. These soil particles will be deposited within the RBS. When water moves downward to reach the ground water, possibly it carries small soil particles as well and finally these particles will be settled at the lower layer. This downward movement of water is highly possible in the soils with more porosity such as loamy soils (Haghnazari et al., 2015; Childs et al., 1993; Ma et al., 2016). This increases the SOC stock in the lower layer than surface layer. Secondly, unfortunately we have done the soil textural analysis only for the surface layer soil and we have assumed similar soil texture in the lower layer since texture will not change much within the given RBS. There is the possibility for changes in soil texture between surface and lower layer when it comes to RBS due to flooding. Water flow can suspend particles in the water column when they move through soil profile, or impulse them along the bottom of a watercourse (Southard, 2006).

Further, at 30 – 60 cm soil layer, YCC and YCL showed interesting results than other buffer sites at the lower layer. YCC showed numerically higher values both in SOC % and SOC content compared to YCL. This may be due to past land management practices that existed prior to this study. For example, most of the lands in the GRW are privately owned and previous management practices of the land are not known. This can make an impact on initial SOC quantity especially in the young vegetated areas. A long period of time is needed before to make even a small difference on SOC after the introduction of perennial vegetation over annual crops. Therefore, the observed difference in SOC at the lower soil layer for YCC and YCL could be due to inherent presence of SOC when RBS were established. In this context, it should also be noted
here that all RBS were established on previously cultivated conventional agricultural lands. Hence, past cropping and land management history is important to firmly conclude SOC accumulation at lower layers. This study therefore has now set a baseline values for all young and mature sites for further investigations on long-term SOC sequestration quantifications.

3.5.2.3 Comparison of mean SOC content between RBS and adjacent agricultural field

Interestingly, all RBS in the upper layer (0-30 cm) and all mature sites in the lower layer (30-60 cm) have higher SOC content compared to its respective adjacent cropped lands. The quantity of remaining SOC, at all tested depths, mainly depend on the amount of plant residue inputs and its decomposition rate. For example, Jenkinson and Rayner (1997) have described the plant residue decay based on first-order kinetics. Subsequently, first-order kinetics has been successfully applied to describe the decomposition and accumulation of SOM in the C related model predictions by several researchers (Six et al., 2002; Parton et al., 1987; Schimel and Weintraub, 2003). They all have stated that SOM is directly proportional to the total C inputs, hence SOC can be increased with increasing C inputs. In this study, soils under RBS receive continuous C inputs through litter fall, fine root turnover, root decay, root exudates, understory annual and perennial plant inputs etc. Whereas, OM additions in agricultural lands are mainly from crop residues, cover crop residues and through organic manure application during the cultivation. Thereby, cropped land might gain low C inputs per unit area basis when compared with inputs in RBS. These differences in OM or organic C inputs in both tested systems could have resulted in low SOC quantities in adjacent agricultural fields. Further, literally RBS is undisturbed whereas agricultural land is disturbed while cropping; tillage practices, crop residue or organic manure incorporation in to the soil. This soil disturbance enhances decomposition rate by mixing up soil and by providing conducive environmental factors (C substrate, aeration,
moisture, temperature etc.) for the decomposition, and thereby reduces the SOC content (Beyaert and Paul Voroney 2011; Van Eerd et al., 2014; Congreves et al., 2017; Congreves et al., 2015).

In addition to the abiotic factors, decomposition is also said to be highly influenced by the quality of the organic matter. In the literature it has been cited that the chemical recalcitrance of residue, substances such as lignin and suberin are resistant to decay by microbial population and therefore the litter quality associated with RBS can influence SOC accumulation (Rasse et al., 2005; Lorenz et al., 2007; Kleber, 2010; Simpson et al., 2012).

In the upper soil layer of RBS studied, annual SOC change showed increasing rates with age influenced by the respective vegetation type and soil type. However, in MDC [60 years] (1.14 Mg C ha$^{-1}$ yr$^{-1}$) and YDC [8 years] (3.25 Mg C ha$^{-1}$ yr$^{-1}$), SOC annual accumulation rate was lower in MDC compared to YDC. This implies that annual SOC accumulation rate will start to decline after some year of existence especially in the case of deciduous trees as they have the potential to grow faster in their early growth stage and then decline. In the lower layer, though all mature sites showed significantly higher SOC compared to the adjacent agricultural soils, young sites namely YCL, YDC and YCC had lower SOC than the adjacent agricultural fields / soils. Therefore, annual SOC change rate cannot be compared among mature and young RBS. The reason for this lower SOC in the young RBS can be the effect of past soil erosion closer to the streams (Olson et al., 2016a; Olson et al., 2016b; Xiao et al., 2018; Stacy et al., 2015), previous land management practices like ploughing close to the edge of the field and tilling etc., before establishing RBS.

3.5.2b.4 Effect of RBS on mean SOC stock in surface and subsurface soil

SOC is shown to decrease with increasing depth across all the mature RBS and in the young RBS established in clayey soils. This is expected, as it is well known that SOC decreases with soil
depth (Montes-Pulido et al., 2017; Hobley and Wilson, 2016; Bai et al., 2016). Even though, this has been studied in agricultural (Montes-Pulido et al., 2017; Zhao et al., 2015) and forest soils (Liu et al., 2017; Park and Ro, 2018), it has not been well-researched in RBS. Further, though there is no significant difference was observed, young sites with loamy soils (YDL and YCL) showed numerically higher SOC content in the lower layer. This is most likely due to past management practices leaving inherent SOC amounts in the surface and flooding conditions that could have resulted in depositing organic matter in the lower layers in loamy soils. However due to the lack of base-line data, the extent of RBS’ ability to accumulate SOC is unknown in this layer. In addition, SOC content was calculated using bulk density data from SOC %. We observed that bulk density was higher comparatively in the lower layer in both YDL and YCL. This could too also be another reason for high SOC in the deeper layer.
CHAPTER FOUR

DETERMINATION OF SOIL ORGANIC CARBON STABILIZATION BY QUANTIFYING ORGANIC CARBON IN LIGHT FRACTION AND HEAVY FRACTION IN RIPARIAN BUFFER SYSTEMS AS INFLUENCED BY VEGETATION TYPE AND AGE

4.1 INTRODUCTION

Soil organic matter (SOM) is a component of soil, comparatively only 1-10 % of the total soil mass, of which, 58% is SOC. It undergoes a series of physical, chemical and biological transformation processes that cause complex interactions between soil minerals and SOM. It contains microbes and their by-products, plant and animal residues in various stages of decomposition, and humus (Paul and Clark, 1996; Brady and Weil, 2010). SOC concentration not only plays a vital role in maintaining soil fertility, but also important to maintain the global C balance via C cycling (Eswaran et al., 1993). Approximately, 70% of the global C stock is SOC, which is three times more than the C in the aboveground terrestrial vegetation and two times more than the atmospheric C pool (Lal, 2005). During last century, increase in atmospheric CO$_2$ specially by anthropogenic activities like deforestation and fossil fuel burning have led to the breakdown of global C balance (Lal et al. 1998). Therefore, measures should be taken to avoid or mitigate the adverse effects of increased atmospheric CO$_2$ such as global warming or climate change. One such recent initiative called “4 per 1000” launched by France in 2015. This initiative attempts to increase the SOC at an annual growth rate of 0.4% or 4‰ per 1000 in the soil C stocks, which would halt the CO$_2$ increase in the atmosphere through human activities (https://www.4p1000.org/). With proper management strategies SOC level can be enhanced through improved C sequestration in terrestrial vegetation and subsequently in the soil. However,
this depends on several factors including soil properties, crop types, cropping practices, tillage practices and climate (Lal, 2004; Gauder et al., 2016).

