The Nature of Soil Organic Carbon Stocks in a Tree-Based Intercropping System in Southwestern Ontario

by

Robyn Coleman

A Thesis
presented to
the University of Guelph

In partial fulfillment of requirements
for the degree of
Master of Science
in
Environmental Sciences

Guelph, Ontario, Canada
© Robyn Coleman, January 2015
ABSTRACT

THE NATURE OF SOIL ORGANIC CARBON STOCKS IN A TREE-BASED INTERCROPPING SYSTEM IN SOUTHWESTERN ONTARIO

Robyn Coleman  
Advisor:  
University of Guelph, 2014  
R. Paul Voroney

This thesis provides an update on the nature of the soil organic carbon pools in a tree-based intercropping system 25 years after establishment. Soil samples were collected in Summer 2012, Fall 2012 and Spring 2013 to quantify soil organic carbon stocks within soils planted with hybrid poplar (*Populus* spp.), red oak (*Quercus rubra*), black walnut (*Juglans nigra*) and Norway spruce (*Picea abies*). Results showed that spatial distribution of dissolved organic carbon was associated with litterfall distribution of each tree species. Cores were collected in Summer 2012 to investigate the spatial orientation of soil organic carbon content (SOC) of soils planted with white cedar (*Thuja occidentalis*), black walnut and Norway spruce. SOC content of soils surrounding walnut, cedar and spruce were 1.62%, 1.73% and 1.58%, respectively. Higher SOC content could be attributed to the higher density tree spacing of cedar (1 m). Soils contained 2.06% SOC within the conventional cropping system.
Acknowledgements

First and foremost I would like to thank my advisor Dr. Paul Voroney for his constant guidance and patience. You were always there for words of encouragement when I needed it. My committee member and the project manager Dr. Naresh Thevathasan who spent countless hours towards this project execution. I would like to thank Dr. Andy Gordon’s dedication towards the Guelph Agroforestry Research Plot and his commitment towards our project. Thank you to my committee members Dr. Richard Heck and Dr. Maren Oelbermann for their time and assistance. To Dr. Shelley Hunt as my external examiner and Dr. Gard Otis as the chair for my defense. I am grateful to have worked with all of you.

I would like to extend my appreciation to other SES department members including Peter Smith, Peter von Bertoldi, Idris Mohammed, Dr. Gary Parkin, Dr. Kari Dunfield, Susantha Jayasundra, Linda Wing, Linda Bissell, Marie Vickery, Jo Scarrow and Virginia Warren.

To my mentors Alex Woodley, Yuki Audette, Renaldo Belfon and Houngjie Zhang, thank you for all your guidance and answering my limitless questions. My fellow AGGP (Agroforestry Greenhouse Gas Project) members Danny Jefferies, Amy Wotherspoon and Karla Graungaard field work, meetings conferences would not have been the same without you.
My support system, thank you for pep talks, encouragement and for coming to the Grad Lounge with me when I needed it. Lia Maldaner, Carlos Maldaner, Alassane Sissoko, Therese Festin, Justin Adams, Karen Thompson, Carolyn Dykstra, Nicola Day, Elke Eichelmann, Phil Formusa, Andy Marshall and Christine George, I cannot thank you enough for your support. I am also very grateful for the field and laboratory assistance from Tara Mathur, Nathan Jenkins, Sarah Pratt, Melissa Vekeman, Trevor Goulet, Jennifer Bernard, William Woodley and Laura Soderman.

Finally, this project would not be possible without the financial support from Agriculture and Agri-Food Canada’s Agriculture Greenhouse Gas Program.
# Table of Contents

Abstract .............................................................................................................................................. i
Acknowledgements ......................................................................................................................... ii
Table of Contents .......................................................................................................................... iv
List of Tables ................................................................................................................................... vi
List of Figures ................................................................................................................................. vii

## Chapter 1 Introduction ............................................................................................................. 1
  1.1 Background ............................................................................................................................... 1
  1.2 Research Context ....................................................................................................................... 1
  1.3 Objectives and Hypothesis ......................................................................................................... 2
  1.4 Thesis Outline .......................................................................................................................... 2
  1.5 References ............................................................................................................................... 3

## Chapter 2 Literature Review ................................................................................................... 4
  2.1 The Carbon Cycle ..................................................................................................................... 4
  2.2 Soil Organic Matter ................................................................................................................... 4
    2.2.1 Soil Microbial Biomass ....................................................................................................... 5
  2.3 Agroforestry ............................................................................................................................ 6
    2.3.1 Introduction of Agroforestry in the Tropics ....................................................................... 6
    2.3.2 Canadian Agroforestry Practices ...................................................................................... 7
  2.4 University of Guelph, Agroforestry Research Site ................................................................. 9
  2.5 Conclusions ............................................................................................................................. 11
  2.6 References ............................................................................................................................... 12

## Chapter 3 Seasonal differences in soil microbial biomass carbon in a
  tree-based intercropping system in southwestern Ontario .......................................................... 15
  3.1 Introduction .............................................................................................................................. 15
  3.2 Materials and Methods ........................................................................................................... 17
    3.2.1 Site Description and Management .................................................................................... 17
    3.2.2 Soil Sampling and Preparation ........................................................................................ 20
    3.2.3 Laboratory Analysis .......................................................................................................... 22
      3.2.3.1 Microbial Biomass Carbon and Dissolved Organic Carbon ........................................ 22
      3.2.3.2 Organic Carbon .......................................................................................................... 24
3.2.4 Statistical Analysis ......................................................... 24

3.3 Results and Discussion ......................................................... 25
   3.3.1 Microbial Biomass Carbon .............................................. 25
   3.3.2 Organic Carbon .......................................................... 32
   3.3.3 Dissolved Organic Carbon .............................................. 38
   3.3.4 Microbial Biomass Carbon and Organic Carbon Ratio ......... 44

3.4 Conclusions ........................................................................... 45

3.5 References ............................................................................ 46

Chapter 4 Spatial distribution of soil organic carbon within a tree-based intercropping system in southwestern Ontario
   4.1 Introduction ......................................................................... 49

4.2 Materials and Methods ........................................................... 51
   4.2.1 Site Description and Management ..................................... 51
   4.2.2 Soil Sampling and Preparation ......................................... 51
   4.2.3 Laboratory Analysis ...................................................... 55
   4.2.4 Statistical Analysis ...................................................... 56

4.3 Results and Discussion ........................................................... 56

4.4 Conclusions ........................................................................... 69

4.5 References ............................................................................ 71

Chapter 5 Summary and Future Research ........................................ 73
   5.1 References ............................................................................ 76
List of Tables

Table 3.1 - Microbial biomass carbon (mg MB-C kg⁻¹ soil) in direction east and west and with distance 2.0 m and 6.0 m from the tree rows of poplar, spruce, walnut and cedar………………………………………………………………………………………………30

Table 3.2 - Soil organic carbon (%) at direction east and west and with distance 2.0 m and 6.0 m from the tree rows of spruce, walnut and cedar…………………...34

Table 3.3 - Dissolved Organic Carbon (mg C kg⁻¹ soil) at direction east and west and with distance 2.0 m and 6.0 m from the tree rows of poplar, spruce, walnut and cedar……………………………………………………………………………41

Table 4.1 - Soil organic carbon (%) in directions east and west, and with distance from the tree row in spruce, walnut and cedar tree-based intercropping system in Southwestern Ontario…………………………………………………………..64
List of Figures

Figure 3.1 - Location of the University of Guelph’s Agroforestry Research Station (43°16’N 89° 26’W)……………………………………………………………………………………………………………………………18

Figure 3.2 - Schematic diagram of the experimental design of the University of Guelph’s Agroforestry Research Station in Guelph, Ontario………………..19

Figure 3.3 - Schematic diagram of sampling locations 2 m and 6 m east and west of the tree base collected from spruce, oak, walnut and poplar in summer 2012, fall 2012 and spring 2013 at the Guelph Agroforestry Research Site, Guelph ON)……………………………………………………………………………………………………………………………21

Figure 3.4 - Systematic contour sampling along a grid within the conventional cropping system (Control)…………………………………………………………22

Figure 3.5 - Soil microbial biomass C (mg MB-C kg⁻¹) across all tested tree species, sample locations and seasons (summer 2012, fall 2012 and spring 2013) at east and west direction from tree rows into the cropping alleys. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers…………………..27

Figure 3.6 - Soil microbial biomass (mg MB-C kg⁻¹ soil) at distances 2.0 m and 6.0 m from tree rows into the cropping alleys across all tested tree species (spruce, walnut, oak and poplar) and seasons (from samples collected in summer 2012, fall 2012 and spring 2013). Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers…………………………………………………………27

Figure 3.7 - Soil microbial biomass (mg MB-C kg⁻¹ soil) sampled at 2 and 6 m on both sides of the tree row a) east b) west across all tested tree species (poplar, spruce, oak and walnut). Boxes represent 25th to 75th percentile of sampled values, horizontal line represent sample medians, and dots indicate data outliers…………………………………………………………………………………………………………………………………………28

Figure 3.8 - Soil microbial biomass (mg MB-C kg⁻¹ soil) in soil samples collected from intercropped poplar, spruce, walnut, oak and from the conventional cropping field, across all locations and seasons. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers…………………………………………………………………………………………………………………………………………29

Figure 3.9 - Soil microbial biomass C (mg MB-C kg⁻¹ soil) of soil samples collected in summer 2012, fall 2012 and spring 2013 from tree row into the crop row of poplar, spruce, walnut and oak. Boxes represent 25th to 75th percentile of
sampled values, horizontal lines represent sample medians, and dots indicate data outliers. 

Figure 3.10 - Soil organic carbon (%) at direction east and west from tree row into crop row of spruce, walnut and oak and poplar from samples collected in summer 2012, fall 2012 and spring 2013. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and the dot indicates a data outlier.

