Quantification of Carbon Gains and Losses for Five Tree Species in a 25-year-old Tree-Based Intercropping System in Southern Ontario, Canada

by

Amy Wotherspoon

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ABSTRACT

QUANTIFICATION OF CARBON GAINS AND LOSSES FOR FIVE TREE SPECIES IN A 25-YEAR-OLD TREE-BASED INTERCROPPING SYSTEM IN SOUTHERN ONTARIO, CANADA

Amy Wotherspoon
University of Guelph, 2013

Co-Advisors:
Professor A. M. Gordon
Professor R. P. Voroney

This thesis is an investigation of above- and belowground carbon (C) pools and fluxes of five tree species in a 25-year-old tree-based (TBI) system as compared to an adjacent sole-cropped conventional agricultural system at the University of Guelph's Agroforestry Research Station (43° 16'N 89° 26'W) (established 1987). This study compared C found in above- and belowground biomass, soil organic carbon, litterfall, litter decomposition and soil respiration. These components were combined in a C model for comparison of C sequestration potential. In intercropping systems planted with hybrid poplar (*Populus* spp.), red oak (*Quercus rubra*), black walnut (*Juglans nigra*), Norway spruce (*Picea* abies) and white cedar (*Thuja occidentalis)*, net C sequestration was quantified to be approximately 3.4, 3.0, 2.5, 3.7 and 2.7 t C ha^{-1} year^{-1}. In comparison, the adjacent sole-cropping system, planted with soybean (*Glycine max)* was found to have a net C sequestration of –1.4 t C ha^{-1} year^{-1}.

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1.0 Chapter 1 – Introduction and Literature Review

1.1 Introduction

The evolution of agriculture in temperate ecosystems has been marked by large gains in land area coverage, high productivity and larger machinery. This intensification of agriculture has resulted in the removal of trees from cropland, the degradation of soil quality and the use of chemical fertilizers, all of which are large contributors to climate change. Policy makers are searching for ways to reverse the negative impacts of agriculture and to mitigate their influence on climate change by turning to sustainable land-use management practices such as agroforestry. The Millennium Ecosystem Assessment (2005) and the International Assessment of Agricultural Science and Technology for Development (2008) have recognized the importance and multifunctional role of agroecosystems in support of the many ecosystem services they provide.

The World Agroforestry Centre (WAC) describes agroforestry as a land-use system that deliberately combines woody perennials on the same land management unit as agricultural crops and/or livestock. Together, these two units provide ecological and economic interactions (Gordon and Newman, 1997). Tree-based intercropping (TBI) is a form of agroforestry where trees are planted in widely spaced rows amongst crops and combine the production of agriculture with trees, which was the focus of this study. Production of trees along with crops allows for both short and long term benefits. In the short term, trees can provide annual income from fruits and nuts, and in the long term provide benefits of timber production and carbon (C) sequestration in biomass and soil. The additional benefit of C sequestration allows TBI systems to mitigate climate change in three ways. The first is that trees can directly sequester atmospheric CO_2 into biomass to reduce atmospheric concentrations. The second is from the reduction of greenhouse gases (GHG) associated with the production of inorganic fertilizers and nonpoint source

pollution. Trees within TBI systems return organic matter (OM) to the soil and provide natural nutrient to the crops below, reducing the need for fertilizers. Trees also have deeper root systems, which allow them to take up excess nutrients not taken in by the crops, reducing nonpoint source pollutants. Lastly, planting trees in TBI systems can alleviate pressure on natural forests by providing additional sources of timber and can therefore offset GHG emissions associated with deforestation.

The realization of the benefits of TBI systems in temperate regions has increased in the past few decades, although adoption of TBI systems is still low. While these benefits have been documented (including minimizing soil erosion, reduced runoff to nearby waterways, and enhanced biodiversity (Gordon and Newman, 1997)), government policy still limits the accessibility of TBI systems to landowners. This includes, but is not limited to, the lack of tax incentives to address the ecological and social benefits (Dyack et al., 1999) and the complexity of adopting and managing the systems not completely understood by the majority of landowners (Matthews et al., 1993). Previously, a large amount of data has been collected in southern Ontario that demonstrates the ecological and biological benefits of TBI systems (to be discussed later on) and supports the acceptance of TBI and agroforestry practices (Jose, 2009; Montagnini and Nair, 2004; Schroeder, 1994; Simpson et al., 2008; Thevathasan and Gordon, 2004; Thevathasan et al., 2004). More recently, the social and economic benefits have been addressed in Quebec in order to surpass the limitation encountered by tax policies and wary landowners (De Baets et al., 2007).

The goals of this study, currently not addressed anywhere else in Canada, are the long term C sequestration potentials of five tree species in a 25-year-old TBI system in southern Ontario. The overall null hypothesis for this study is that tree-based intercropping systems and

sole cropping systems sequester the same amount of carbon per year. The alternative hypothesis is that tree-based intercropping systems and sole cropping systems do not sequester the same amount of carbon per year. The main objectives of this study were to 1) quantify above- and belowground C pools of five tree species in a 25-year-old TBI system, 2) determine the quantity and quality of the tree C and crop residue inputs, both above- and belowground for five tree species in a 25-year-old system as compared to a conventional agricultural system, and 3) develop a carbon model for five tree species in a 25-year-old TBI system compared to a conventional agricultural system using findings from objectives 1 and 2.