Soil microbial decomposition of fresh crop residues results in organic C being oxidised to CO$_2$ or stabilized as humic substances. To better understand carbon stabilization in soil, scientists have developed multicompartment turnover models such as ROTHC (Jenkinson and Rayner 1977) and CENTURY (Parton et al. 1987). They have classified the SOM into three compartments or pools in terms of C mineralization rates, stabilization and mean residence times (van Veen and Paul 1981; Paustian et al. 1992). Further, SOC pools can be characterized by physical and chemical fractionation methods. Physical fractionation helps to separate soil particles based on their size. They are classified as coarse (250–2000 μm), medium (53–250 μm) and fine (<53 μm) (Christensen., 2001; Cambardella and Elliott., 1993). Stability of SOC are related to the content of silt and clay particles in the soil. SOC in silt and clay particles are said to be more stable than in coarse particles due to the physical protection capacity of the soil particles (Hassink et al., 1997). Density fractionation method separates SOC into light fraction (LF) and heavy fraction (HF) by using a denser liquid (specific gravity between 1.6 to 2.0 g cm$^3$). Commonly used density fractioning solutions are NaI (Gregorich at al., 1997) and sodium polytungstate (Tan at al., 2007). LF is not firmly associated with soil particles mostly contains labile organic matter and sensitive to management practice changes. Typically, LF has high C:N ratio and relatively low turnover time (Cambardella and Elliott., 1993). HF consist of highly decomposed material and closely associated with mineral particles hence has higher specific density than LF. It has low C concentration, less sensitive to land management changes and high turnover time (Jiang and Dou., 1987). The nature of LF and HF interprets that stabilization of SOC increases with increasing SOC in the HF.
In this study density fractionation method was executed. NaI was used as a density separating solution with a specific gravity of 1.7 g cm$^{-3}$ to assess the degree of SOC stabilization in different RBS within the Grand River Watershed (GRW) by quantifying SOC concentration in LF and HF. The objective of this study was to find the effect of different vegetation type (deciduous and coniferous) and age (young < 15 years and mature ≥ 15 years) on SOC stabilization in one soil type (clay soil), and to compare with SOC stabilization associated with adjacent agricultural fields.

4.2 MATERIALS AND METHOD

4.2.1 Soil sampling

Soils sampling from the RBS and adjacent agricultural fields were collected as was previously described in section 3.3.2a. SOM fraction and its C content determination was performed for samples collected from only four sites [Both RBS (MCC, MDC, YDC and YCC) and adjacent agricultural land] namely Sites 7, 8, 13a and 13b (i.e., only sites having clay soils).

4.2.2 Soil sample preparation for SOM fractionation

Soil samples kept in cold storage (−20°C) were thawed overnight and were then sieved through 2 mm sieve. All recognizable litter residues and roots were removed by hand-picking, and therefore not included in the light fraction.

4.2.3 Light fraction isolation by density fractionation method

Light fraction (LF) organic matter was isolated from the soil using density fractionation as described by Gregorich and Beare 2008. Soil moisture content was obtained by drying a 10 g subsample of the soil at 105°C for 24 h. A 25 g sample of field moist soil was placed into a 250 ml centrifuge bottle (polypropylene, wide-mouth) and 100 ml of sodium iodide (NaI) solution.
was added. The specific gravity of the NaI solution used was 1.7 g cm\(^{-3}\), as determined with a Precision® hydrometer (Thomas Scientific, Swedesboro, NJ). This minimizes contamination of the LF with organo-mineral particles, which can have densities of 1.7 to 2.0 g cm\(^{-3}\) (Sollins et al. 1984; Janzen et al. 1992). The bottles were capped, placed upright and shaken on an end-to-end platform shaker for 1 h at 160 rpm. The suspension was allowed to settle for 48 h at room temperature. The particulate LF floating on the surface of the NaI solution was recovered using a water-jet vacuum filtration system. The vacuum filtration system (aspiration unit) consisted of a Millipore® Sterifil® filtration system fitted with a 0.45 μm Whatman® nylon membrane filter (Whatman International Ltd, Maidstone, England) and attached to a 8-mm (i.d.) Tygon® tubing and a 2-mL pipette tip cut at an angle of 45°. The LF was washed using 100 ml of 0.01 M CaCl\(_2\) (reduces clogging of filter) followed by 100 ml of distilled water, and then transferred into a Pyrex® petri-dish. The LF was dried at 60°C for 16 h and then weighed.

The residual organo-mineral fraction (heavy fraction), was washed by adding 100 mL of 0.05 M CaCl\(_2\) (to flocculate mineral particles), shaking for 1 h, followed by further washing with deionized water (centrifuging at 8000 × g for 20 min) three times. This fraction was then air dried, ground and sieved to get <0.250mm in size for C analysis.

4.2.4 Organic C determination in SOM fractions (LF and HF)

Total and inorganic C content in the HF (TC\(_{HF}\) and IC\(_{HF}\)) were analyzed by using the method as described for SOC determination in the previous chapter, 3.2.3c. HF moisture content was obtained by drying a 10 g subsample of the HF at 105°C for 24 h for the moisture correction. Organic C content in the HF (OC\(_{HF}\)) was computed by the equation 12 and organic C content in the LF (OC\(_{LF}\)) was derived by using the equation 13.
Equation 12: Determination of OC_{HF}

\[ \text{OC}_{HF} (\text{g C / 100g soil}) = [\text{TC}_{HF} (\text{g C / 100g soil}) - \text{IC}_{HF} (\text{g C / 100g soil})] \]

Equation 13: Determination of OC_{LF}

\[ \text{OC}_{LF} (\text{g C / 100g soil}) = [\text{SOC} (\text{g C / 100g soil}) - \text{OC}_{HF} (\text{g C / 100g soil})] \]

Where, SOC – Soil organic carbon of soil sample obtained from equation 8 (section 3.2.3c).

4.3 STATISTICAL ANALYSES

The experiment was conducted as a two-factor factorial with completely randomized design (CRD) design. Two considered factors were vegetation type (deciduous and coniferous) and vegetation age (young and mature) at two levels. The statistical software used was SAS 9.4 (SAS Institute, Cary, NC). Data were tested for normality using the Shapiro-Wilk test and evaluated by analysis of variance (ANOVA) using prog GLM. Proc Mixed was used to obtain the least square means. Least significant difference at P < 0.05 was used to determine significant differences between treatment means.
4.4 RESULTS

4.4.1 Mean organic carbon concentration of LF (OC_{LF}) and HF (OC_{HF}) at both depths.

According to the Tukey’s multiple range test with two factor (vegetation type and age) factorial experiment, no significant difference was observed in the two-factor interactions and main factor levels for both OC_{LF} and OC_{HF} content among the four treatment combinations (buffer types) at both the depths (Figure 12).