Figure 3.11 - Soil organic carbon (%) at distances 2.0 m and 6.0 m from tree row into the crop row across all tested tree species and seasons. Boxes represent 25th to 75th percentile of sampled values and horizontal lines represent sample medians.

Figure 3.12 - Soil organic carbon (%) at 2 and 6 m from the tree row on either sides (East and West) across all tested tree species. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and the dot indicates a data outlier.

Figure 3.13 - Soil organic carbon (%) associated with tested tree species and within the conventional cropping field across all seasons. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and the dot indicates a data outlier.

Figure 3.14 - Soil organic carbon (%) as influenced by sampling time across all tested tree species. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and the dot indicates a data outlier.

Figure 3.15 - Dissolved organic carbon (mg C kg⁻¹ soil) at direction east and west from tree rows of spruce, walnut and oak and poplar from samples collected from soils in the adjacent crop (soybeans) in summer 2012, fall 2012 and spring 2013. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.

Figure 3.16 - Dissolved organic carbon (mg C kg⁻¹ soil) in soil at distances 2.0 m and 6.0 m from rows of spruce, walnut, oak and poplar. Soil samples were collected in summer 2012, fall 2012 and spring 2013. Boxes represent 25th to 75th percentile of sampled values and horizontal lines represent sample medians.

Figure 3.17 - Dissolved organic carbon (mg C kg⁻¹ soil) at sampled distances a) west and b) east of tree rows. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.
Figure 3.18 - Dissolved organic carbon (mg C kg\(^{-1}\) soil) for soil samples collected adjacent to tree rows of poplar, spruce, walnut and oak and within the conventionally cropped field. Samples were collected in summer 2012, fall 2012 and spring 2013. Boxes represent 25\(^{th}\) to 75\(^{th}\) percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.

Figure 3.19 - Dissolved organic carbon (mg C kg\(^{-1}\) soil) of soil samples collected in summer 2012, fall 2012 and spring 2013 from the cropped area of poplar, spruce, walnut and oak. Boxes represent 25\(^{th}\) to 75\(^{th}\) percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.
Figure 4.1 - A schematic diagram of soil cores extractions surrounding spruce and walnut plots………………………………………………………………………………53

Figure 4.2 - A schematic diagram of soil cores extractions surrounding the cedar plots………………………………………………………………………………54

Figure 4.3 - Systematic contour sampling along a transect within the conventional cropping system. Sampling locations are represented by M1-M6………55

Figure 4.4 - Soil organic carbon (%) at sampled depths 0-10 cm and 10-20 cm (data from spruce, walnut and cedar trees combined). Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers…………………………………………………………57

Figure 4.5 - Soil organic carbon (%) at 0-10 cm depth of sampled directions west and east of tree rows (data averaged across spruce, walnut and cedar tree species). Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers…………………………………………………………59

Figure 4.6 - Soil organic carbon (%) at depth 10-20 cm at sampled directions west and east of tree rows (data averaged across spruce, walnut and cedar tree species). Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers…………………………………………………………59

Figure 4.7 - Soil organic carbon (%) at a depth of 0-10 cm at sampled distances a) west and b) east of tree rows. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers…………………………………………………………61

Figure 4.8 - Soil organic carbon (%) at a depth of 10-20 cm at sampled distances a) west and b) east of tree rows. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers…………………………………………………………62

Figure 4.9 - Soil organic carbon (%) at depth of 0-10 cm at sampled distances a) north and b) south of trees (spruce and walnut) in rows. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and the dot indicates a data outlier………………………………………………65

Figure 4.10 - Soil organic carbon (%) at depth of 10-20 cm at sampled distances a) north and b) south of trees (spruce and walnut) in rows. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers………………………………………………66
Figure 4.11 - Soil organic carbon (%) at depth of 0-10 cm within the crop rows and within the spruce and walnut tree rows. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and the dot indicates a data outliers..........................................................67

Figure 4.12 - Soil organic carbon (%) at depth of 10-20 cm within the crop rows and within the spruce and walnut tree rows. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers..........................................................67

Figure 4.13 - Soil organic carbon (%) at both depths combined of all sampling locations within the spruce, cedar and walnut rows and within the conventional agriculture control. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers..........................................................68
CHAPTER 1.0 GENERAL INTRODUCTION

1.1 BACKGROUND

A global population increase has necessitated intensification of agricultural production resulting in an increasing transformation of forest and grasslands into agricultural lands (Peichl et al. 2006). A consequence of this transformation is a reduction in soil organic carbon (SOC) stocks. Transition from forest to cultivation has led to a 30% loss of SOC in temperate soils and 50-75% loss in tropical soils within the first 20 years of cultivation (Oelbermann and Voroney 2011).

Current land management practices have altered the stability of SOC and have resulted in an increased release of CO$_2$ to the atmosphere (Lal 2004; Ramnarine 2010; Stoécio et al. 2010). Alternative land management practices have been suggested in order to conserve soil C and reduce the contribution of agriculture to global carbon emissions; these include agroforestry (Shroeder 1994; Oelbermann 2002; Peichl et al. 2006). Tree-based intercropping is a form of agroforestry that incorporates trees into the agriculture system to allow for the production of trees and crops within the same area of land (Thevathasan et al. 2004).

1.2 RESEARCH CONTEXT

The research conducted for this study was a component of a much larger project “Tree-based intercropping: An agroforestry land-use for greenhouse gas mitigation in Canadian agricultural systems”. This project was funded by Agriculture and Agri-Food Canada through the Agriculture Greenhouse Gases Program (AGGP). The goal of the AGGP project is to study greenhouse gas mitigation along a 25-year-old tree-based intercropping chronosequence in Eastern Canada. This project was led by the School of
Environmental Sciences (SES), University of Guelph, in partnership with Universities of Sherbrooke, Laval, Montreal, Toronto, Alberta and McGill, where similar studies were undertaken in younger tree-based intercropping systems.

1.3 OBJECTIVES AND HYPOTHESES

This study aims to provide an update on the soil carbon sequestration potential 25 years after establishment of a tree-based intercropping system, by measuring the spatial distribution of soil organic carbon constituents in surface soils sampled from the area surrounding five different tree species. The null hypotheses for this study are: (i) there is no difference in soil organic carbon stocks between the tree-based intercropping system and the conventional cropping system, (ii) there is no difference in the soil organic carbon stocks surrounding each tree species, and (iii) there is no spatial variation of soil organic carbon at distance, depth and direction of soils surrounding each tree species.

Soil microbial biomass, dissolved organic carbon and total soil organic carbon content in surface soils were measured throughout the 2012 growing season. Soil organic carbon was measured within a systematic spatial grid surrounding the tree base. This research aimed to develop a best approach for carbon stabilization, as influenced by different tree species, within a tree-based intercropping system.

1.4 THESIS OUTLINE

Chapter 2 of this thesis provides a review of the literature pertaining to the carbon cycle, soil organic matter and soil microbial biomass. The literature review also provides a history of agroforestry and current practices of agroforestry in Canada. Chapter 3 focuses on seasonal variation of soil organic matter constituents including soil microbial biomass and dissolved organic carbon. Chapter 4 contains research on the spatial
orientation of soil organic carbon within a tree-based intercropping system. Lastly, Chapter 5 provides concluding remarks for the research presented in this thesis.

1.5 REFERENCES


Chapter 2.0 LITERATURE REVIEW

2.1 THE CARBON CYCLE

Changes to land management practices have had a major impact on increasing atmospheric carbon (CO₂) emissions. Out of the total anthropogenic carbon (C) emissions contributing towards global warming, ~14% of emissions is associated with agricultural practices (Liang et al. 1998; Lal 2004; Peichl et al. 2006; IPCC 2007; Ramnarine 2010; Sanderman and Baldock 2010). Soils contain a large reservoir of organic carbon (SOC) 2500 Pg C, which is more than 2 times greater than that of the terrestrial plant vegetation (610 Pg C) and the atmospheric pools (760 Pg C) combined (Lal 2004; Ramnarine 2010). Carbon is exchanged from the atmosphere to the biosphere during plant photosynthesis where carbon dioxide and water are converted to sugars and oxygen. Some of this carbon is returned back to the atmosphere during plant respiration as carbon dioxide and the remainder becomes plant biomass (Horwath 2007). Organic carbon is deposited into the pedosphere from plant litter and root debris and some ~60% is converted to CO₂ during the microbial decomposition (Horwath 2007). The process of C being removed from the atmosphere by growing plants and having plant residues deposited in soil for transformation into a stable SOC reservoir is referred to as carbon sequestration (Lal 2004; Nair 2012).

2.2 SOIL ORGANIC MATTER

Soil organic matter (SOM) is a complex mixture of organic substances, comprised of plant, animal and microbial residues at various stages of decomposition. SOM is an important indicator of soil quality, productivity and sustainability as it contributes to enhancing soil chemical, physical and biological properties. About 10% of the earth’s
total soil C (140 to 170 Pg C) is stored as stable organic matter in agricultural soils (Lal 2004; Ramnarine et al. 2011).

SOM can be assessed by measurement of the SOC carbon content. The size of the SOC pool is determined by the balance between C additions from non-harvested portions of plants and organic amendments, and C losses during the decomposition of organic matter (Oelbermann and Voroney 2007). During soil formation, SOM content increases to approach a steady state when plant C inputs equal C losses. However, current agricultural practices have disturbed this steady state and caused a decline in SOC content. Growing of annual crops and removal of plant biomass during agricultural production has decreased amounts of plant C inputs returned to the soil (Janzen 2006). Furthermore, tillage practices expose to microbial decay processes organic matter otherwise protected within macro-aggregates (Oelbermann and Voroney 2011). Nevertheless decomposition of plant residues and SOM is essential as it promotes soil biological activity and provides nutrients for subsequent plant uptake (Janzen 2006). Since the purpose of agriculture is to grow crops to harvest for food and fiber, to increase both the amount of carbon sequestered and to continue to harvest crops, plant C inputs (biomass not removed during harvest) must be carefully managed and returned back to the soil.