1.2 Literature Review

1.2.1 Introduction

Agroforestry is a collective term that encompasses the practice of managing trees and agriculture on the same land unit (Thevathasan et al., 2004). Currently agroforestry has been established for a variety of ecological, environmental and economic benefits in both tropical and temperate regions. In tropical regions, agroforestry is popularly practiced due to limitations in land area for both crops and trees. However, in temperate regions, agroforestry is a relatively new land-use practice used in the hopes of mitigating some of the effects of climate change brought on by unsustainable agricultural practices. An understanding of the benefits of agroforestry systems is an important component to increase their adoptability in the temperate region. With higher adoptability, landowners, policy makers and government will realize their importance and implement a way in which landowners can receive monetary benefit for both their agricultural production and their environmental services.

In this review of the literature, the ecological benefits of agroforestry will be presented, as well as their ability to mitigate climate change both in the tropical and temperate regions. The future of agroforestry as an important component in the carbon market will also be discussed, as well as the current limitations that should be addressed in order to enhance our understandings of their components and their global C sequestration potential.

1.3 The Development of Agroforestry

1.3.1 Origin

The World Agroforestry Centre defines agroforestry as "a dynamic, ecologically based, natural resources management system that, through the integration of trees on farms and in the agricultural landscape, diversifies and sustains production for increased social, economic and environmental benefits for land users at all levels." The Association for Temperate Agroforestry

(AFTA) also adds that it "optimized the benefits from biological interactions when trees and/or shrubs are deliberately combined with crops and/or livestock."

Agroforestry has been incorporated into agricultural and forestry practices in many developing countries mostly since the 1980s and 1990s as a practical land-use solution to addressing factors such as deforestation, soil degradation and the loss of biodiversity (Nair et al., 2008). Prior to then, practices such as homegardening and silvopasture had been practiced in Southeast Asia and Spain as far back as 13,000 – 900 BC and 4,500 years ago, respectively (Nair et al., 2008). More recently, in temperate regions agroforestry has been seen as a solution to land use change involving fragmentation of natural vegetation and forests for land development and agriculture, loss of biodiversity, increase of exotic species, soil erosion, reduction in water quality and environmental pollution (Nair et al., 2008). Along with North America, China, Australia, New Zealand and countries in southern Europe are now understanding the ecological and economic benefits of agroforestry and demonstrating a variety of ways they can be used in various geographical areas (Nair et al., 2008).

1.3.2 Up until Now

Higher demands of productivity and larger machinery from farming practices have led to the eventual exclusion of trees from cropland (Gray, 2000). As a result, there has been the loss of biodiversity, forest resources and wildlife in addition to environmental hazards such as erosion, nonpoint aquatic pollution and greenhouse gas emissions (Nair et al., 2008). Clearing land for agriculture has also resulted in high levels of CO_2 that have been (and continue to be) released into the atmosphere through degradation and deforestation, aided by unsustainable farming practices and land clearing for agriculture (Rivest, 2009). With these loses, came the development of temperate agroforestry in an attempt to reintroduce trees into agricultural

landscapes to remediate ecological and environmental damages. Various types of agroforestry exist to meet various ecological demands, and are characterized by how they address these challenges and by association and combination of their use of plant species, management, and environment and socioeconomic factors (Nair et al., 2008). Agroforestry types that dominate temperate regions are presented in Table 1.

Table 1. Major agroforestry practices in temperate regions (adapted from Nair et al., 2008)

Agroforestry practice	Description
Alley cropping (also known as tree-based intercropping)	Trees planted in single or grouped rows in herbaceous (agricultural or horticultural) crops in the wide alleys between the tree rows.
Forest farming	Utilizing forested areas for producing specialty crops that are sold for medicinal, ornamental or culinary uses.
Riparian buffer strips	Strips of perennial vegetation (tree/shrub/grass) planted between croplands/pastures and streams, lakes, wetlands, ponds, etc.
Silvopasture	Combining trees with forage (pasture or hay) and livestock production.
Windbreaks	Row trees around farms and fields, planted and managed as part of crop or livestock operation to protect crops, animals, and soil from wind hazards.

Agroforestry systems in tropical regions are a great example of the potential benefits of these systems, not only in their protection and sustainability of crops, livestock, soil and water, but also by providing a variety of agricultural revenues including alternative production of timber and non-timber products such as fruits, syrups and medicinal products. They also enhance conventional agricultural landscapes by promoting diversity of neighbouring flora and fauna species, and sequester atmospheric carbon.

Tree-based intercropping (TBI) is a form of agroforestry and was chosen as the focus of this study. TBI is the practice of growing agricultural crops between alternating rows of trees and other crops, which allows for greater structural and functional diversification (Clinch et al., 2009). These systems allow for interspecific competition that creates a more complex ecosystem than if agricultural crops were grown on a conventional monocropped landscape.

1.3.3 Room for Growth

By adopting agroforestry practices into conventional agricultural systems, we can help to mitigate some of the changes that have developed from unsustainable farming practices. Given that agroforestry has risen from ecological foundations, it has many ecosystem services and benefits that make it an attractive land use management strategy (Nair et al., 2008), and those will be discussed further in this review. In order to ensure agroforestry systems continue to provide these services and benefits, we have to ensure their adoptability rates. Presently, adoption rates are low, particularly in temperate regions, due to the focus on sustainability rather than short-term monetary gain (Rivest, 2009). Jose (2009) points out that due to large variability in the estimates and lack of consistent and uniform methods of research, comparisons of carbon sequestration potential of agroforestry systems is difficult.