Figure 12: Mean organic carbon concentration in (a) light fraction (OC_{LF}) and (b) heavy fraction (OC_{HF}) of SOM (g C/100g soil) in the soils taken from riparian buffer systems sampled in Grand River Watershed, southern Ontario in 2017-2018. (Buffer types; MDC-Mature deciduous clay, MCC- Mature coniferous clay, YDC- Young deciduous clay and YCC – Young coniferous clay). LF_30 and LF_60 represent light fraction at 0-30 cm and 30–60 cm depth, respectively whereas, HF_30 and HF_60 represent heavy fraction at 0-30 cm and 30–60 cm, respectively. In each depth, means followed by the same letters are not significantly different according to a Tukey’s multiple range test (P≥0.05). Error bars indicate the standard error of the mean (n=3)

Among all the sites, numerically higher mean OC_{LF} was observed in RBS with mature deciduous trees (MDC = 2.832 ± 0.4578 g C/100 g soil) followed by YDC (1.253 ± 0.4578 g C/100 g soil), YCC (1.194 ± 0.4578 g C/100 g soil) and MCC (0.902 ± 0.4578 g C/100 g soil) at the upper layer, whereas, YDC (1.009 ± 0.3106 g C/100 g soil) had higher mean OC_{LF} followed
by MDC (0.708 ± 0.3106 g C/ 100 g soil), YCC (0.508 ± 0.3106 g C/ 100 g soil), MCC (0.412 ± 0.3106 g C/ 100 g soil) at the lower layer (Figure 12a). In the case of OC_{HF}, similar trend was observed in C content at both depths (i.e., MDC>MCC>YCC>YDC), 2.897 ± 0.2544, 2.763 ± 0.2544, 2.483 ± 0.2544 and 2.436 ± 0.2544 g C/ 100 g soil, respectively at 0-30 cm and 2.612 ± 0.3342, 2.552± 0.3342, 2.265 ± 0.3342 and 1.643 ± 0.3342 g C/ 100 g soil, respectively at 30-60 cm (Figure 12b).

### 4.4.2 Effect of soil depth on mean organic carbon concentration in light fraction (OC_{LF}) and heavy fraction (OC_{HF}).

Figure 13 shows impact of soil depth (0 – 30 cm and 30 – 60 cm) on OC_{LF} and OC_{HF} in selected buffer systems.

![Graph showing mean organic C concentration in light and heavy fraction](image)

**Figure 13:** Comparison of mean organic carbon concentration in (a) light fraction [OC_{LF}] (g C/ 100g soil) and in (b) heavy fraction [OC_{HF}] between 0-30 cm and 30-60 cm depths in soils collected from riparian buffer systems sampled in Grand River Watershed, southern Ontario in 2017-2018. (Buffer types; MDC- Mature deciduous clay, MCC- Mature coniferous clay, YDC- Young deciduous clay and YCC – Young coniferous clay). LF_{30} and LF_{60} represent light fraction at 0-30 cm and 30 – 60 cm depth, respectively whereas, HF_{30} and HF_{60} represent heavy fraction at 0-30 cm and 30 – 60 cm, respectively. In each buffer type, means followed by the same letters are not significantly different according to a Tukey’s multiple range test (P≥0.05). Error bars indicate the standard error of the mean (n=3).
In the case of \( \text{OC}_{\text{LF}} \), a significant difference was observed in MDC and MCC between upper and lower layers, and the upper layer had significantly higher OCLF compared to the lower layer based on the Tukey’s multiple range test at 95% confidence interval. Even though there were no significant difference observed in the young buffer systems (YDC and YCC), \( \text{OC}_{\text{LF}} \) was numerically higher in the upper layer (Figure 13a).

Results in this study suggest that there were no significant difference observed in \( \text{OC}_{\text{HF}} \) between the two soil layers in all tested buffer sites. However, \( \text{OC}_{\text{HF}} \) was numerically higher in the upper layer in each buffer system studied.

4.4.3 Comparison of mean organic carbon concentration (g C/100 g soil) in light fraction (\( \text{OC}_{\text{LF}} \)) and heavy fraction (\( \text{OC}_{\text{HF}} \))

\( \text{OC}_{\text{LF}} \), \( \text{OC}_{\text{HF}} \) and their proportion of contribution to total SOC are given in Table 8. At both depths (0-30 and 30-60 cm), MDC, MCC and YCC buffer systems exhibits significantly higher C concentration \((p < 0.05)\) in HF than LF. In the YDC buffer system, no significance difference was observed, however C concentration was numerically higher \((P \geq 0.05)\) in HF than LF (Table 8).

With respect to mature trees (deciduous and coniferous tree-buffers) having the same age class, coniferous had 75.21% of the SOC in HF and only 24.79% of the SOC in LF whereas, deciduous had 48.67% SOC in HF and 51.33% SOC in LF. However, both deciduous and coniferous, in the young age-class, had nearly 67% SOC in HF and 33% in the LF in the upper layer. In the lower soil layer, percentage of SOC in LF in the MDC, MCC, YDC and YCC buffer systems were 21.33, 13.90, 38.05 and 18.32 % whereas SOC in HF were 78.67, 86.10, 61.95 and 81.68 %, respectively (Table 8).
Table 8: Comparison of mean organic carbon concentration between heavy fraction (OC<sub>HF</sub>) and light fraction (OC<sub>LF</sub>) in riparian buffer systems<sup>a</sup>

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>SOM fractions</th>
<th>Site 08</th>
<th>Site 07</th>
<th>Site 13</th>
<th>Site 13b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MDC</td>
<td>MCC</td>
<td>YDC</td>
<td>YCC</td>
</tr>
<tr>
<td>0-30</td>
<td>LF</td>
<td>2.8 (±0.38)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.9 (±0.14)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3 (±0.54)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2 (±0.14)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HF</td>
<td>3.0 (±0.38)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7 (±0.14)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4 (±0.54)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5 (±0.14)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>SOC</td>
<td>5.8</td>
<td>3.6</td>
<td>3.7</td>
<td>3.7</td>
</tr>
<tr>
<td>30-60</td>
<td>LF</td>
<td>0.7 (±0.37)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.4 (±0.14)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>1.0 (±0.54)&lt;sup&gt;x&lt;/sup&gt;</td>
<td>0.5 (±0.14)&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HF</td>
<td>2.6 (±0.37)&lt;sup&gt;x&lt;/sup&gt;</td>
<td>2.6 (±0.14)&lt;sup&gt;x&lt;/sup&gt;</td>
<td>1.6 (±0.54)&lt;sup&gt;x&lt;/sup&gt;</td>
<td>2.3 (±0.14)&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>SOC</td>
<td>3.3</td>
<td>3.0</td>
<td>2.6</td>
<td>2.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Proportion to SOC (%)</th>
<th>0-30</th>
<th>30-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF</td>
<td>48.67</td>
<td>21.33</td>
</tr>
<tr>
<td>HF</td>
<td>51.33</td>
<td>78.67</td>
</tr>
</tbody>
</table>

<sup>a</sup>This study was conducted in the Grand River Watershed, southern Ontario in 2017-2018.