### 2.2.1 Soil Microbial Biomass

Soil microbial biomass (SMB) is the living component of organic matter and is comprised of bacteria, fungi, protozoa, algae and some nematodes but excludes living organisms greater than 5000 µm³ (earthworms and plant roots). The SMB-C accounts for 1-5% of SOC and as such is a significant portion of the active fraction of SOM. The SMB has a rapid turnover time (<5 y), therefore it responds quickly to changes in soil management (Kandeler 2007; Ramnarine 2010). Long before management effects on
SOC are detectable, measurements of SMB-C can indicate stresses within the soil (Kandeler 2007; Voroney et al. 2008; Ramnarine et al. 2010). While plant residues are the major source of soil C inputs, it is the products of microbial decay that are responsible for the formation of stable SOC and the humification process. Plant residues provide soil microorganisms substrates for growth and it is their components that are slowly transformed to stable SOC (Liang et al. 2010; Miltner et al. 2012).

2.3 AGROFORESTRY

The integration of trees within agricultural land management is considered to increase conservation of soil C as the addition of litter from trees increases plant biomass-C inputs (Thevathasan and Gordon, 1997; Oelbermann and Voroney 2007; Clinch et al. 2009; Lancombe et al. 2009). Agroforestry is a land management practice in which trees are included within the production of cropping or animal pasture systems (Buttoud 2013). Almost half the world’s agricultural landscape has at least 10% tree cover, indicating the interest and benefit of including trees (Garrity 2012; Buttoud 2013). However, most of this integration of trees with crop production has been within tropical ecosystems.

2.3.1 Introduction of Agroforestry in the Tropics

Integrating trees within cropping systems has always been a practice for smallholder farms within the tropics (Garrity 2012). In 1975, John Bene and a team of researchers from the Canadian International Development Research Centre (IDRC) completed a review of forestry within the tropics. Since industrialization, population pressure and demand for food had destroyed large forested areas of the tropical landscape. They came to the conclusion that first priority should be integrating trees with crops and animals into a combined production system. Agroforestry was suggested to tackle issues
of nutrient leaching and erosion, as well as to mitigate further formation of unproductive lands. Although trees had already been integrated within several tropical ecosystems, they recommended that there was need for more research and an internationally financed organization. As a result, the International Centre for Research in Agroforestry (ICRAF) was established in 1978 (Bene et al. 1977; King 1987; Steppler 1987).

In the 1980s, agroforestry was specifically directed towards the functionality of the land and for meeting the needs of the community. For example, in wind-prone areas, trees were established for use as shelterbelts; in areas where fuel wood was scarce, the emphasis of the land management system in agroforestry was directed towards fuel wood production (Nair 1987). There was still a serious gap in knowledge when it came to experimental data which ICRAF was working towards bridging. (Steppler 1987; Nair 1987). In the 1990s, the scientific community became involved in addressing research questions towards agroforestry as it is implemented in practice.

2.3.2 Canadian Agroforestry Practices

The abundance and availability of arable land within Canada has not warranted Canadian farms to adopt agroforestry practices to the same extent as in the tropics (Reynolds et al. 2007). In contrast, European settlers practiced land clearing to create farmland and for the harvest of lumber (Reynolds et al. 2007; Thevathasan et al. 2012). Although not due to land availability, inclusion of trees into agricultural practices is used to address various environmental demands and is included into today’s definition of agroforestry. Planting crops within forests was part of traditional ecological knowledge of aboriginal peoples. Windbreaks and shelterbelts (trees planted in a row within or around field boundaries) have been included to protect against wind erosion in southern Ontario, Atlantic Canada and the Prairie provinces. Windbreaks have also been established to
reduce odor within livestock production facilities (feedlots) in western Canada and to reduce dust in British Columbia (Reynolds et al. 2007; Thevathasan et al. 2012). Establishment of riparian buffer strips (tree, shrub and grass buffers between farm fields and water sources) is recommended to address concerns related to non-point source of pollution (nutrients, herbicides and pesticides) entering lakes and streams from potato production in the Atlantic Provinces. Since the Walkerton water enquiry, Riparian Buffer Strips have been included within the Best Management Practices of manure management within Ontario farming systems (Government of Ontario 2003). Forest farming (utilizing both native and managed forests for food and other non-timber products), although not widely practiced in Canada, is used for mushroom and ginseng production in the Atlantic Region. Silvopastoral systems (integrating trees, forages and livestock) are used within the Atlantic region for forest grazing. Alley cropping or tree-based intercropping, in which agricultural crops are grown between alternating rows of trees and other crops, has been used in fruit production within British Columbia to help recover start-up costs and to improve soil conditions (Thevathasan and Gordon 2004; Reynolds et al. 2007; Thevathasan et al. 2012).

Although forms of agroforestry have been in place in Canada for some time, it is a relatively new researched topic within temperate regions. Agroforestry has become an important agriculture system in order to counter some of the effects of climate change brought on by unsustainable agricultural practices. Climate change mitigation strategies have become a focal point at international meetings since the 1990’s. Research confirming the ability of soil to sequester carbon has encouraged adoption of SOM conservation practices, and agroforestry land-use systems are amongst the measures suggested by the International Panel on Climate Change (IPCC) (Peichl et al. 2006; Oelbermann and Voroney 2007; IPCC 2007; Nair 2012;). Agroforestry systems have
higher organic matter input in comparison to monocropping systems as trees provide additional plant C inputs (as litterfall, through fall, stem flow and root turnover) in combination with residues provided by the agricultural crops (Oelbermann and Voroney 2011).

2.4 UNIVERSITY OF GUELPH, AGROFORESTRY RESEARCH SITE

The University of Guelph’s Agroforestry Research Station consists of 30 hectares of farmland located in Wellington County (43 16’N 89 26’W) (Oelbermann 2002). The field site is on a west-facing side slope of a drumlin with an average slope of 6%. The elevation of the field is 346.2 m maximum and 330.7 m minimum above sea level. The mean annual temperature is 7.2° C, with an annual precipitation of 830 mm of which 340 mm falls during the growing season (May to August). The average frost-free period is 136 d (Peichl et al. 2006; Oelbermann and Voroney 2007; Reynolds et al. 2007).

The site was converted into a long-term tree-based intercropping station in 1987. Tree species of various hardwood genera (Juglans, Quercus, Fraxinus, Acer, and Populus) and coniferous genera (Picea, Thuja, and Pinus) were planted. The tree rows were oriented along the NW-SE axis of a drumlin (referred to N-S in the thesis) (Thevathasan and Gordon, 2004). Each tree species was planted in blocks consisting of eight trees. Locations of blocks of each tree species were made through a randomized complete block design. The tree rows were either 12.5 or 15 m apart. Annual crops planted between tree rows included corn (Zea mays), barley (Hordeum vulgare L.), soybean (Glycine max L. Merr.) and winter wheat (Triticum aestivum L.) in rotation. A conventional cropping system is located adjacent to the agroforestry research plots and has been under the same soil and crop management systems and was used as the control treatment (Peichl et al. 2006; Oelbermann and Voroney 2007; Reynolds et al. 2007).
The soil has been classified as an Albic Luvisol according to the FAO soil system classification (Peichl et al. 2006) or a Gray Brown Luvisol according to the Canadian System of Soil Classification (Oelbermann and Voroney 2007). The soils are underlain by 18 to 20 m unconsolidated glacial till above bedrock. The surface soil belongs to the sandy loam textural class containing 65% sand, 25% silt and 10% clay. The depth of the Ap horizon ranges from 28 to 53 cm. The pH of the top 30 cm soil is 7.4 compared to 7.7 at a depth of 30-40 cm (Oelbermann 2002). The drainage has been labeled as naturally imperfect to moderately well drained (Oelbermann 2002). Much of the site is tile-drained. The site has been given a Canada Land Inventory Rating for agriculture of 3 (Peichl et al. 2006).

Before agroforestry was established at this site, the field was moldboard plowed to a depth of 20 cm in late autumn after crop harvest. This was followed in the spring by seedbed preparation with a disc plow to a depth of 10 cm (Oelbermann and Voroney 2007). In 1991 a disc plow was used to till the cropped field and the site has been under no-till cultivation since 1996.

In 2002, samples were collected every 1 m to 12 m perpendicular to the tree rows into the cropped area in 2 depth classes (0-5 cm and 5-20 cm), which was planted to barley (Peichl et al. 2006). Samples were acquired in the same depth classes at an adjacent conventional cropping site, which was different from the conventional site used in this study. A significantly higher concentration of soil C across all distances within poplar samples (3%) was found when compared to both the spruce (2.5%) and barley (2.4%) (Peichle et al. 2006) Oelbermann and Voroney (2007) collected samples at different distances and depths surrounding the poplar trees in fall 2001. Sampling transects were located 1.0, 3.5, 6.3, 9.0 and 11.5 m from the tree row east and west at depths 0-10, 10-20 and 20-40 cm. They measured carbon using the Dumas method and
the Tracemass® Isotope Ratio Mass Spectrometer. Within the 0-10 cm depth profile they found no significant difference in SOC content (19 mg C g⁻¹) at 1 m in comparison to 6.3 m (15 mg C g⁻¹). They found that SOC declined from 17 mg C g⁻¹ in the top 20 cm to 8 mg C g⁻¹ at 20-40 cm. Previous to this study, no-till agriculture had been established and the variation between depth could have been attributed to this change in tillage management. They concluded that results from this study showed the potential to sequester C, but changes in SOC from agroforestry systems should be measured over a long period of time (Oelbermann and Voroney 2007).