1.4 The Benefits of Agroforestry

1.4.1 Soil Productivity and Protection

By incorporating trees into conventional agricultural landscapes there is opportunity to improve soil conditions, productivity and ensure sustainability. Trees improve soil fertility as a result of the addition of OM in the form of litterfall, promoting the presence of microbial biomass and earthworm populations (Price and Gordon, 1999). Lee and Jose (2003) found higher soil organic matter (SOM) and microbial biomass in pecan and cotton alley cropping systems compared to a cotton monoculture system. Udawatta et al. (2008) found improved soil

conditions in the form of improved soil aggregate stability, soil C, N and soil enzyme activity in soils under agroforestry when compared to row crops. Improved soil quality will enhance crop production and allow for continuing harvesting without sooner onset of land degradation, which would require more land clearing of natural forests for agriculture.

1.4.2 Soil Erosion and Water Quality

Tree roots aid in reducing surface runoff and soil erosion, which indirectly impacts nearby ecosystems. In conventional agriculture, less than half of the available nitrogen in applied fertilizer is taken up by the crops (Allen et al., 2004); agroforestry can reduce the losses by 1) providing windbreaks that reduce soil erosion caused by wind and water (Nair et al., 2008), and 2) by deep tree roots recover soil nutrients which would otherwise accumulate in nearby bodies of water, which can result in eutrophication (Jose, 2009). By having deeper and more extensive roots than crops, trees act as a 'safety net' to allow for more uptakes of nutrients from the soil, not used by crops, that would otherwise be lost from the system through leaching (Allen et al., 2004). A variety of recent research has shown the benefits of agroforestry systems on reducing nonpoint source pollution (Allen et al., 2004; Lee and Jose, 2003; Udawatta et al., 2008). Riparian buffers are commonly used for these benefits to reduce sediment losses into waterways. Allen et al. (2004) and Lacombe (2007) found a pecan – cotton and hybrid poplar – barley alley cropping system had a 72 and 80% reduction, respectively, of leached nitrates in groundwater. This reduction in soil erosion and water recharge reduces habitat loss and degradation of nearby water ecosystems (Jose, 2009).

1.4.3 Biological Diversity

In addition to improving water quality, TBI systems can improve surrounding biological diversity by providing habitat and connecting otherwise segregated landscapes. Thevathasan and Gordon (2004) found a higher number and species of birds due to the connectivity of tree rows

that mimic natural forest settings. Temperate TBI studies have found higher diversity and numbers of predators and agricultural pests than when compared to conventional agricultural systems, reducing the need for pesticides (Howell, 2001; Stamps and Linit, 1998). For these reasons, TBI systems also provide ecological benefits that go beyond monetary value but enhance our landscapes beyond that of traditional agriculture.

1.4.4 Food Security

Landscapes that incorporate trees into agriculture can support food production in addition to conventional crops. This is done directly by planting trees or bushes within intercropping systems that can support an annual fruit or nut production, such as black walnut trees. Intercropping systems indirectly support food security by ensure soil productivity through the SOC stock. By increasing the SOC stock by 1 ton, agricultural soils have the potential to increase crop yield from 20 – 40 kg ha^{-1} for wheat, 10 – 20 kg ha^{-1} for maize and 0.5 – 1 kg ha^{-1} for cowpeas (Lal, 2004).

1.5 Agroforestry for Climate Change Mitigation

1.5.1 Carbon Storage

Agroforestry systems not only provide a variety of ecosystem services, but can also be used as a tool to mitigate climate change by providing a long term C sinks from the sequestration of atmospheric CO_2. The difference of C gained from photosynthesis and lost from respiration results in C that will be immobilized for a significantly long period of time (Thevathasan et al., 2004). As global deforestation is estimated to occur at 17 million ha yr^{-1} with emissions of 1.6 Pg C ha^{-1} yr^{-1} (Pg=10^{15} g = billion tons) (Nair et al. 2008), we can no longer rely on our natural forests to sequester the majority of atmospheric C. Agroforestry systems with high tree densities can sequester C into above and belowground woody biomass, as well as in the soil. (Nair et al., 2009) estimate that aboveground C sequestration can range from 0.29 to 15.21 Mg ha^{-1} y^{-1}

depending on a variety factors from the surrounding ecosystem including site characteristics, species, stand age, and management practices.

In temperate ecosystems, Dixon et al. (1994) estimate carbon sequestration potential (CSP) within temperate ecosystems to be between 15 and 198 Mg C ha^{-1} (mode being 34 Mg C ha^{-1}). CSP of various types of temperate agroforestry systems in the United States as projected in the current literature are listed in Table 2. Overall, Nair and Nair (2003) proposed an estimate of 90 Tg C yr^{-1} in all agroforestry systems through the United States.