<sup>a-b</sup> Means followed by the same letter along the column at 0-30 cm are not significantly different according to a Tukey's multiple range test (P≥0.05) and numbers in the parentheses are standard error of the mean (n=3).

<sup>x-y</sup> Means followed by the same letter along the column at 30-60 cm are not significantly different according to a Tukey's multiple range test (P≥0.05) and numbers in the parentheses are standard error of the mean (n=3).
4.4.4 Effect of land use (RBS vs. agricultural fields) on mean organic carbon concentration in light fraction (OC\textsubscript{LF}) and heavy fraction (OC\textsubscript{HF})

Table 9 interprets the effect of land use on both OC\textsubscript{LF} and OC\textsubscript{HF} (g C/100 g soil). Tukey’s multiple range test at P = 0.05 was conducted in each selected study site to find the impact of land use (riparian buffer systems vs. adjacent agriculture) on C concentration in both LF and HF.

In the case of OC\textsubscript{LF} (g C/100 g soil), significantly higher (P < 0.05) C concentration was observed in the MDC and MCC buffer systems (2.8 ± 0.25 and 0.9 ± 0.14) than their respective adjacent agriculture fields (0.6 ± 0.25 and 0.1 ± 0.14) in the 0 - 30 cm depth and no significant different (P ≥ 0.05) was observed between different land use in the 30 - 60 cm soil depth. However, all buffer systems had numerically higher OC\textsubscript{LF} than adjacent agriculture lands in both depths (Table 9).

As far as OC\textsubscript{HF} (g C/100 g soil) is concerned, significantly higher (P<0.05) C concentration was exhibited in the MCC and YCC buffers than adjacent agricultural lands in both soil depths. However, like OC\textsubscript{LF}, all buffers showed numerically higher OC\textsubscript{HF} than adjacent agriculture fields in both soil depths except YDC buffer and adjacent agriculture field (Table 9).
Table 9: Comparison of mean organic carbon concentration in heavy fraction (OC$_{HF}$) and light fraction (OC$_{LF}$) between riparian buffer systems and adjacent agricultural fields$^a$

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Land Use</th>
<th>OC$_{LF}$ (g C/100g soil)</th>
<th>OC$_{HF}$ (g C/100g soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>MDC</td>
<td>2.8 (±0.24)$^a$</td>
<td>3.0 (±0.32)$^a$</td>
</tr>
<tr>
<td></td>
<td>MCC</td>
<td>0.9 (±0.14)$^a$</td>
<td>2.7 (±0.12)$^a$</td>
</tr>
<tr>
<td></td>
<td>YDC</td>
<td>1.3 (±0.50)$^a$</td>
<td>2.4 (±0.32)$^a$</td>
</tr>
<tr>
<td></td>
<td>YCC</td>
<td>1.2 (±0.28)$^a$</td>
<td>2.5 (±0.13)$^a$</td>
</tr>
<tr>
<td>Adjacent</td>
<td>Wheat</td>
<td>0.62 (±0.24)$^b$</td>
<td>2.1 (±0.32)$^a$</td>
</tr>
<tr>
<td>Agriculture</td>
<td>0.1 (±0.13)$^b$</td>
<td>1.3 (±0.12)$^b$</td>
<td>0.3 (±0.14)$^x$</td>
</tr>
<tr>
<td></td>
<td>Clover</td>
<td>0.6 (±0.50)$^a$</td>
<td>0.8 (±0.51)$^x$</td>
</tr>
<tr>
<td></td>
<td>Clover</td>
<td>0.9 (±0.28)$^a$</td>
<td>1.4 (±0.28)$x$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Land Use</th>
<th>OC$_{LF}$ (g C/100g soil)</th>
<th>OC$_{HF}$ (g C/100g soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>MDC</td>
<td>0.62 (±0.24)$^b$</td>
<td>2.6 (±0.32)$x$</td>
</tr>
<tr>
<td></td>
<td>MCC</td>
<td>0.1 (±0.13)$^b$</td>
<td>2.6 (±0.12)$x$</td>
</tr>
<tr>
<td></td>
<td>YDC</td>
<td>0.6 (±0.50)$^a$</td>
<td>1.6 (±0.32)$x$</td>
</tr>
<tr>
<td></td>
<td>YCC</td>
<td>0.9 (±0.28)$a$</td>
<td>2.570 (±0.3210)$^a$</td>
</tr>
<tr>
<td>Adjacent</td>
<td>Wheat</td>
<td>0.5 (±0.25)$x$</td>
<td>2.0 (±0.32)$x$</td>
</tr>
<tr>
<td>Agriculture</td>
<td>0.5 (±0.14)$x$</td>
<td>1.0 (±0.32)$y$</td>
<td>1.0 (±0.12)$y$</td>
</tr>
<tr>
<td></td>
<td>Clover</td>
<td>0.8 (±0.51)$x$</td>
<td>2.1 (±0.32)$x$</td>
</tr>
<tr>
<td></td>
<td>Clover</td>
<td>1.4 (±0.28)$x$</td>
<td>1.3 (±0.13)$y$</td>
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</tbody>
</table>

$^a$This study was conducted in the Grand River Watershed, southern Ontario in 2017-2018. (MDC- Mature deciduous clay, MCC- Mature coniferous clay, YDC- Young deciduous clay and YCC- Young coniferous clay)

a-b Means followed by the same letter along the column for LF and HF at 0-30 cm are not significantly different according to a Tukey's multiple range test (P≥0.05) and numbers in the parentheses are standard error of the mean (n=3).

x-y Means followed by the same letter along the column for LF and HF at 30-60 cm are not significantly different according to a Tukey's multiple range test (P≥0.05) and numbers in the parentheses are standard error of the mean (n=3).
4.5 DISCUSSION

4.5.1 Mean organic carbon concentration in light fraction ($OC_{LF}$) and heavy fraction ($OC_{HF}$) at both depths.

Results exhibit that both $OC_{LF}$ and $OC_{HF}$ were numerically higher in the MDC buffer than MCC buffer system among the mature vegetation, and YDC buffer had higher than YCC buffer among young vegetation at 0 – 30 cm soil depth. Where as in the 30- 60 cm, similar results were observed among the mature vegetation but, in the young vegetation buffer systems, both $OC_{LF}$ and $OC_{HF}$ were numerically higher in YCC buffer than YDC buffer. This might be due to firstly, the amount of total SOC concentration and secondly, may be influenced by the biomass C sequestration rate in the buffer systems studied. Referring to SOC concentration (%), higher SOC was observed in MDC followed by YDC, MCC and YCC at 0-30 cm depth (Figure 3), whereas at 30 – 60 cm depth, SOC % trend was MDC>YCC>MCC>YDC (Figure 7). In relation to biomass C sequestration, MDC sequestered more C than MCC among mature buffer systems having similar age whereas, YDC sequestered more C than YCC among young buffer systems in their biomass at the same age of growth (Table 5). Increase in biomass C production ultimately enhances the SOC stock through increased litter availability (above ground and below ground). SOC is influenced by aboveground and belowground biomass production and associated inputs through litter fall, root exudation and the addition of plant residue (Hu et al., 2013; Zhang et al., 2014). When litter reaches the soil biodegradation of fresh litter occurs and thereby contributing to increase in SOC content (Chenu et al., 2018).