### 2.5 CONCLUSIONS

Land management practices such as agroforestry land-use systems that focus on increasing soil organic carbon have been identified as one way to reduce levels of atmospheric CO₂. The addition of tree litter by trees grown in agroforestry systems has shown potential to sequester C in the soil in the short-term, however the long-term C sequestration potential has not been extensively researched within the temperate region. SOC is an important measure as it represents the soil reservoir of C in the global C cycle, and SOM pools must be included within these measures. Dissolved organic carbon can be an indicator of plant C inputs and C sequestration within the soil profile and therefore has potential for long-term storage. Microbial biomass is important as an early indicator of management effects on additional C inputs and transformations of SOC.
2.6 REFERENCES


**IPCC. 2007.** Climate change 2007: Impacts, adaptation and vulnerability. Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change. Pages 976 in M. L. Parry, O. F. Canziani, J. P. Palutikof, P. J. van der Linden and C. E. Hanson, eds. Cambridge University Press, Cambridge, UK.

**Janzen, H. H. 2006.** The soil carbon dilemma: shall we hoard it or use it? Soil Biology and Biochemistry. 38: 419–424.


CHAPTER 3:

Seasonal differences in soil microbial biomass carbon in a tree-based intercropping system located in Southwestern Ontario

3.1 INTRODUCTION

Soil microbial biomass (SMB) is comprised of the living organisms, mainly microorganisms, and the carbon within the SMB (SMB-C) accounts for 3-5% of soil organic carbon (SOC). This pool of organic carbon is considered to be a major portion of the active fraction of soil organic matter and with a turnover time of 1-5 years, responds quickly to changes in management practices. The size and activity of the SMB is controlled by soil and plant management factors and its measurement is useful in determining the response of soil microbiota to management (Kandeler 2007; Ramnarine 2010).

Measurements of SMB-C provide an indicator of soil carbon (C) inputs and help to determine the soil’s role as either a source or sink of carbon (Oelbermann 2002). Microbes build up their biomass by decomposing plant residue-C and are involved in its transformation to stable SOM (Liang et al. 2010; Miltner et al. 2012).

The largest pool of global terrestrial C is stored within the soil (2500 Pg C), whereas the atmospheric pool contains 760 Pg. Close to ten percent of total soil C is stable organic matter (140 to 170 Pg C) (Lal 2004; Grandy and Neff 2008; Ramnarine 2010). Considering that soils are a major C reservoir, changes in their C levels could have a major impact on the concentrations of atmospheric CO₂. The storage of SOC represents the balance of carbon inputs from plant litter and organic amendments and losses during decomposition as CO₂ is released to the atmosphere (Oelbermann and Voroney 2007).
Changes in agricultural cropping systems alter both the quantity and quality of carbon inputs from plant residues and the stability of SOM (Lal 2004; Ramnarine 2010). Alternative land management practices have been suggested to conserve soil C and reduce the role of agriculture in global carbon emissions; including agroforestry (Shroeder 1994; Oelbermann 2002; Peichl et al., 2006).

Agroforestry has been recognized as an alternative land use system for its role in capturing atmospheric carbon dioxide and for its potential to store SOC (Nair 2012). Tree-based intercropping (TBI) is an agroforestry system that incorporates trees into agricultural practices, and supports the production of trees and crops together within the same field area (Thevathasan et al. 2004). The integration of trees within agricultural land management systems has been shown to increase soil C by supplying increased C inputs (Oelbermann and Voroney 2007). Above-and below-ground tree components such as branches, leaves and roots provide organic C inputs to the soil (Oelbermann 2002) from the annual autumnal litterfall from the trees and from root production (Thevathasan and Gordon 1997).

Previous studies in Ontario have examined the variability of SOC in TBI systems and compared TBI systems to conventional agriculture. Bambrick et al. (2010) collected soil samples from under Norway spruce and hybrid poplar in fall 2007 at 0.75 m and 5.0 m from the tree row at 2 depths (0-5 cm and 5-20 cm). They reported no significant difference in the SOC content at distance from the hybrid poplar into the intercropped field. However, they did find greater SOC content 0.75 m from spruce trees in comparison to the cropping area 5.0 m from tree.

SOC in soils at the University of Guelph Agroforestry Research Site have been studied extensively over the past 25 years. The focus of this current study concerned soil microbial biomass carbon (SMB-C) as it is the products of microbial decay that are
responsible for the formation of stable SOC and the humification process. This study aimed to quantify the long-term effects of a temperate tree-based intercropping system on soil microbial biomass 25 years after the site was established. The goal of the research was to determine measures of SMB-C in soils under and near four tree species: Red Oak (*Quercus rubra*), Norway Spruce (*Picea abies*), Hybrid Poplar (*Populus* sp), and Black Walnut (*Juglans nigra*). It is hypothesized that the size of the SMB-C in soils under the tree-based intercropping system would not be different from those in the adjacent conventionally cropped system. Deviations from this null hypothesis will be tested.

### 3.2 MATERIALS AND METHODS

#### 3.2.1 Site Description and Management: University of Guelph Agroforestry Research Station

The Agroforestry Research Station at the University of Guelph consists of a 30 ha site located in Wellington County (43 16’N 89 26’W) (Oelbermann 2002). The site was set out as a long-term tree-based intercropping experiment in 1987. Fifteen tree species were established and planted in rows in blocks of eight trees. Tree rows are oriented along a NE-SW direction on the westerly slope of a drumlin (referred to N-S) (Reynolds et al. 2007) shown in Figure 3.1. Trees were spaced at either 1 m, 3 m or 6 m within the rows, depending on tree species, and either 12.5 m or 15 m between the rows. Figure 3.2 displays the spacing of tree species examined within this study. The annual crops planted in between the tree rows were corn (*Zea mays*), soybean (*Glycine max*), winter wheat (*Triticum aestivum*) or barley (*Hordeum vulgare*) in rotation. At the time of this study the annual crop planted was soybean.

The mean annual temperature at the site is 7.2ºC, with an annual precipitation of 830 mm, 340 mm of which falls during the growing season (May to August). The average
frost-free period is 136 days (Oelbermann and Voroney 2007). The soils have been classified as Gray Brown Luvisols (Oelbermann and Voroney 2007). The depth of the A-horizon ranges from 28 to 53 cm and the soil pH within the top 30 cm is 7.4 (Oelbermann 2002).

Figure 3.1 Location of the University of Guelph’s Agroforestry Research Station (43°16’N 89°26’W)
Figure 3.2 Schematic diagram of the experimental design of the University of Guelph’s Agroforestry Research Station in Guelph Ontario
3.2.2 Soil Sampling and Preparation

Soils from adjacent to the rows of four different tree species were sampled in summer 2012, fall 2012 and spring 2013. The tree species were Norway spruce, red oak, poplar hybrid and black walnut. Soil samples were collected within the adjacent area cropped to soybeans (*Glycine max*) at distances of 2 m and 6 m, both east and west, from each selected tree, as displayed in Figure 3.3. Three replicates of each tree species were selected at random and soil samples were collected at locations at distance from each tree. Samples were collected from a nearby conventionally managed (control) field in fall 2012 and spring 2013. The soil samples from the control field were collected based on a systematic contour sampling along a grid as displayed in Figure 3.4. These sampling locations were selected in conjunction with soil cores collected for analysis of soil structure (CT-scanning) (Jefferies, 2014). Soil samples were collected from the area surrounding each core to a depth of 15 cm. Each sample was mixed and a sub-sample of ~500 g of soil stored in polyethylene bags was kept at 4°C until analyzed for SMB-C.

In preparation for SMB-C measurements, plant roots and other residues were removed by handpicking and the soil was passed through a 2-mm sieve. Soil water content (SWC) was adjusted to ~20% volumetric water content and pre-incubated for 7 d in the laboratory at 22°C to allow soil microbes to recover from the soil preparation treatment and for their activity to stabilize.
Figure 3.3 Schematic diagram of sampling locations 2 m and 6 m east and west of the tree base collected from spruce, oak, walnut and poplar in summer 2012, fall 2012 and spring 2013 at the Guelph Agroforestry Research Site, Guelph ON
Figure 3.4 Systematic contour sampling along a grid within the conventional cropping system (Control). Sampling locations labeled as M1-M6.

3.2.3 Laboratory Analysis

3.2.3.1 Microbial Biomass Carbon and Dissolved Organic Carbon

Soil microbial biomass carbon (SMB-C) was measured using the chloroform(CHCl$_3$)-fumigation extraction method adapted from Voroney et al. (2008). Six 15 g subsamples of the pre-incubated soils were weighed into 80 mL sealable glass containers. Three replicates of each sample were fumigated and three samples were left un-fumigated. The soils to be fumigated were placed in a glass vacuum desiccator lined with moistened paper towels and a beaker containing boiling chips and 50 mL CHCl$_3$. The desiccator was evacuated for 5 min until CHCl$_3$ bubbled; it was then left sealed for 24 h in a dark room at 22°C.
The un-fumigated samples were immediately extracted with 60 mL of 0.05 M K$_2$SO$_4$ (Makarov et al. 2013; Voroney et al. 2008). The soil extracts were shaken for 1 h at 160 rpm and filtered through 1.0 µm glass fibre filter paper (Whatman®).

Measurements of the organic carbon in the un-fumigated soil extracts were used for estimates of dissolved organic C (DOC) (Morris 2008; Makarov et al. 2013). SOC in extracts was measured as described below.

After soil fumigation, the desiccator vacuum was slowly released, the beaker of CHCl$_3$ and paper towel removed and the desiccators evacuated for 20 min; this was repeated 3 times to remove residual CHCl$_3$ vapour. The fumigated subsamples were then extracted, shaken, filtered, and analyzed, as outlined for the un-fumigated subsamples (Joergensen and Olfs 1998; Voroney et al. 2008; Ramnarine 2010).