Table 2. Carbon sequestration potential of various types of agroforestry systems based on land area coverage throughout the United States

Type of Agroforestry	Land area coverage	Carbon sequestration potential (Tg C $year^{-1}$)	Source
Silvopastoral systems	70 million ha of land in the US grazed by livestock	9.0 Tg	(Montagnini and Nair, 2004)
Windbreaks	5% of the 85 million ha of field area in the north central United Stations	2.9 Tg	National Agroforestry Centre, 2000
Riparian buffers	3.2 million km of land proposed by the US department of agriculture (30 m wide)	1.5 Tg	(Montagnini and Nair, 2004)
All agroforestry systems through the United States		90 Tg	(Nair and Nair, 2003)

In Ontario, Peichl et al. (2006) estimated that a 13-year-old TBI system planted with hybrid poplar and barley was able to sequester 13 t C ha^{-1}, and 1 t C ha^{-1} when planted with Norway spruce and barley compared to – 3 t C ha^{-1} in a barley monoculture system. In the same system, the root system of a 12 year old hybrid poplar was excavated and determined to be 2.2 Mg C ha^{-1}, converting to 0.2 Mg C ha^{-1} y^{-1} at a planting density of 111 trees ha^{-1} (Gordon and Thevathasan, 2005).

1.5.1.1 Tropics and Worldwide

In tropical ecosystem, the greatest C sequestration potential lies in aboveground biomass (Montagnini and Nair, 2004). With the help of agroforestry systems incorporated into degraded pastures, croplands and grasslands, soil stocks are estimated to only increase 5 to 15 Mg C ha^{-1} (Palm and 17 others., 2000). C sequestration within vegetation is estimated at 50 Mg C ha^{-1} over a 20-year period and future projections range from 1.5 to 3.5 Mg C ha^{-1} yr^{-1} (Watson et al., 2000). Oelbermann et al. (2005) found that in a Costa Rican agroforestry system planted with *E. poeppigiana*, annual aboveground C input ranged from 1.0, 1.4 to 4.0 Mg C ha^{-1} y^{-1}, for a 4, 10 and 19 year old stand, respectively. They determined that from the 4 and 10-year-old alley cropping systems, *E. poeppigiana* roots had an annual coarse root increment of 0.2 and 0.4 Mg C ha^{-1} y^{-1}, respectively. Due to tree productivity in the tropics, pruning is an important component of management practices and depends on tree function, age and species (Oelbermann et al., 2004). Nygren (1995) estimates that C input from *E. poeppigiana* clones in Costa Rica range from 2.3 to 5.2 Mg C ha^{-1} y^{-1} at a tree density of 625 trees ha^{-1}.

Table 3. Current estimates of global carbon sequestration potential

Area of land	Carbon Sequestration Potential	Source
585 – 1,215 million ha (Africa, Asia and Americas)	1.1 – 2.2 Pg C over 50 years	(Dixon, 1995a)
1,023 million ha	1.9 Pg C over 50 years	(Dixon, 1995a; Nair et al., 2009)
Existing agroforestry practices	17,000 Mg C yr^{-1} by 2040	(IPCC, 2000)
630 million ha of unproductive croplands and grasslands	586,000 Mg C yr^{-1} by 2040	(Jose, 2009)

Worldwide, the IPCC currently estimates global adoption of agroforestry to be less than 400 million hectares that is sequestering 0.72 Mg C ha^{-1} yr^{-1}, which would have sequestered 45

Tg C yr^{-1} by 2010 (1 Tg=10^{12} g or 1 million tons) (Watson et al., 2000). Current estimates for future global CSP currently found in the literature are presented in Table 3.

1.5.1.2 Carbon Storage in Soil

Belowground, to a depth of 1m, SOC stock consists of 1550 Pentagrams (Pg), and soil inorganic C consists of approximately 750 Pg (Batjes, 1996). With the loss of 1 Pg organic C lost from the soil through deforestation and poor agricultural management practices, atmospheric CO_2 can increase by 0.47 ppm (Lal, 2001) in addition to nutrient-poor soil for crops (Nair et al., 2009). Current soil conservation practices are based on an approximate loss of 4.5 to 11.2 Mg ha^{-1} $year^{-1}$ (Hudson, 1995). Thevathasan and Gordon (1997) have found that SOC in TBI systems in Ontario has increased by more than 1% due to litterfall inputs and fine root turnover. In a 13-year-old Ontario intercropping system with hybrid poplar and soybean SOC content were found to be 1.8% and 1.6% in the alley crop and sole crop system, respectively, to a depth of 20 cm (Oelbermann et al., 2006). Oelbermann et al. (2006) also found that the average annual accumulation rate for the TBI system ranged from 30 g m^{-2} y^{-1} (at 20 cm depth) to 39 g m^{-2} y^{-1} (at 40 cm depth). At the same site, when the system had reached 20 years of age, SOC stocks were found to be 57.0, 50.9 and 50.8 Mg C ha^{-1} under poplar hybrid and Norway spruce TBI systems and a conventional soybean system, respectively. The sustainability of these SOC stocks is not only an important component of C sequestration but also ensures soil fertility and crop production.

1.5.2 On marginal or degraded land

Agroforestry is an important land-use system as it can be an important sector in utilizing some of the 140 million ha of degraded or marginal land that currently exists in North America (Dixon et al., 1994). Of this land, between 50 and 57 million ha is situated in Canada, and is suitable for agroforestry (Thevathasan and Gordon, 2004). Thevathasan et al. (2004) state that of

degraded land available in Canada, 20 – 25 million hectares of land can be adopted as agricultural land classes 1 to 4 which can provide new land available for agroforestry systems. The IPCC has recommended agricultural land be converted into agrosilvicultural systems, as a remedial measure to mitigate climbing CO_2 emissions (IPCC, 2001). Incorporating fast-growing tree species that can sustain poor soil conditions, such as hybrid poplar can provide a new opportunity to reforest and reutilize land that would otherwise be left untouched. As trees sequester CO2, it allows for an increase in water use efficiency in photosynthesis which could allow for trees to establish in semiarid areas where forested areas would not have been possible before (Vitousek, 1991).