In our study, the range of $OC_{LF}$ is 0.902 to 2.832 and 0.412 to 0.708 g C /100 g soil at 0-30 and 30 – 60 cm depth, respectively (Figure 12a). The range of $OC_{HF}$ is 2.436 to 2.897 and 1.643 to 2.612 g C /100 g soil at 0-30 and 30 – 60 cm depth, respectively (Figure 12b).
However, in all RBS studied $OC_{HF}$ is greater than $OC_{LF}$. Shang et al., (2014) have postulated same range of $OC_{LF}$, 1.4 to 13.1 g·C·kg$^{-1}$ soil (0.14 to 1.31 g C / 100 g soil) in four different forest types including deciduous and coniferous trees. They have also stated that C in LF only represents 21 to 37% of the total SOC. This indicates that majority of the SOC is in the HF which is well protected and stabilized by creating organo-mineral complexes with soil mineral particles in the RBS (Jiang and Dou., 1987). This organo-mineral interaction reduces the accessibility for enzymes and micro-organisms thereby increasing the turn over time for decay (Jenkinson et al., 1987; Van Veen and Kuikman, 1990).

In mature RBS, microbial decomposition of SOM would have progressed resulting in microbially-derived new end products, which are chemically more complex and resistant to further microbial degradation. This chemical recalcitrant nature of new microbial products will be adsorbed by the mineral surfaces and create organo-mineral complexes (Tisdall and Oades 1982). Hence, stabilization of SOC occurs with aging by the compilation of both, chemical and physical stabilization process. Although, SOC stability increases with vegetation age irrespective of the vegetation type, according to our study, RBS with deciduous trees have more potential to sequester and protect C in the soil than coniferous trees.

4.5.2 Effect of depth on mean organic carbon concentration in light fraction ($OC_{LF}$) and heavy fraction ($OC_{HF}$)

Both $OC_{LF}$ and $OC_{HF}$ were greater in the top layer than in the lower layer in all RBS (Figure 13) studied. Regarding the percentage of $OC_{LF}$ and $OC_{HF}$ to total SOC, $OC_{LF}$ percentage was higher in the upper layer compared to lower soil layer. Conversely, $OC_{HF}$ percentage was higher in the lower soil layer than upper layer (Table 8). This scenario most likely due to continuous new litter inputs from above ground plant residues in the upper soil layer resulting in
more SOC in the upper layer than lower layer. In addition, below ground decaying roots and root exudates also provide C to soil. However, plant root biomass and density are higher in the upper layer compared to lower layer in the RBS (Abernethy and Rutherfurd., 2001). This allows more decomposable labile plant material left in the upper layer which is not undergone complete bio degradation, referred as LF (Cambardella and Elliott., 1993). Heavy fraction (HF) is considered relatively resistant SOC having longer turnover rate. Deeper soil layers generally have old organic matter, and also in more quantity, which has undergone several decay stages. This process contributes to forming chemically recalcitrant organic C pool, and this pool of C interacts with soil mineral particles and forms organo-mineral complexes. Therefore, as observed in this study, the OC$_{HF}$ percentage to total SOC was higher in the lower layer.

4.5.3 Comparison of mean organic carbon concentration in light fraction (OC$_{LF}$) and heavy fraction (OC$_{HF}$)

SOC associated with OC$_{HF}$ was greater than OC$_{LF}$ in all buffer systems studied at both soil depths. However, the percentage contribution to total SOC, by both fractions, was different in each buffer systems and also at both depths. Considering both, mature coniferous and deciduous buffer systems having same age, 75% of SOC was present in HF in the MCC buffer system and nearly 50% of the SOC present in HF in the MDC buffer system in the top layer (Table 8). Shang et al., (2014) also found that 63.5 to 78.8 % of SOC was in OC$_{HF}$ in forest soils. This scenario might be due to: (i) the annual C inputs, (ii) decomposition rate of the residue, (iii) rate of SOC (humus) decomposition and (iv) combination of all above factors (Paustian et al., 2000). Deciduous trees shed their leaves annually during the winter period, whereas coniferous trees shed their needles throughout the year and the needles take longer time to decompose but, they do contribute to recalcitrant SOC (OC$_{HF}$) (Prescott, 2000; Albers et al., 2004). This may be
the reason as to why there was 75% \( OC_{HF} \) in MCC buffer systems as recorded in this study. This litter quality difference coupled with microclimatic conditions imposed by RBS could contribute to differential allocation of SOC to HF and LF. In addition, depending on the proximity of the buffer to the adjacent crop fields, during surface runoff events, fresh crop residues can be added to both coniferous and deciduous RBS. These fresh crop residue inputs can also alter SOC allocations and can contribute to labile C pools in both systems. The fact that the study recorded \( OC_{LF} \) in both systems can be partially attributed to crop residue inputs. Even though crop residue inputs were not quantified in this study, crop residues were observed when soil samples were collected and also during debris separation in the lab. There were relatively more crop residues in MDC RBS than observed in MCC RBS.

In the young buffer system, percentage of \( OC_{LF} \) to total SOC ranged nearly 13 to 33% in both buffer system and at both soil depths. That is within the range 4 – 60% suggested by Boone, (1994) and 21.2 to 36.5% by Shang et al., (2014). Annual litter inputs from young buffer systems are comparatively less than the mature buffer systems. This could have resulted in low OCLF in both tested RBS.

4.5.4 Effect of land use (RBS Vs. agricultural fields) on mean organic carbon concentration in light fraction \( (OC_{LF}) \) and heavy fraction \( (OC_{HF}) \)

Both \( OC_{LF} \) and \( OC_{HF} \) were higher in RBS than adjacent agricultural fields in both soil depths. LF tends to decompose faster than the HF, which is more chemically complex and therefore basically more resistant to microbial degradation (Gregorich and Janzen, 1996). RBS are mostly undisturbed whereas agricultural fields are disturbed during land preparation, cropping, fertilizer application and harvesting. LF is a labile pool and sensitive to crop management practices (Gregorich et al., 1996). Hence, LF in the agriculture mostly affected and
depleted in agriculture fields than RBS resulting in more $OC_{LF}$ and $OC_{HF}$ in the tested RBS. Ramnarine et al, 2015 have also postulated higher level of $OC_{LF}$ in no-till soil (undisturbed) compared to conventional till soil (disturbed soil).