Soluble organic C in the extracts was measured using a Shimadzu® TOC analyzer (Model TOC-5000A, Shimadzu Corporation, Kyoto, Japan). Soil microbial biomass C was calculated as the difference between the OC of the fumigated and the un-fumigated subsamples:

\[
\text{Microbial Biomass C} = (C_F - C_{UF}) / k_{EC}
\]

Where \( C_F \) = organic C in fumigated soil

\( C_{UF} \) = organic C in un-fumigated soil

\( k_{EC} = 0.35 \) = efficiency of extraction of microbial biomass C

(Voroney et al. 2008)
3.2.3.2 Organic Carbon

Carbonate removal from soil was performed using a H$_2$SO$_3$ method described by Wotherspoon et al. (in press), adopted from Shaw (1959), Skjemstad and Baldock (2008), and Ramnarine et al. (2011). Soils that had been ground to <0.125 mm were weighed and transferred (~1.00 g) to 20 mL glass vials. Soil mass change due to the acid treatment was determined by recording vial weights, initial soil mass and final soil mass. Soils were moistened with 500 µL of nano-pure water and placed on a hot plate set to 65°C. 1 mL of 0.73 M H$_2$SO$_3$ was added hourly by micropipette until the effervescence reaction stopped (to a minimum of 6 mL and maximum of 8 mL). Once samples had stopped reacting with the acid, they were removed from the hot plate and placed in a glass vacuum desiccator (7.5 L) together with a beaker containing NaOH pellets as a desiccating agent (adapted from Shaw 1959). The desiccator was vacuum-sealed using a vacuum pump and samples were left overnight. The following day the NaOH pellets were removed and the samples were dried at 50°C for approximately 48 h. The samples were cooled to room temperature and weighed for final soil mass. A sample of the soil (~0.30 g) was measured for SOC content using a LECO CR-12, combusted at 1300°C.

3.2.4 Statistical Analysis

Normality of data was confirmed through visual inspection of Q-Q plots. An analysis of variance (p = or <0.05) was used to test the differences of soluble SOC and microbial biomass C means as influenced by tree species and seasons. A Welch’s t-test was used to determine the correlation of variables between the tree species and seasons.
and distances between the trees. Pearson’s correlation coefficient was used to determine the correlation between soil microbial biomass and organic carbon values.

3.3 RESULTS AND DISCUSSION

3.3.1 Soil Microbial Biomass Carbon

A factorial analysis showed no significant differences (at $\rho >0.05$) of SMB-C at directions east and west (Figure 3.5) or distances 2.0 m and 6.0 m (Figure 3.6) from the tree rows. Figure 3.7 displays the mean SMB-C at each sampling point with no significant differences (at $\rho >0.05$). The comparable spatial values of SMB-C in the nearby alley-cropped area adjacent to each tree may be an indication of tree maturity in that as trees age their litterfall is dispersed in a more homogeneous pattern (Thevathasan and Gordon 2004). The uniform SMB-C provides evidence of the relatively uniform annual inputs of plant C. These results were consistent with walnut, spruce, oak and poplar where there was no variation within sampling locations. Table 3.1 and Figure 3.8, show that a significantly higher (at $\rho >0.05$) SMB-C was found in the areas adjacent to spruce when compared to all other species as well as the conventional control plot. The spruce species were planted at higher density of 3.0 m spacing between trees instead of 6.0 m spacing of all other species assessed within this study, which could have contributed to higher microbial communities. Further, the Norway Spruce was the only coniferous species studied. According to Kubertová et al. (2009), deciduous species should be more favorable to microbial decomposers than coniferous species. Part of that study sought to examine the effect of tree species on fungal species richness and community structure in litter during litter decay in the Morvan Mountains, France. They
found that the spruce species showed the most distinct fungal community structure when compared to beech, oak and Douglas-fir species. However, they found many fungal species that were present only in litter of a mixed tree species sample. Greater enzyme diversity and niche complementarity are generally anticipated to increase microbial species richness (Vesterdal et al. 2013; Kubertová et al. 2009).

Spruce are often recommended for use as shelterbelts (Kort and Turnock 1999) and would have created a barrier for the transportation of litterfall by wind in this study. This wind barrier effect may have contributed to greater diversity of litter accumulating under the spruce trees from the deciduous tree species on the site. It could also suggest greater crop growth in the area adjacent to the spruce species due to higher soil moisture contributed by higher snow accumulation (Dingman 2008) and also enhanced solar radiation closer to the spruce trees in summer due to its conical canopy shape.
Figure 3.5 Soil microbial biomass C (mg MB-C kg\(^{-1}\)) across all tested tree species, sample locations and seasons (summer 2012, fall 2012 and spring 2013) at east and west direction from tree rows into the cropping alleys. Boxes represent 25\(^{th}\) to 75\(^{th}\) percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.

Figure 3.6 Soil microbial biomass (mg MB-C kg\(^{-1}\) soil) at distances 2.0 m and 6.0 m from tree rows into the cropping alleys across all tested tree species (spruce, walnut, oak and poplar) and seasons (from samples collected in summer 2012, fall 2012 and spring 2013). Boxes represent 25\(^{th}\) to 75\(^{th}\) percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.
Figure 3.7 Soil microbial biomass (mg MB-C kg\(^{-1}\) soil) sampled at 2 and 6 m on both sides of the tree row a) east b) west across all tested tree species (poplar, spruce, oak and walnut). Boxes represent 25\(^{th}\) to 75\(^{th}\) percentile of sampled values, horizontal line represent sample medians, and dots indicate data outliers.
Figure 3.8 Soil microbial biomass (mg MB-C kg⁻¹ soil) in soil samples collected from intercropped poplar, spruce, walnut, oak and from the conventional cropping field, across all locations and seasons. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.
Table 3.1 Microbial biomass carbon (mg MB- C kg soil\(^{-1}\)) in direction east and west and with distance 2.0 m and 6.0 m from tree rows in poplar, spruce, walnut and cedar

<table>
<thead>
<tr>
<th></th>
<th>Poplar</th>
<th>Spruce</th>
<th>Oak</th>
<th>Walnut</th>
<th>Conventional</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>172(^A)</td>
<td>264.2(^B)</td>
<td>186(^A)</td>
<td>183.7(^A)</td>
<td>179.5(^A)</td>
</tr>
<tr>
<td></td>
<td>(± 11.21)</td>
<td>(± 21.71)</td>
<td>(± 12.09)</td>
<td>(± 9.44)</td>
<td>(± 31.01)</td>
</tr>
<tr>
<td></td>
<td>West</td>
<td>West</td>
<td>West</td>
<td>West</td>
<td></td>
</tr>
<tr>
<td>2.0 m</td>
<td>178(^a)</td>
<td>222(^a)</td>
<td>202(^a)</td>
<td>192(^a)</td>
<td></td>
</tr>
<tr>
<td>6.0 m</td>
<td>154(^a)</td>
<td>309(^a)</td>
<td>184(^a)</td>
<td>177(^a)</td>
<td>(± 36.30)</td>
</tr>
<tr>
<td></td>
<td>(± 26.75)</td>
<td>(± 39.79)</td>
<td>(± 22.09)</td>
<td>(± 14.05)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(± 13.53)</td>
<td>(± 48.01)</td>
<td>(± 36.30)</td>
<td>(± 27.63)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>East</td>
<td>East</td>
<td>East</td>
<td>East</td>
<td></td>
</tr>
<tr>
<td>2.0 m</td>
<td>194(^a)</td>
<td>279(^a)</td>
<td>167(^a)</td>
<td>204(^a)</td>
<td></td>
</tr>
<tr>
<td>6.0 m</td>
<td>168(^a)</td>
<td>241(^a)</td>
<td>194(^a)</td>
<td>165(^a)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(± 28.52)</td>
<td>(± 37.93)</td>
<td>(± 17.92)</td>
<td>(± 19.02)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(± 21.13)</td>
<td>(± 51.14)</td>
<td>(± 24.39)</td>
<td>(± 18.32)</td>
<td></td>
</tr>
</tbody>
</table>

Standard errors are given in parenthesis (n=5). Values followed by the same upper-case letters, comparing species are not significantly different at \(\rho=0.05\). Soil sampling locations for each species followed by the same lower case letters, are not significantly different at \(\rho=0.05\).
Overall there was no seasonal variation in SMB-C content (Figure 3.9). However there were significant differences (at $p<0.05$) in the tree species comparison across tested seasons for spruce, poplar and walnut. The same trees were not selected within each season, and therefore were at different locations within the field, and this may have had an impact on measurement variability. Within the conventional treatment (control) the same locations were chosen for the fall and spring samplings and there was no significant difference (at $p>0.05$) attributed to the seasonal comparison. The varying topography of these sampling sites could have impacted the SMB-C content as the SMB-C content was highest within samples collected at the toe slope of the conventional cropping system. The downslope transport of water and nutrients from the slope impacts soil chemistry, vegetation and productivity (Seibert et al. 2007).
Figure 3.9 Soil microbial biomass C (mg MB-C kg\(^{-1}\) soil) of soil samples collected in summer 2012, fall 2012 and spring 2013 from tree row into the crop row of poplar, spruce, walnut and oak. Boxes represent 25\(^{th}\) to 75\(^{th}\) percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.