Thevathasan and Gordon (2004) found that the amount of land required to immobilize 9 t CO_2 ha^{-1} yr^{-1} would be less than 6 million ha of pastureland when planted with fast growing tree species. While agroforestry systems on degraded land have lower carbon sequestration potential than those on fertile humid sites (Jose, 2009), they still have the potential to sequester an additional 17,000 Mg C y^{-1} by 2040 (IPCC, 2000). Dixon and Turner (1991) estimate that with the remediation of this land, temperate systems have the ability to sequester 4.9 x 10^9 Mg C year-1 in aboveground pools. Of this, 1.9 x 10^9 Mg C $year^{-1}$ could be incorporated into aboveground components that would then help to conserve existing SOC by providing OM back to the soil surface (Oelbermann et al., 2004).

With the incorporation of trees on degraded land, not only would site quality be improved but crop production would be enhanced, and provide the ecological services previously mentioned to continue to enhance the surrounding environment.

1.5.3 Reduction of Greenhouse Gases

In addition to ecosystem services, agroforestry aids in the reduction of greenhouse gases in multiple ways. Firstly, planting trees into agricultural landscapes provide an alternative to the deforestation of natural forests and help to sequester atmospheric CO_2 that is lost during deforestation (Nair et al., 2008). Currently, agriculture is responsible for 75% of deforestation that occurs globally, resulting in the emissions of 2,200 – 6,600 million t CO_2 per year, which account for 30 – 50 % of agricultural emissions and 4 – 14% of global emissions (Blaser and Robledo, 2007; van der Werf et al., 2009; Vermeulen et al., 2012). Within tropical ecosystems, it is estimated that per one hectare of agroforestry, 5 – 20 ha of deforestation could be offset (Dixon, 1995). This will not only reduce CO_2 emissions caused by deforestation but also help to conserve natural forests that provide diversity and their own range of environmental services.

Secondly, agroforestry reduces GHGs by providing a source of nutrients for nearby crops, reducing the need for additional synthetic fertilizers. By providing sources of C and N from annual litterfall, landowners can reduce their use of supplemental N to enhance crop production while providing additional nutrients to the soil. This then reduces N_2O emissions from the production and use of chemical fertilizers and lowers application rates. Deep tree roots also reduce the amount of N in the root zone of crops and therefore reduce N_2O emissions caused by denitrififcation – approximately 2.5 % of leached N (Oenema, 1999). Any additional N fertilizer within the root zone can be taken up by the tree to promote growth and further sequestration of atmospheric CO_2 into above and belowground biomass. The amount of N_2O reduced will be proportional to the amount of land that is covered by trees in a specific agroforestry system (Thevathasan et al., 2004). Modeled data from Thevathasan (1998) indicates that nitrate lost from the system can be reduced by 50 % with the use of TBI systems compared to conventional agriculture. Thevathasan and Gordon (2004) estimate that N_2O emission in TBI

systems could be reduced by a potential 0.69 kg N_2O ha^{-1} in relation to emissions from conventional systems.

1.5.4 The Carbon Market

Interest in agroforestry systems may have peaked with the introduction of the Kyoto Protocol and the encouragement of forestry plantations as C sinks. As the biological and ecological benefits of agroforestry systems have been realized and documented, countries are looking for government incentives to promote their establishment and provide monetary compensation for the environmental services they provide. Currently, the FAO State of Food and Agricultural Report (2007), has shown support in providing financial benefits to land owners and farmers who choose to make those sustainable and responsible decisions. This will help to increase adoptability of land-use practices that provide environmental services.

In the temperate region, the idea of a cap and trade carbon market has developed where "the total amount of carbon emissions is limited by a mandatory cap, and carbon-emitting industries are allowed to meet their targets with some combination of carbon emission reduction technologies and the purchase of carbon offsets" (Montagnini and Nair, 2004). Given the current research on their abilities to sequester C, agroforestry systems seem like a viable option should cap and trade market ever set in place. For the tropics, Montagnini and Nair (2004) list several examples how private companies, NGOs and development agencies are working to pay for environmental services through carbon trading for sustainable goods (such as coffee), and the establishment of agroforestry and woodlots to offset CO_2 emissions caused by other technologies or industries. Some companies quote $12 per Mg for C sequestration potentials at 26 Mg ha^{-1} (Montagnini and Nair, 2004), and others at $3.30 per ton on fallowed land and $62.50 per ton on land with high value agricultural crops (Shivley et al., 2004).

1.6 Future Work

Due to low rates of adoptability, many areas of agroforestry are still open for future research. Currently, most long-term data on the C sequestration potential of agroforestry systems is limited to computer modeling such as CO_2Fix and CENTURY programs that use tree growth data from forest systems, in which trees have different biophysical characteristics (Kürsten and Burschel, 1993). This is due to the relatively short-term existence of agroforestry systems and the lack of older age systems present in North America. Nair (2011) also suggests that at a system level, high quality quantitative data is difficult to obtain due to their specificity to region, climate, and management. In order to overcome these limitations, further research needs to be conducted in a variety of different regions with precise, accurate and standardized methodologies. Montagnini and Nair (2004) suggest that the lack of this empirical evidence is also a major limitation to realizing the C sequestration potential of agroforestry systems. Oelbermann et al. (2002) suggest that allometric equations would also be useful in assessing the C sequestration potential of agroforestry systems, since trees grown in agroforestry systems have different branching morphologies compared to those found in natural forest systems and growth is highly influenced by density (Oelbermann, 2002). This would then help to increase the accuracy of computer modeling to better represent agroforestry systems.