C in both fractions expresses the SOC stock. SOC stock is directly influenced mainly by the C inputs. C inputs in the RBS and adjacent agriculture field are different in residue quantity and quality, which also could have contributed to significant difference between two different land-use systems. At a bigger scale therefore, planting RBS along all degraded agricultural streams in Ontario will not only enhance carbon sequestration in the perennial tree components but also in the soils as recorded in this study. It is intrigued to know that just within the Grand River watershed there is 11,000 linear km of degraded streams, and there are close the 35 Conservation Authorities (CAs) [https://en.wikipedia.org/wiki/Conservation_authority_(Ontario,_Canada)] in Ontario covering large portions of Ontario watersheds. Planting RBS in all watersheds in Ontario should have a significant impact on Ontario’s climate mitigation initiatives, and also enhancing water quality simultaneously.
CHAPTER FIVE

DETERMINATION OF SOIL INORGANIC N (NO$_3^-$ AND EXCHANGEABLE NH$_4^+$) CONTENT IN RIPARIAN BUFFER SYSTEMS AS INFLUENCED BY SOIL TEXTURE, VEGETATION TYPES AND AGE

5.1 INTRODUCTION

Worldwide, intensive agriculture is being promoted in order to meet the required food production for the increasing global population (Godfray and Garnett, 2014; Tilman et al., 2011). The reason for this intensification is mainly due to the limitation of the arable land area around the world. The intensive agricultural operations warrant high inputs such as, the use of inorganic fertilizers and agrochemicals like pesticides and herbicides excessively. Inappropriate use of fertilizers and other agrochemicals can cause detrimental effects to the terrestrial ecosystems and also enhance non-point source pollution in the aquatic ecosystems. Especially, when high quantity of nitrate and phosphates reach aquatic resources through runoff it results in eutrophication (Vought et al., 1995). To reduce this detrimental effect of excessive nutrient contamination, riparian buffer systems (RBS) act as a nutrient filtering medium between the terrestrial and aquatic ecosystems (Pinay and Decamps, 1988; Osborne and Kovacic, 1993; Mankin et al., 2007; Aguiar et al., 2015). The mechanism by which nutrients are removed by the RBS are mainly through plant uptake/assimilation, immobilization, and adsorption to the soil surface. The extent to which nutrients are removed by the RBS can be influenced by soil type, vegetation type and age, porosity and moisture content (Lowrance, 1992). In this context, presence of inorganic nitrogen (NO$_3^-$ and NH$_4^+$) in the riparian zone, where water content is high (at times anaerobic conditions) coupled with high C content, as seen in this study, could enhance greenhouse gas (GHGs) emissions (CH$_4$ and N$_2$O) in RBS (Lloyd, 1995). However, there is a
paucity of data available on the effects of soil type, vegetation type and age on removal of inorganic N from the riparian zones. Therefore, the objective of this study was to quantitatively assess the in-situ soil inorganic N content within the tested RBS as influenced by the above mentioned factors. This can help us to design RBS in order to optimize net carbon sequestration in RBS by enhancing C capture in vegetation and in soils and at the same time to reduce GHG emissions influenced by soil type, vegetation type and age.

5.2 MATERIALS AND METHODS

5.2.1 Soil sampling

Soils samples from the RBS and adjacent agricultural field were collected as described in section 3.3.2a, soil sampling procedure.

5.2.2 Soil extractions

Soil samples were taken to the laboratory for inorganic nitrogen extractions. In the laboratory, 20 g subsamples were extracted with 60 ml of 2 M KCl and were shaken for one hour in an Orbit Shaker (Lab-Line Instrument, Inc., Melrose Park, Illinois). The samples were then filtered using P5 Fisherbrand filter paper with medium porosity at slow flow rate. An additional 10 g of soil was weighed, and oven dried at 105°C to determine the moisture content for moisture correction. All data were adjusted to an oven-dry basis as described in Maynard and Kalra (1993) and Thevathasan (1998).

5.2.3 Analysis of Soil Nitrate and Ammonium

Soil extractions were collected in clean vials and analyzed for nitrate (NO$_3^-$) and ammonium (NH$_4^+$) using a Technicon Autoanalyzer II (Technicon Industrial Systems 1978, Maynard and Kalra, 1993). For each inorganic N measurement, two different procedures were used. In the case of nitrate analysis, a copper- cadmium column was used to reduce nitrate to
nitrite (NO$_3^-$ to NO$_2^-$), and nitrate concentrations were measured calorimetrically (Maynard and Kalra, 1993). To determine ammonium concentration, the Berthelot reaction was utilized, where ammonia, sodium salicylate, sodium nitroprusside and sodium hypochlorite reacts to produce a green-coloured solution. The degree of colour saturation is calorimetrically read to obtain the ammonium concentration (Technicon Industrial Systems 1978). Sub samples were used for moisture content determination for the moisture correction (CFM$_2$) as described in the section 3.3.2c (equation 9).

Extracted samples were analysed within 24 hours; when this was not possible, samples were frozen until a later date. Frozen samples can be stored indefinitely (Maynard and Kalra, 1993).

Equation 14: Actual inorganic N (NO$_3^-$ and NH$_4^+$) determination

$$\text{NO}_3^- (\text{mg NO}_3^- - \text{N} / \text{kg soil})_{\text{actual}} = [\text{NO}_3^- (\text{mg NO}_3^- - \text{N} / \text{kg soil}) \times \text{CFM}_2]$$

Equation 15: Inorganic N determination on mass basis per hectare land

$$\text{NO}_3^- (\text{kg C ha}^\text{(-1)})) = [10,000m^2 \times 0.3m] \times [\text{bulk density (Mg m}^\text{(-3)})] \times [\text{mg NO}_3^- - \text{N} / \text{kg soil}]_{\text{actual}}].$$

Same equations described above were used for NH$_4^+$ content determination.

5.3 STATISTICAL ANALYSIS

The experiment was conducted as a three-factor factorial with completely randomized design (CRD) design. Three considered factors were soil type (clay and loam) vegetation type (deciduous and coniferous) and vegetation age (young and mature) at two levels. The statistical software used was SAS 9.4 (SAS Institute, Cary, NC). Data were tested for normality using the Shapiro-Wilk test and evaluated by analysis of variance (ANOVA) using prog GLM. Proc Mixed
was used to obtain the least square means. Least significant difference at P < 0.05 was used to
determine significant differences between treatment means.

5.4 RESULTS

5.4.1 Soil inorganic nitrogen content (NO$_3^-$ and NH$_4^+$) in RBS

Mean soil exchangeable NH$_4^+$-N and NO$_3^-$-N content for each RBS are shown in
Figure 14 and 5.2 respectively. According to the Tukey’s multiple range test at 95% confidence
interval there was no significant difference observed in three-factor (expresses different buffer
systems) and in any of the two-factor interaction for both mean NH$_4^+$-N and NO$_3^-$-N content at
both depths (0-30 and 30-60 cm).

Figure 14: Mean NH$_4^+$-N content (kg N ha$^{-1}$) in the riparian buffer systems sampled at 0 – 30 cm and 30-60 cm soil depth within Grand River Watershed, southern Ontario in 2017-2018. (Buffer types; MDL- Mature deciduous loam, MCL- Mature coniferous loam, MDC- Mature deciduous clay, MCC- Mature coniferous clay, YDL- Young deciduous loam, YCL- Young coniferous loam, YDC- Young deciduous clay and YCC- Young coniferous clay). Error bars indicate the standard error of the mean (n=5).
Figure 15: Mean soil NO$_3$-N content (kg N ha$^{-1}$) in the riparian buffer systems sampled at 0 – 30 cm and 30-60 cm soil depth in Grand River Watershed, southern Ontario in 2017-2018. (Buffer types; MDL- Mature deciduous loam, MCL- Mature coniferous loam, MDC- Mature deciduous clay, MCC- Mature coniferous clay, YDL- Young deciduous loam, YCL- Young coniferous loam, YDC- Young deciduous clay and YCC- Young coniferous clay). Error bars indicate the standard error of the mean (n=5).