3.3.2 Organic Carbon

There were no significant differences (at \(\rho > 0.05\)) in SOC concentrations with distance or direction from the tree rows (Figures 3.10, 3.11 and 3.12). The similarity in concentrations may be attributed to the maturity of the trees. The highest SOC concentration was found surrounding the poplars with significant differences (at \(\rho > 0.05\)) when compared to both spruce and oak (Table 3.2; Figure 3.13). These results are consistent with those reported by Wotherspoon (2014) who found higher surface SOC contents in soils adjacent to poplars when compared to soils adjacent to oaks, walnuts and
spruces. Significantly higher (at $\rho > 0.05$) SOC concentrations were also found within the conventional cropping system when compared to the TBI system.
### Table 3.2 Soil organic carbon (%) at direction east and west and with distance 2.0 m and 6.0 m from the tree rows of poplar, spruce, walnut and cedar

<table>
<thead>
<tr>
<th>Species</th>
<th>West 2.0 m</th>
<th>West 6.0 m</th>
<th>East 2.0 m</th>
<th>East 6.0 m</th>
<th>Conventional 2.0 m</th>
<th>Conventional 6.0 m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poplar</td>
<td>1.96&lt;sup&gt;A&lt;/sup&gt; (+0.06)</td>
<td>1.76&lt;sup&gt;B&lt;/sup&gt; (+0.06)</td>
<td>1.68&lt;sup&gt;A&lt;/sup&gt; (+0.08)</td>
<td>1.93&lt;sup&gt;A&lt;/sup&gt; (+0.15)</td>
<td>1.89&lt;sup&gt;AB&lt;/sup&gt; (+0.04)</td>
<td>2.38&lt;sup&gt;C&lt;/sup&gt; (+0.16)</td>
</tr>
<tr>
<td>Spruce</td>
<td>2.06&lt;sup&gt;a&lt;/sup&gt; (+0.10)</td>
<td>1.62&lt;sup&gt;a&lt;/sup&gt; (+0.11)</td>
<td>1.74&lt;sup&gt;a&lt;/sup&gt; (+0.10)</td>
<td>1.76&lt;sup&gt;B&lt;/sup&gt;(+0.11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oak</td>
<td>1.68&lt;sup&gt;a&lt;/sup&gt; (+0.10)</td>
<td>1.74&lt;sup&gt;a&lt;/sup&gt; (+0.07)</td>
<td>1.97&lt;sup&gt;a&lt;/sup&gt; (+0.08)</td>
<td>1.76&lt;sup&gt;B&lt;/sup&gt;(+0.11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walnut</td>
<td>1.89&lt;sup&gt;AB&lt;/sup&gt; (+0.04)</td>
<td>2.38&lt;sup&gt;C&lt;/sup&gt; (+0.16)</td>
<td>1.76&lt;sup&gt;B&lt;/sup&gt;(+0.11)</td>
<td>1.76&lt;sup&gt;B&lt;/sup&gt;(+0.11)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Standard errors are given in parenthesis (n=5). Values followed by the same upper-case letters, comparing species are not significantly different at $p=0.05$. Soil sampling locations for each species followed by the same lower case letters, are not significantly different at $p=0.05$. 
Figure 3.10 Soil organic carbon (%) at direction east and west from tree row into crop row of spruce, walnut and oak and poplar from samples collected in summer 2012, fall 2012 and spring 2013. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and the dot indicates a data outlier.

Figure 3.11 Soil organic carbon (%) at distances 2.0 m and 6.0 m from tree row into the crop row across all tested tree species and seasons. Boxes represent 25th to 75th percentile of sampled values and horizontal lines represent sample medians.
Figure 3.12 Soil organic carbon (%) at 2 and 6 m from the tree row on either sides (East and West) across all tested tree species. Boxes represent 25$^{th}$ to 75$^{th}$ percentile of sampled values, horizontal lines represent sample medians, and the dot indicates a data outlier.
Significantly higher (at $\rho >0.05$) SOC concentrations were found in the soil samples collected in the spring compared to those taken in fall and summer. Since measurements would not be able to detect seasonal differences in SOC concentrations, these differences in the SOC concentrations can only be attributed to the different sampling locations. This variation was consistent within seasonal samplings of spruce and oak. The conventional cropping system did not vary between seasons. The sample locations within the conventional cropping system field did not change between seasons.
and could therefore suggest the influence of topography on the dispersion of SOC concentration within this field site.

Figure 3.14 Soil organic carbon (%) as influenced by sampling time across all tested tree species. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and the dot indicates a data outlier.

3.3.3 Dissolved Organic Carbon

Dissolved organic carbon (DOC) is readily used by soil microbes as it is a highly labile C pool (Solinger et al. 2001; Neff and Asner 2001; Morris 2008). Because it is soluble in water, it can be leached to deeper soils depths where it makes an important contribution to soil C in deeper horizons and to the activity of soil microorganisms (Neff and Asner 2011). A factorial analysis showed no significant differences (at $p > 0.05$) of dissolved organic carbon at distance from trees (Figure 3.17) but a significantly higher (at
dissolved organic carbon content to the east of tree rows compared to the west (Figure 3.16). Typical westerly winds within southwestern Ontario would suggest that higher amounts of litterfall would be distributed east of the tree rows. Wotherspoon (2014) found higher annual litterfall east of tree rows of walnut and poplar species, but higher west of spruce and oak tree rows.

Within this study, the DOC showed the same pattern as Wotherspoon’s (2014) litterfall data at the same site. As presented in Table 3.3, the soils west of the walnut and poplar rows had significantly higher (at $\rho > 0.05$) DOC contents than those soils east of the tree rows. The DOC of soils adjacent to spruce and oak rows tended to be higher than those west of the trees. Distance from the tree played a significant role in the DOC pool in both spruce and oak. Spruce had higher DOC at 2.0 m compared to 6.0 m, while oak had higher DOC at 6.0 m.

A significantly higher (at $\rho > 0.05$) DOC content was found in soils sampled from the conventional cropping system when compared to the tree-based intercropping system. There were no differences in the soil DOC contents of walnut, poplar and spruce but it was significantly lower (at $\rho > 0.05$) in soils adjacent to the oak. This is consistent with litterfall data reported by Wotherspoon (2014) who found the lowest litterfall adjacent to oak trees. There was a significant difference (at $\rho > 0.05$) in DOC contents between all seasons with the highest DOC in the fall. This was expected as the greatest input of plant material is in fall from both tree litterfall and crop residues.
**Figure 3.15** Dissolved organic carbon (mg C kg\(^{-1}\) soil) at direction east and west from tree rows of spruce, walnut and oak and poplar from samples collected from soils in the adjacent crop (soybeans) in summer 2012, fall 2012 and spring 2013. Boxes represent 25\(^{th}\) to 75\(^{th}\) percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.

**Figure 3.16** Dissolved organic carbon (mg C kg\(^{-1}\) soil) in soil at distances 2.0 m and 6.0 m from rows of spruce, walnut, oak and poplar. Soil samples were collected in summer 2012, fall 2012 and spring 2013. Boxes represent 25\(^{th}\) to 75\(^{th}\) percentile of sampled values and horizontal lines represent sample medians.
Table 3.3 Dissolved Organic Carbon (mg C kg soil\(^{-1}\)) at direction east and west and with distance 2.0 m and 6.0 m from the tree rows of poplar, spruce, walnut and cedar

<table>
<thead>
<tr>
<th>Species</th>
<th>East 2.0 m</th>
<th>East 6.0 m</th>
<th>West 2.0 m</th>
<th>West 6.0 m</th>
<th>East 2.0 m</th>
<th>East 6.0 m</th>
<th>West 2.0 m</th>
<th>West 6.0 m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poplar</td>
<td>27.7(^b) (± 0.82)</td>
<td>27.49(^b) (± 1.09)</td>
<td>21.89(^a) (± 0.93)</td>
<td>27.85(^c) (± 1.02)</td>
<td>33.54(^c) (± 1.79)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spruce</td>
<td>25.15(^a) (± 1.19)</td>
<td>25.72(^a) (± 1.51)</td>
<td>33.25(^b) (± 1.56)</td>
<td>24.56(^c) (± 2.62)</td>
<td>20.53(^a) (± 1.87)</td>
<td>25.29(^b) (± 1.87)</td>
<td>27.36(^a) (± 1.73)</td>
<td>21.85(^c) (± 2.35)</td>
</tr>
<tr>
<td>Oak</td>
<td>26.61(^a) (± 1.32)</td>
<td>25.73(^a) (± 2.30)</td>
<td>17.63(^a) (± 1.18)</td>
<td>22.32(^a) (± 1.79)</td>
<td>27.20(^a) (± 1.73)</td>
<td>33.93(^a) (± 1.50)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Standard errors are given in parenthesis (n=5). Values followed by the same upper-case letters, comparing species are not significantly different at \(p=0.05\). Soil sampling locations for each species followed by the same lower case letters, are not significantly different at \(p=0.05\).
Figure 3.17 Dissolved organic carbon (mg C kg\(^{-1}\) soil) at sampled distances a) west and b) east of tree rows. Boxes represent 25\(^{th}\) to 75\(^{th}\) percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.
Figure 3.18 Dissolved organic carbon (mg C kg$^{-1}$ soil) for soil samples collected adjacent to tree rows of poplar, spruce, walnut and oak and within the conventionally cropped field. Samples were collected in summer 2012, fall 2012 and spring 2013. Boxes represent 25$^{th}$ to 75$^{th}$ percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.
Figure 3.19 Dissolved organic carbon (mg C kg\(^{-1}\) soil) of soil samples collected in summer 2012, fall 2012 and spring 2013 from the cropped area of poplar, spruce, walnut and oak. Boxes represent 25\(^{th}\) to 75\(^{th}\) percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.

3.3.4 Proportion of Microbial Biomass Carbon to Total Organic Carbon

SMB-C is considered to account for 1-5\% of SOC. Within the TBI system the SMB-C accounted for 1.1\% of the SOC whereas within the conventional cropping system the SMB-C was 0.7\% of the total SOC concentration. Sampling locations within the
conventional cropping system were selected based on their topographic locations. However, samples collected from the toe-slope of the conventional cropping system had higher SMB-C contents than those from the TBI system, and SMB-C accounted for a higher proportion of the SOC, with values of 1.7% in the spring and 2.3% in the fall. Transport of water and nutrients to lower slope positions may have increased available C supplies to the SMB-C.

3.4 Conclusions

The soil can act as either a source or sink of carbon, and measurements of SMB-C can provide an early indication of the effects of management practices on SOC (Oelbermann, 2002). The TBI system increased the proportion of SOC present as SMB-C suggesting that this management is providing additional carbon inputs. Within the tree-based intercropping system the spatial location of soil samples did not have a significant effect on either the SOC or SMB-C. However, direction from the tree row did affect DOC levels, which can be attributed to increased litterfall on the east side of the poplar and walnut rows and the west side of spruce and oak rows. The orientation of tree rows and the spacing of tree species within the study site could affect the transportation of litterfall by wind and therefore the placement of litterfall according to tree species and location.
3.5 REFERENCES


CHAPTER 4:

Spatial distribution of soil organic carbon within a tree-based intercropping system in southwestern Ontario

4.1 INTRODUCTION

Alternative agricultural land management practices can impact net emissions of CO₂ from soils. Soils hold a large reservoir of carbon (C): 2500 Pg C, which is greater than that of the vegetation (610 Pg) and the atmospheric pools (760 Pg) combined (Lal 2004; Ramnarine 2010). Soil organic carbon (SOC) content is the resulting balance between C additions from non-harvested portions of crops and organic amendments and C losses from the decomposition of soil organic matter (SOM) (Oelbermann and Voroney 2007).