Further data are required to enhance computer models, including empirical data from belowground biomass, given that aboveground biomass is easier and therefore more often measured. Knowledge gaps still remain in the accumulation of C belowground, both in biomass roots and soil organic carbon (SOC). Oelbermann et al. (2004) suggest that research should focus on SOC stocks and their accumulation as a result of crop and residue input and also their stabilization from the conversion of forest to agroforestry systems. Where levels of SOC have been quantified, there also lacks base-line data for comparative purposes between SOC of

agroforestry systems and conventional agriculture. These results would help to further quantify the accumulation of belowground C sequestration.

This study will attempt to address some of the limitations listed above including studying a long-term agroforestry site at 25 years of age, with five varieties of tree species, and C pools and fluxes both above- and belowground. By quantifying as many pools and fluxes as possible with precision and accuracy, it allows for the use of empirical data over computer modeled data and therefore more accurate C sequestration potentials that can help increase the adoptability of agroforestry systems.

1.7 Summary

Agroforestry is a sustainable land-use practice that in the past has been more prevalent in tropical and developing countries compared to temperate regions. However, with the intensification of agriculture, temperate regions are losing viable land and emitting GHGs propagating climate change. In order to mitigate these damages, temperate regions are looking to agroforestry as a way to encourage sustainable agriculture practices, improve land quality and mitigate climate change, collectively. Agroforestry provides a variety of ecological services that slow land degradation caused by agriculture while at the same time acting as a long-term sink for atmospheric CO_2. Together, these components make agroforestry a promising land-use practice in addition to reducing pressure on natural forests for C sequestration and timber harvesting. The continuing research on the C sequestration potentials of the different types of agroforestry using a variety of species shows promising results for their use as a sustainable land-use practice and to mitigate climate change.

1.8 Materials and Methods

1.8.1 Site Description

This study was carried out in 2012 and 2013 at the University of Guelph Agroforestry Research Station; 30 ha of agricultural land established in 1987 in southwestern Ontario (43°32'28" N, 80°12' 32" W). The site has a mean annual temperature of 7.2°C, 136 mean annual frost free days and mean precipitation of 833 mm, 344 mm of which fall during the growing season (Oelbermann, 2002; Simpson, 1999; Simpson et al., 2008). This site has a Canadian Land Index of 3 and soils that are Albic Luvisols with a sandy loam texture (65% sand, 25% silt, 10% clay), a pH of 7.4, and an A-horizon depth ranging between 28 and 52 cm (Abohassan, 2004; Oelbermann, 2002; Thevathasan, 1998). The site was located to the west of a side-slope of a drumlin with a 6 % average slope (Abohassan, 2004).

Before the establishment of the TBI system, the land was under a hay and crop production with declining yields and bedrock exposure due to extensive soil erosion (Oelbermann, 2002). Between 1987 and 1994, twelve tree species were planted at a density of 111 trees ha^{-1} in groups of eight with four replicates with a within-row and between-row spacing of 6 and 15 m, respectively (Figure 1) (Peichl et al., 2006; Simpson, 1999; Simpson et al., 2008). Trees that were studied included hybrid poplar (*Populus* spp.), Norway spruce (*Picae abies),* red oak (*Quercus rubra*), black walnut (*Juglans nigra)*, and white cedar (*Thuja occidentalis*). All deciduous trees were pruned to a height of 3 m during 2000 – 2001, and spruce trees were pruned in 2002 to a height of 2 m (Abohassan, 2004).

Trees were intercropped with a variety of agricultural crops including corn (*Zea mays* L.), and soybean (*Glycine max* L.) since 2003 and winter wheat and barley (*Hordeum vulgare* L) between 1987 and 2002 (Peichl et al., 2006). At the time of this study (2012), the annual crop

was soybean. Inorganic fertilizers were used for nutrient additions and maize, soybean and wheat receive 150 – 170 Kg N ha-1, 0 – 20 kg N ha-1 and 75 kg N ha-1, respectively (Oelbermann, 2002). The field is imperfect to moderately well drained and is tilled annually to 15 cm each autumn (since 1996) (Oelbermann, 2002). Previously, tillage practices included moldboard plowing to 20 cm depths in the fall, followed by seedbed preparation with discs to a depth of 10 cm in the spring. In 1991, the moldboard plow was replaced by disking corn stubbles to a depth of 2.5 cm in the fall for rodent control purposes(Oelbermann, 2002), followed by no till between 1991 and 1996.

An adjacent conventional agricultural field planted with the same crop, same soil properties and undergoing the same practices was used for comparison of TBI to conventional agricultural practices. Both sites were used for all experimental measurements including SOC, soil respiration, and litter decomposition. Above- and belowground biomass, tree biomass C and litter fall were measured at the intercropping system only, as there are no trees present in the conventional agricultural system.