In the case of mean NH$_4^+$-N content (kg N ha$^{-1}$), significant difference was observed in soil type (P=0.0031) and vegetation age (P=0.0459) at 0-30 cm among the main factor effect. Whereas, only soil type was significantly differed (P=0.0024) at 30-60cm soil depth. RBS in clay soil showed significantly higher NH$_4^+$-N content than loamy soils at both depths and young RBS showed significantly higher amount of NH$_4^+$-N than mature vegetation at 0-30 cm depth (Figure 16). At 0-30cm soil depth, MCC buffer system had numerically higher mean NH$_4^+$-N content (32.87 ±10.674) followed by MDC (19.04 ±10.674), MCL (19.02 ±10.674) and MDL (12.65 ±10.674) among the mature buffer systems. Among young buffer systems, YDC had numerically higher NH$_4^+$-N content (57.25 ±10.674) than YCC (49.48 ±10.674), YDL (20.98 ±10.674) and YCL (15.11 ±10.674). At 30-60 cm soil depth, mean NH$_4^+$-N content in the soil associated with buffer systems MCC, MDC, MCL, MDL, YDC, YCC, YCL and YDL were

In the case of mean NO₃⁻-N content (kg N ha⁻¹), only vegetation type had significant difference at both, 0-30 cm (P=0.0173) and 30-60cm (P=0.0192) soil depth among the main factor effects. Deciduous tree buffers had significantly higher content of NO₃⁻-N in soils than coniferous buffers at both depths (Figure 17). In our study, mean NO₃⁻-N content of buffer
systems MDC, MCC, MDL, MCL, YDC, YCC, YDL and YCL were 8.92 ±1.222, 6.89 ±1.222, 9.304 ±1.222, 10.48 ±1.222, 11.14 ±1.354, 8.475 ±1.222, 11.22 ±1.354 and 6.37 ±1.222, respectively at 0-30 cm soil depth. In the 30-60 cm depth NO$_3$-$N$ contents of buffer systems MDC, MCC, MDL, MCL, YDC, YCC, YDL and YCL were 7.58 ±1.087, 4.90 ±1.087, 8.60 ±1.087, 9.05 ±1.087, 8.84 ±1.087 and 5.64 ±1.087, respectively (Figure 15).

Figure 17: Main factor effects on soil mean NO$_3$-$N$ content (kg N ha$^{-1}$), in riparian buffer systems sampled at a) 0-30 cm and b) 30 – 60 cm soil depth in Grand River Watershed, southern Ontario in 2017-2018. Means followed by the same letter are not significantly different according to a Tukey’s multiple range test (P≥0.05) Error bars indicate the standard error of the mean (n=20).
5.5 DISCUSSION

Though, there was no interaction effect among treatment factors, overall mean NH$_4^+$-N content was observed higher in RBS with clay soils than loamy soils, coniferous trees than deciduous trees and young trees than mature trees. Mean NH$_4^+$-N content ranged 12.65 – 57.25 kg N ha$^{-1}$ at 0-30 cm soil depth and 10.50 – 67.49 kg N ha$^{-1}$ at 30-60 cm soil depth (Figure 14). Mean NO$_3^-$-N content varied 6.37 – 11.22 kg N ha$^{-1}$ and 4.90 – 8.60 kg N ha$^{-1}$ at 0-30cm and 30-60 cm soil depth, respectively (figure 15). Overall, NH$_4^+$ ion content was higher than NO$_3^-$ content in all RBS. Conversely, Pinay et al, (1995) have reported higher NO$_3^-$ content than NH$_4^+$ ion content in riparian soils. Their study reported 2 – 11 kg N ha$^{-1}$ of NH$_4^+$ ion content and 30 – 100 kg N ha$^{-1}$ of NO$_3^-$ content. This variation in available N concentration in riparian soils might be due to soil type, soil compaction, organic matter removal and sampling date (Li et al., 2003).

NH$_4^+$-N accumulation was noted by Hefting et al, (2004) during wet condition and Pinay et al, have reported increased NH$_4^+$-N after flood events. Soil moisture and temperature are important factors that influence nitrogen mineralization process (Thevathasan and Gordon, 1997; Thevathasan, 1998) and therefore can have a direct impact on NH$_4^+$-N and NO$_3^-$-N contents in the soil. Higher NH$_4^+$-N can also be associated with soils that are more moist and drier soils can display more NO$_3^-$-N (Pinay et al., 1993). It is also possible that NO$_3^-$ in soil solution could have been utilized by the understory vegetation in the tested RBS. A vegetation survey conducted in parallel in RBS (Enoch Ofosu, personal Communication,, 2019) revealed dense understory vegetation in RBS, and these annuals and perennials have the tendency or preference to uptake NO$_3^-$-N more than NH$_4^+$-N (Nadelhoffer et al., 1984).

Further, results indicate that NH$_4^+$ content significantly differed only among soil types [Clay>loam] whereas, NO$_3^-$ concentration showed significant difference among vegetation types.
[deciduous > coniferous] at both depths. The observed results could indicate that clay soils having more surface area can hold the NH$_4^+$ ion in their negatively charged internal and external surface area compared to loamy soils (Brady and Weil, 2010).

Besides, soil exchangeable NH$_4^+$ content was numerically higher in coniferous buffer systems than in deciduous buffers. Conversely, NO$_3^-$ ion content was higher in deciduous buffers than coniferous buffers. The soil surface of deciduous RBS had numerous annual leaf litter inputs from both trees in the RBS and also from the understory vegetation. Therefore, mineralization and nitrification of organic N from these litter inputs could have contributed to relatively and numerically higher NO$_3^-$ -N in deciduous RBS compared to coniferous RBS. The decomposition of needles in the coniferous RBS is slow and therefore, mineralization and nitrification rates also would be comparatively low compared to deciduous RBS (Prescott, 2000; Albers et al., 2004). Further, mineralization rate will be limited by the proportion of organic compounds like protein, chitin and lignin. Lignin and chitin are more resistant to degradation by the soil micro-organisms compared to protein. Since coniferous needles consist more of lignin and chitin, decomposition process could be slow resulting in low NO$_3^-$ content than seen in deciduous buffers (Esperschütz et al., 2013; Krishna and Mohan, 2017).