Agroforestry is an agricultural practice intended to contribute additional biomass and consequently carbon to soil. Tree-based intercropping (TBI) is a system of agroforestry that incorporates trees into agricultural practices, for the production of tree resources (lumber, woody biomass, etc.) and crops together within the same field (Thevathasan et al. 2004). Leaves and branches provide increased organic matter inputs in comparison to cropping systems lacking trees (Lal 2004; Peichl et al. 2006; IPCC 2007; Horwath 2007; Oelbermann and Voroney 2007; Clinch et al. 2009; Lancombe et al. 2009).

Much of the available data on agroforestry systems in Canada has come from a study site located in Guelph, Ontario (Thevathasan and Gordon 2004; Oelbermann et al. 2006; Peichl et al. 2006; Oelbermann and Voroney 2007; Reynolds et al. 2007; Bambrick et al.
Results from this site have shown variability in the spatial distribution of SOC at distance from tree rows as the trees have matured (Oelbermann et al. 2006; Bambrick et al. 2010). Peichl et al. (2006), Oelbermann and Voroney (2007) and Thevathasan et al. (2004) reported increased levels of SOC in soils closer to tree rows. Oelbermann and Voroney (2007) also indicated relatively higher SOC contents adjacent to poplar trees compared to within the conventionally cropped field nearby. In soil samples collected from this research site near Norway spruce and hybrid poplar trees in fall 2007, at distances of 0.75 m and 5 m from tree rows and at 2 depths (0-5 cm and 5-20 cm), Bambrick et al. (2010) reported no significant difference in the SOC content in the intercropped field as a function of distance from the hybrid poplars. They found greater SOC content at a distance of 0.75 m than at 5 m from the spruce tree rows. After 25 years of establishment of trees at this site, Wotherspoon et al. (2013) found higher SOC concentrations in surface samples (0-20 cm) in the conventionally cropped fields, but greater amounts at deeper soil depths (20-40 cm) in the TBI system.

The current study, conducted 25 years after the site was established, examined the long-term effects of a temperate TBI system on the constituents of SOC. The goal of the research was to measure the long-term effects of TBI systems on carbon storage within SOM. Soil organic carbon was measured at distance from three tree species: Black Walnut (Juglans nigra), White Cedar (Thuja occidentalis) and Norway Spruce (Picea abies).
4.2 MATERIALS AND METHODS

4.2.1 Site Description and Management: University of Guelph Agroforestry Research Station

The Agroforestry Research Station at the University of Guelph consists of a 30 ha site located in Wellington County (43 16’N 89 26’W) (Oelbermann 2002). The site was set out as a long-term tree-based intercropping experiment in 1988.

Fifteen tree species were planted in row sections of eight trees per species. Tree rows are oriented along the NE-SE axis of a drumlin (referred to N-S) (Reynolds et al. 2007). Trees were spaced at either 1 m (cedar) 3 m (spruces) or 6 m (all other tree species) within the rows, and either 12.5 m or 15 m between the rows, depending on tree species. The crops planted in between the tree rows were corn (Zea mays), soybean (Glycine max), winter wheat (Triticum aestivum) or barley (Hordeum vulgare) in rotation. In the year of this study, the annual crop was soybean.

The mean annual temperature at the site is 7.2°C, with an annual precipitation of 830 mm, 340 mm of which falls during the growing season (May to August). The average frost-free period is 136 days (Oelbermann and Voroney 2007). The soil has been classified as a Gray Brown Luvisol (Oelbermann and Voroney 2007). The depth of the A-horizon ranges between 28-53 cm and the soil pH within the top 30 cm is 7.4 (Oelbermann 2002).

4.2.2 Soil Sampling and Preparation

Soils adjacent to Black Walnut (Juglans nigra), White Cedar (Thuja occidentalis) and Norway Spruce (Picea abies) were taken in the spring and summer 2012 (May-August 2012) using a Concord® hydraulic soil corer. In order to quantify carbon
sequestered at distance and depth from trees, soil cores from around Black Walnut and Norway Spruce were obtained in a grid surrounding each tree to a depth of at least 40 cm and where possible up to 60 cm. Soil samples were taken at distances of 0.5 m, 1 m, 2 m, and 3 m within the tree row and perpendicular to the tree rows in both easterly and westerly directions at distances of 1.5 m, 3 m, 4.5 m and 6 m. At locations 3 m north and south of trees, cores were taken perpendicular to the tree row at distances 1.5 m, 3 m, 4.5 m and 6 m east and west. Three replicates were taken from the soils near cedar trees at 1.5 m, 3 m, 4.5 m and 6 m east and west of the row, as shown below in Figure 4.2. Cores were obtained from an adjacent field managed as a conventional cropping system with the same crop rotation. Soil samples were collected in a transect which extended along the contour of the drumlin as shown below in Figure 4.3.
Figure 4.1 A schematic diagram of soil cores obtained from spruce and walnut plots.
Figure 4.2 A schematic diagram of soil cores obtained from the cedar plots.
In preparation for soil organic carbon measurements, each core was divided into segments of 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm. Each soil sample was air dried and then passed through a 2 mm sieve while hand picking and removing the root and plant material. Each soil sample was homogenized and ground to <0.125 mm.

4.2.3 Laboratory Analysis

Since calcareous soils contain inorganic carbon (IC), it must be removed prior to measurements of SOC. Carbonate removal was performed using the H$_2$SO$_3$ digestion method described by Wotherspoon et al. (in press), adopted from Shaw (1959), Skjemstad and Baldock (2008), and Ramnarine et al. (2011). Soil samples from all depths were weighed (~1.000g) in a 20 mL glass vials. Soil mass change due to loss of
carbonates during acid digestion was determined by recording vial weight, initial soil mass and final soil mass. Soils were moistened with 500 µL of nanopure water, placed on a hot plate set to 65°C and 0.73 M H$_2$SO$_3$ was added, 1 mL hourly (to a minimum of 6 mL and maximum of 8 mL) by micropipette until the effervescence reaction stopped. Samples were then removed from the hot plate and placed in a glass vacuum desiccator (7.5 L) together with a beaker containing NaOH pellets as a desiccating agent (Shaw 1959). The desiccator was vacuum-sealed using a vacuum pump and samples were left overnight. The following day the NaOH pellets were removed and the samples were dried at 50°C for approximately 48 h. The samples were cooled to room temperature, weighed for final soil mass and the soil was manually reground. SOC from a ~0.3000 g subsample, was measured using a LECO CR-12 and combusted at 1300°C.

4.2.4 Statistical Analysis

SOC data were assessed for differences due to sampling direction and depth for each tree species and for comparison to the conventionally cropped field. An ANOVA and Tukey’s t-test for multiple comparisons was used to analyze differences of means between tree species and at different depths. Significant differences of means were calculated using a Welch’s t-test to identify and compare intra-species differences among SOC sampling directions and distances.

4.3 RESULTS AND DISCUSSION

Due to analytical limitations only data from the top 20 cm are presented. A factorial analysis showed statistically significant differences (at $\rho >0.05$) within soils due
to depth, direction from tree row, and surrounding tree species. SOC concentrations were significantly (at \( \rho > 0.05 \)) higher in samples taken from 0-10 cm compared to those from 10-20 cm within the tree-based intercropping site, as shown in Figure 4.4. In the conventionally cropped field, mean SOC concentrations tended to be higher but were not statistically (at \( \rho > 0.05 \)) higher in soil samples from 10-20 cm depth. These trends are consistent with previous studies on the same site by Oelbermann and Voroney (2007) and Wotherspoon (2014).

![Figure 4.4](image)

**Figure 4.4** Soil organic carbon (%) at sampled depths 0-10 cm and 10-20 cm (data from spruce, walnut and cedar trees combined). Boxes represent 25\(^{th}\) to 75\(^{th}\) percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.
A multiple comparisons test indicated that soil samples collected from west of the tree rows had significantly (p<0.05) higher SOC concentrations (1.55%) than those taken from east (1.48%) of the tree row. This trend was found in soils from both 0-10 cm and 10-20 cm as shown in Figure 4.5 and 4.6, respectively. By species, SOC differed (at ρ >0.05) east and west of the walnut trees; this was not the case for soils taken from east and west of the spruces and cedars. Non-significant interactions of direction and species as well as direction and depth were reported by Wotherspoon (2014) in five tree species: poplar, spruce, walnut, cedar and oak. The increased number of soil samples collected from each tree in our study could have attributed to the significant findings east and west of the walnut tree rows.

Differences in SOC with direction from the tree rows were expected in that SOC concentrations should be highest east of the tree due to litterfall distribution from westerly winds. My results suggest that litterfall distribution from the trees does not have a significant on SOC concentration.

Topography results in downslope transport of water and nutrients, which impacts effective precipitation and soil chemistry and consequently the SOC within sites (Seibert et al. 2007). During the experimental design of the tree-based intercropping site, locations of blocks of each tree species were made through a randomized complete block design. Topography was not considered during the experimental design and differences in SOC that can be attributed to the TBI system in this study were confounded by influences from site topography.
Figure 4.5 Soil organic carbon (%) at 0-10 cm depth of sampled directions west and east of tree rows (data averaged across spruce, walnut and cedar tree species). Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.