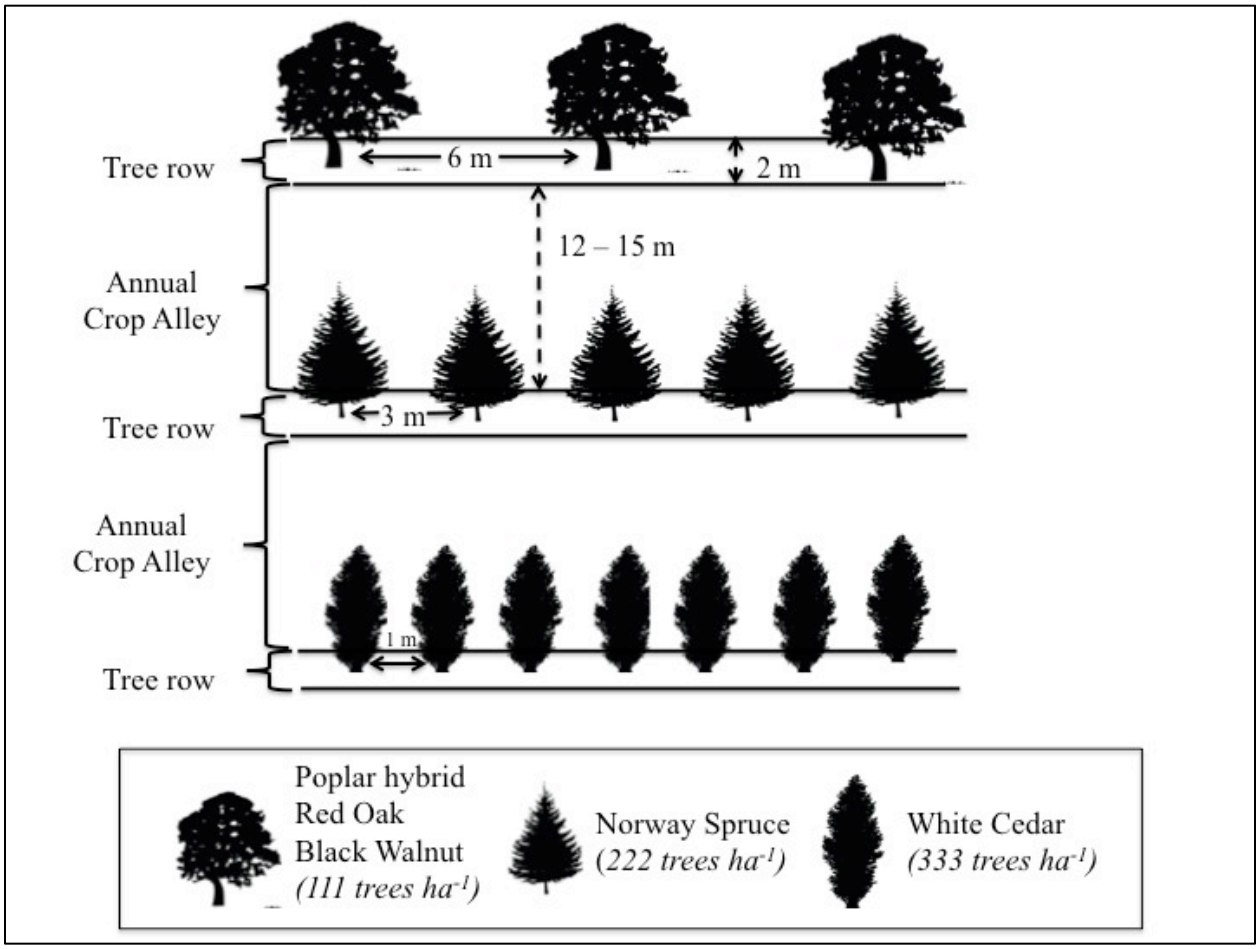

Figure 1. Experimental design of 25-year-old tree-based intercropping system at the Guelph Agroforestry Research Station in Guelph, Ontario

2.0 Chapter 2 – Carbon stocks

2.1 Introduction

A 30% increase in atmospheric carbon dioxide (CO_2) since 1750 has been recorded and it is predicted to give rise to another 50% increase towards the latter part of this century (IPCC, 2001). The conversion of agricultural land into agroforestry systems, a land-use practice where trees are planted among agricultural crops in the hopes of sequestering atmospheric CO_2, in addition to other benefits, is one of the many recommendations put forward by the International Panel on Climate Change (IPCC) in order to reverse this trend. Tree-based intercropping (TBI) is one of the temperate agroforestry systems, where trees are considered to be long-term carbon (C) sinks through sequestration of atmospheric CO_2 into permanent tree components and as SOM (Bambrick et al., 2010; Dixon, 1995a; Montagnini and Nair, 2004; Peichl et al., 2006; Sampson, 2001; Thevathasan and Gordon, 2004). In TBI systems, various tree species and planting densities are utilized to maximize both economic and environmental benefits. In tropical latitudes, agroforestry systems have carbon sequestration potentials (CSP) between 21 and 240 t C ha^{-1} (Adesina, 2004; Dixon, 1995a; Montagnini and Nair, 2004; Schroeder, 1994; Swisher, 1991), whereas in temperate latitudes, CSP between 10 and 208 t C ha^{-1} have been reported (Dixon, 1995a; Kort and Turnock, 1999; Montagnini and Nair, 2004; Schroeder, 1994; Turnock, 2001). The range in values and differences in systems found at different latitudes are likely due to tree species, density and cutting cycle (every 10 – 20 years in the tropics; every 20 – 50 years in temperate systems).