Both inorganic N contents were higher in young buffers than mature buffers. This can be due to higher soil temperature contributing to higher soil mineralization rates (Thevathasan and Gordon, 1997) due to open canopy in the RBS, and less plant uptake due to low understory vegetation (Enoch Ofosu pers. Comm., 2019). Mature buffers also have well established root systems than young buffers and hence they could absorb more of available nitrogen from the soil compared to young RBS.
CHAPTER SIX

CONCLUSION

The first objective of the study was to determine the effect of different soil textures (clay and loam), vegetation types (deciduous and coniferous) and age [mature (≥ 15 years) and young (< 15 years)] on system level C sequestration in RBS in the Grand River Watershed (GRW), Ontario. Altogether eight different sites (eight different buffer systems; MDC, MCC, MDL, MCL, YDC, YCC, YDL and YCL) were selected based on treatment combinations. System level C sequestration includes C sequestration in both above and belowground biomass and in soil. Biomass C sequestration was estimated by non-destruction method using previously published allometric equation. Total biomass C and annual biomass sequestration were two to three times higher in deciduous trees than coniferous trees irrespective of soil texture and vegetation age. However, results also suggest that trees in clay soils sequestered more C in their biomass than trees in loamy soils when compared to trees of similar maturity (age) in the buffer systems.

SOC was quantified at 0-30 cm and 30-60 cm soil depths. Though there were significant difference observed in SOC % among different RBS, no significant difference was seen in SOC content among all RBS at both depths. This clearly indicates the need of bulk density in estimates of SOC stocks. RBS with mature deciduous trees had sequestered more C (numerically) in soils compared to coniferous trees irrespective of soil type and vegetation age at 0-30 cm soil depth. At 0-30 cm soil depth, MDC and YDC have sequestered numerically higher C in soils among mature and young RBS, respectively. Tree residues represented to be the primary input of C entering the soil. According to the results, deciduous trees are producing more litter biomass than coniferous trees. This scenario has led to accumulate more SOC content in RBS with deciduous trees. Additionally, we saw that SOC content was higher in clayey soils.
than loam soil at the top soil layer (0-30 cm). This exhibits the capacity of clayey soils to hold more C on their surface area and produce organo-mineral complexes, thus increases the aggregation stability by resisting microbial decomposition. At 30-60 cm soil depth, though SOC was higher in RBS with deciduous trees than coniferous trees, SOC content was numerically higher in loamy soils than clayey soils irrespective of vegetation age. We are speculating that flooded conditions, during some parts of the year, in RBS could transfer clay particles from top layer to lower layer and thereby enhance SOC in the lower layer in loamy soils.

When looking at system level C sequestration, MDC and MDL showed highest C sequestration in the system compared to MCC and MCL among the mature RBS. Likewise, among the young RBS, highest C accumulation was recorded in YDC and YDL compared to YCC and YCL. This indicates that RBS having deciduous tree have more potential to sequester C when they are established in both clay and loamy soils compared to coniferous (trees) RBS.

GRW has 11,000 km linear length of degraded streams and if RBS are established on both sides, 22,000 km of buffer length can be realized, and this can result in huge landscape level impact on C sequestration. For an example, if we take MDC RBS, the system level C sequestration, calculated from results derived in this study, is 172 Mg C per linear km [ having 3m width of buffer and up to 60 cm soil depth]. Therefore, total C sequestration within the GRW can be up to 3.8 Tg C (172 x 22,000 km Mg C). Montagnini and Nair (2003) have also reported that estimated system level C sequestration potential by RBS that are 30 m wide in USA having a linear length of 0.8 x 10^6 km can be close to 1.5 Tg C y^{-1} by 2025.

The second objective of this study was to determine the influence of RBS on SOC sequestration compared to adjacent agricultural lands. All mature RBS had sequestered significantly higher SOC than adjacent agriculture land whereas young RBS did not show
significant difference. However, all RBS sequestered higher SOC than the respective adjacent agriculture lands. This clearly depicts the positive impact of RBS on SOC sequestration compared to conventional agriculture land-use systems. In addition, annual SOC change in RBS also showed an increasing with age in relation to vegetation type and soil type.

The third objective was to assess the SOC distribution in the upper soil layer (0-30 cm soil depth) and lower soil layer (30-60 cm soil depth). SOC content in the upper soil layer was higher than the lower soil layer in most of the RBS studied.

The fourth objective of the study was to assess the SOC stabilization among different RBS with respect to vegetation type and age. Here, the soil texture was kept constant (clay soil). No significant different was observed in both \( OC_{LF} \) and \( OC_{HF} \) among four buffers studied, which were in clay soil. However, all buffers had numerically higher quantity of \( OC_{HF} \) than \( OC_{LF} \). Above 66% of SOC was as associated with \( OC_{HF} \) in most of the buffers in this study, and also the percentage of C secured in HF was increasing with buffer age. Further, in relation to land-use, both \( OC_{HF} \) and \( OC_{LF} \) were higher in RBS than adjacent agriculture fields indicating that SOC in RBS, influenced by the presence of perennial trees, is contributing to SOC stabilization.

The fifth objective of the study was to assess the soil inorganic N content (\( NH_4^+ \)-N and \( NO_3^- \)-N) in all buffers studied (effect of treatment factors; soil type, vegetation type and age were checked). Results showed that there was no interaction effect among treatment factors, and the mean \( NH_4^+ \)-N content was observed to be higher in RBS in clay soils than loamy soils, coniferous RBS than deciduous RBS, and young RBS than mature RBS. \( NO_3^- \)-N content was seen significantly higher in RBS with deciduous trees than coniferous trees. Overall, \( NH_4^+ \) content was higher than \( NO_3^- \) content in all RBS. The fact that there was less nitrate-N in all tested RBS due to low rates of nitrification in undisturbed perennial plant systems suggests low
availability of residue nitrogen potentially contributing to low nitrous oxide emission (a parallel study has documented low GHGs emissions; Personal Communication, Megan Baskerville, 2019). Low GHG emissions coupled with increased system-level C sequestration can make all RBS to act as net C sinks. Therefore, the establishment of RBS within all watersheds, 35 conservation authorities in Ontario, could significantly contribute to Canada’s climate change mitigation efforts.

Results in this study could have been influenced by external factors such as past management practices, flooding events, sediment loads and environmental factors at the time of this study or sampling time. Specifically, inorganic N levels in the soil can fluctuate based on soil temperature and moisture levels, input litter quality and quantity, crop management associated with adjacent agricultural land-use, and can also vary from one site to another. However, in this study NH$_4^+$-N was seen as the predominant form of inorganic N in all tested RBS and therefore, it can be concluded that inorganic N loading to waterways can be controlled by RBS. The second aspect is, RBS exist in lowland areas whereas agricultural fields are situated in upland areas. Due to erosion or runoff events during the spring time, soluble carbon and crop residue can move from upland to lowland areas, which could influence the SOC values in RBS. However, in this study, the majority of young RBS had numerically lower SOC than the adjacent agricultural fields. This could have been due to the fact that all RBS selected for this study had less than 5% slope and such movement of SOC did not occur. Hence, all reported gains in SOC in comparison to adjacent agricultural fields reported in this study can be attributed to the influence of RBS.
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APPENDIX – Site descriptions

Site No 02
Mature deciduous loam buffer (MDL)
Adjacent agriculture - Wheat

Site No 05
Mature coniferous loam buffer (MCL)
Adjacent agriculture - Soybean
Site No 11

Young deciduous loam buffer (YDL)

Adjacent agriculture - Wheat

Young coniferous loam buffer (YCL)

Adjacent agriculture - Wheat