Figure 4.6 Soil organic carbon (%) at depth 10-20 cm at sampled directions west and east of tree rows (data averaged across spruce, walnut and cedar tree species). Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.
A study in 2002 at the Guelph Agroforestry Research Site (Oelbermann and Voroney 2007) reported numerically higher but not significantly different values at distances closer to tree rows. Wotherspoon (2014) and Bambrick et al. (2010) found that for all tree species, SOC concentrations were numerically higher closer to the tree rows compared to samples taken at distance from the tree rows, but there were no significant differences. As trees age, litterfall should be distributed more uniformly and therefore distance from tree rows should become increasingly less of a factor influencing the distribution of SOC (Thevathasan and Gordon 2004).

It was expected that higher concentrations would occur closer to tree rows due to elevated litterfall closer to trees. The objective of the grid sampling was to analyze spatial distribution of SOC relative to the rows of trees. West of tree rows there were no significant differences (at $\rho >0.05$) in SOC content at different distances, as shown in Figure 4.7 (a) and Figure 4.8 (a). East of tree rows significantly higher (at $\rho >0.05$) SOC concentrations were found at distances 1.5 m within both depths 0-10 cm (1.61%) and 10-20 (1.38%), as displayed in Figure 4.7 (b) and 4.8 (b). Significantly higher (at $\rho >0.05$) SOC concentrations at 1.5 m were found from cedar and walnut tree rows, but not from spruce tree rows. This significant difference at 1.5 m could be attributed to wind direction and leaf litter distribution in the area closest to the tree rows.
Figure 4.7 Soil organic carbon (%) at a depth of 0-10 cm at sampled distances a) west and b) east of tree rows. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.
Figure 4.8 Soil organic carbon (%) at a depth of 10-20 cm at sampled distances a) west and b) east of tree rows. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.
Table 4.1 Soil Organic Carbon (%) in directions east and west, and with various distances from tree (spruce, walnut and cedar) rows in a tree-based intercropping system in southwestern Ontario.

<table>
<thead>
<tr>
<th>Distance from tree row (m)</th>
<th>Spruce</th>
<th></th>
<th>Walnut</th>
<th></th>
<th>Cedar</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>West</td>
<td>East</td>
<td>West</td>
<td>East</td>
<td>West</td>
<td>East</td>
</tr>
<tr>
<td>1.5</td>
<td>1.33</td>
<td>1.40</td>
<td>1.53</td>
<td>1.46</td>
<td>1.58</td>
<td>1.79</td>
</tr>
<tr>
<td></td>
<td>(+ 0.09)</td>
<td>(+ 0.02)</td>
<td>(+ 0.04)</td>
<td>(+ 0.06)</td>
<td>(+ 0.05)</td>
<td>(+ 0.09)</td>
</tr>
<tr>
<td>3.0</td>
<td>1.47</td>
<td>1.54</td>
<td>1.31</td>
<td>1.43</td>
<td>1.28</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>(+ 0.06)</td>
<td>(+ 0.08)</td>
<td>(+ 0.06)</td>
<td>(+ 0.06)</td>
<td>(+ 0.06)</td>
<td>(+ 0.07)</td>
</tr>
<tr>
<td>4.5</td>
<td>1.35</td>
<td>1.36</td>
<td>1.40</td>
<td>1.22</td>
<td>1.55</td>
<td>1.38</td>
</tr>
<tr>
<td></td>
<td>(+ 0.05)</td>
<td>(+ 0.06)</td>
<td>(+ 0.05)</td>
<td>(+ 0.07)</td>
<td>(+ 0.04)</td>
<td>(+ 0.07)</td>
</tr>
<tr>
<td>6.0</td>
<td>1.48</td>
<td>1.29</td>
<td>1.21</td>
<td>1.51</td>
<td>1.56</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>(+ 0.06)</td>
<td>(+ 0.05)</td>
<td>(+ 0.05)</td>
<td>(+ 0.07)</td>
<td>(+ 0.05)</td>
<td>(+ 0.07)</td>
</tr>
</tbody>
</table>

Standard errors are given in parenthesis (n=5).
Within the tree row, north and south distances were only collected in the spruce and walnut species. Soils from within the tree row of cedar could not be collected as cedars are spaced at 1 m distances. There was very little variation of SOC content within tree rows. Although SOC percentage tended to be higher closest to the tree (Figure 4.9 and 4.10), these values were not significantly different (at $\rho >0.05$) from each other. SOC concentrations within the tree row were significantly higher (at $\rho >0.05$) than those in the adjacent cropped area (Figure 4.11 and 4.12).

SOC content of soils from the cropped area adjacent to walnut, cedar and spruce rows was 1.62%, 1.73% and 1.58%, respectively. Slightly higher SOC levels were found in the cropped area adjacent to cedar but they did not differ significantly (at $\rho >0.05$). These results were consistent with Wotherspoon (2014), and could be attributed to the higher density tree spacing (1 m) between cedars. Within the conventional cropping system, the SOC concentration was 2.06% and was significantly higher (at $\rho >0.05$) than near the spruces. The experimental design of the tree-based intercropping system did not consider effects of topography and therefore this higher level in soil carbon content in the conventionally cropped field could have been related to the landscape position rather than to effects of the management system.
Figure 4.9 Soil organic carbon (%) at depth of 0-10 cm at sampled distances a) north and b) south of trees (spruce and walnut) in rows. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and the dot indicates a data outlier.
Figure 4.10 Soil organic carbon (%) at depth of 10-20 cm at sampled distances a) north and b) south of trees (spruce and walnut) in rows. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.
**Figure 4.11** Soil organic carbon (%) at depth of 0-10 cm within the crop rows and within the spruce and walnut tree rows. Boxes represent 25<sup>th</sup> to 75<sup>th</sup> percentile of sampled values, horizontal lines represent sample medians, and the dot indicates a data outlier.

**Figure 4.12** Soil organic carbon (%) at depth of 10-20 cm within the crop rows and within the spruce and walnut tree rows. Boxes represent 25<sup>th</sup> to 75<sup>th</sup> percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.
Figure 4.13 Soil organic carbon (%) at both depths combined of all sampling locations within the spruce, cedar and walnut rows and within the conventional agriculture control. Boxes represent 25th to 75th percentile of sampled values, horizontal lines represent sample medians, and dots indicate data outliers.

4.4 CONCLUSIONS

There was no significant effect of tree species on SOC concentration in this study.

However, there were significant effects of tree species on SOC concentrations with
distance and direction from the tree row. Both walnut and spruce showed higher SOC concentrations at distances 1.5 m from the tree east of the tree rows when compared to increased distances at both the 0-10 cm and 10-20 cm sampling depths; a trend was visible in cedar with highest values 1.5 m from the tree. It is evident that there was high spatial variability within intra-species comparisons in soils sampled east and west of the tree rows. Higher SOC concentrations in the surface soils were found within the tree rows of both spruce and walnut vs. within the crop row, and became increasingly affected by sampling position within the 10-20 cm profile. SOC(%) was highest in the conventional cropping system. However the experimental design of this TBI system relative to the conventional cropping system limited comparison of management system effects on SOC levels.
4.5 REFERENCES


5.0 SUMMARY AND FUTURE RESEARCH

This project sought to provide an update of soil carbon sequestration potential 25 years after establishment of the tree-based intercropping site located in Guelph, ON. We gathered samples in summer 2012, fall 2012 and spring 2013 to measure temporal effects on soil organic carbon pools, including DOC and SMB-C. This would help us better understand the factors controlling spatial variability of soil carbon within the tree-based intercropping site. We obtained soil cores via grid sampling, surrounding several different tree species in order to gain a better understanding of spatial orientation of soil organic carbon. Finally we compared soil samples extracted in the tree-based intercropping system to an adjacent conventional cropping system. Samples within the conventional cropping system were chosen based on a systematic contour design along a grid in order to take into consideration topographic influence of soil organic carbon.

There was a relationship between DOC and the litterfall data collected by Wotherspoon (2014) from the same species. Both the litterfall and the DOC content were highest east of the poplar and walnut tree rows and higher west of the spruce and oak tree rows. The highest DOC contents were found for the fall sampling, which provides evidence of a link between litterfall and DOC. As trees mature, their leaf litter would be distributed more broadly over the cropped area. The highest SMB-C content was found in soils adjacent to the spruce species. Spruce is often recommended as a tree species for shelterbelts. This could suggest greater crop growth surrounding the spruce species due to higher soil moisture contributed by higher snow accumulation (Dingman 2008).

The soil samples collected in the cropped area surrounding cedar, walnut and spruce provided statistically higher SOC (%) of samples west of the tree row in
comparison to east of the row. It was expected that soils collected from a direction east of the tree row would have the highest SOC concentration due to typical wind direction in southwestern Ontario. The orientation of tree rows could have created a barrier for the transportation of litterfall by wind. Accumulation of litter could have occurred in areas other than predicted. Soils collected from east of the tree row had significantly higher (at \( \rho > 0.05 \)) SOC concentrations in samples at distances 1.5 m within both depth classes. SOC concentrations were significantly higher within the spruce and walnut tree rows when compared to the adjacent cropped area. Overall SOC % was highest in the cropped area adjacent to cedar when compared to walnut and spruce and these results are consistent with those reported by Wotherspoon (2014).

Results from this study can be used as a basis to recommend best management practices for tree-based intercropping sites. The increased SMB-C in the cropped area adjacent to the spruce row suggests benefits to crop yield and soil health. The relatively small canopy of cedar may help to minimize shading effects on crop growth.

Further studies will need to be conducted in order to understand the effects of topography on the spatial variation of SOC at this site. This study suggested that 25 years after establishment, this temperate TBI system did not sequester more SOC than the conventional cropping system. The highest SOC within the TBI system was in the cropped area adjacent to the cedar.

It should be noted that if C accumulation or sequestration is considered at the system level, the above and below ground C sequestration in the tree components will always place these systems above conventional agricultural systems in terms of C captured in tree-based intercropping systems. Carbon sequestration in conventional
agricultural systems is limited to the inputs of crop residue left after harvest to maintain SOC contents.

5.1 REFERENCES