Trees return organic material to the soil in the form of litterfall, a component largely absent in conventional agricultural systems, and having a high CSP for soil (Sanderman et al., 2009). This is important, as land clearing for agriculture has resulted in a loss of 40 – 60 % of soil organic carbon (SOC) from native land and a global release of more than 150 Pentagrams

(Pg) of CO_2 into the atmosphere (Sanderman et al., 2009). The top meter of the soil alone stores ~ 1500 Pg as organic carbon (OC) globally and exchanges 60 Pg C year^{-1} with the atmosphere, of which ~750 Pg is CO_2 (Eswaran et al., 1993; Schlesinger, 1997). This documentation of changes in SOC stocks of C due to historical and present land clearing generates the need for the quantification, in agricultural soils, of the potential for sequestering atmospheric C and mitigating GHG with the use of TBI systems (Sanderman et al., 2009).

In recent years, studies have shown the *in situ* potential of TBI systems to sequester C in the temperate region (Bambrick et al., 2010; Oelbermann et al., 2004; Peichl et al., 2006; Thevathasan and Gordon, 2004). However, these studies have only examined the CSP of one or two tree species. An extensive study to evaluate many of the temperate tree species suitable for TBI systems and to document their potential to sequester C at the system level is lacking for the temperate region. Therefore, this study was designed to test the CSP of five tree species that are currently integrated into a 25-year-old TBI system in southern Ontario, Canada. The objectives of this study are, a) to quantify above- and belowground tree Biomass C and b) to quantify the SOC stock. This will help to estimate levels of atmospheric CO_2 that could be sequestered by TBI systems in the above- and belowground C pools for this climatic region of Canada. It may also contribute towards national climate change mitigation goals and the development of C models for TBI systems.

2.2 Methods

2.2.1 Tree Biomass Carbon

To determine the above- and belowground Biomass C of hybrid poplar, red oak, black walnut, Norway spruce and white cedar, three replicates of each species were destructively harvested and weighed. Belowground roots were excavated to a depth of 1.5 m using a 580

Robert Tire Backhoe. All coarse roots, having a diameter greater than that of a pencil thickness (approximately 2 mm), were hand-picked from a soil volume of 24 m^3 [4 m along the tree row (2 m north and 2 m south from the tree trunk) and 4 m into the crop alleys (2 m east and 2 m west from the tree trunk) and to a depth of 1.5 m]. Distances from the tree trunk were determined from a previous study by Gray (2000), who determined that 90% of the roots on this site were within the distances from the trunk given above. The tree components were classified as trunk, primary and secondary branches, twigs (plus needles for spruce and cedar) and roots. Subsamples of these components were taken to determine gravimetric moisture content. Moisture content obtained from the sub-samples was used to convert fresh weights to oven dry biomass, which was then multiplied by C concentration (%) to determine tree component biomass C. Carbon (%) in respective tree components was determined by analyzing the sub-samples in a LECO CR-12 dry combustion Carbon Analyzer (LECO Corporation, MI, USA). Deciduous species were quantified at 111 trees ha^{-1}, but due to reduced spacing between spruce (3 m instead of 6 m) and cedar (1 m instead of 6 m) were classified at 222 and 333 trees ha^{-1} (Figure 1), respectively and therefore all calculations were done using new densities to represent realistic spacing of these trees in a 1 ha TBI system.

2.2.2 Soil Organic Carbon

Soil samples were collected from each tree in both east and west directions, at 0.5, 1.0, 1.5 and 2.0 m distances from the tree trunk and at 0 - 10, 10 - 20 and 20 - 40 cm depths ((4 East locations + 4 West locations) x 3 depths x 3 replicates = 72 samples per tree in total x 3 trees = 216 samples per species) using a metal core (250 cm^3) in the TBI system. Samples were collected in the same spatial pattern in the soybean sole-crop (without sampling from two directions as soil C was assumed to be homogeneous in all directions x 1 replication = 36 samples). Soil samples were stored at -20°C until ready for analysis at which point they were

thawed, air dried for 48 h and passed through a 2 mm sieve to exclude gravels and fine roots. A ~15 g subsample was measured from each air-dried soil sample to determine gravimetric moisture content (oven-dried at 105ºC). Another ~15 g sample was further ground to <0.125 mm with a ball-mill to ensure a homogenized sample for SOC analysis. After being ball-milled, ~1.000 g samples were fumigated with 12 M hydrochloric (HCl) acid for 7 d in order to remove the inorganic C fraction following methods adapted from (Ramnarine et al., 2011), including the use of a correction factor. Preliminary studies as to methods to remove inorganic C can be found in Appendix I. After fumigation, samples were dried at 50°C for 24 h and a ~0.3000 g sample was analyzed for SOC content using the LECO CR-12 dry combustion Carbon Analyzer (LECO Corporation, MI, USA).

SOC stocks (t C ha^{-1}) were obtained from a parallel study conducted at the same site by Borden (2013, pers. comm.) for 0 - 10, 10 - 20 and 20 - 40 cm. Species- and depth-specific bulk densities and corresponding SOC stocks were used to calculate tree-specific SOC stocks to 40 cm soil depth.

2.3 Statistical Analysis

All treatments were tested for statistical parameters using SAS 9.3 (SAS Institute Inc. Cary, NC). Data was assessed for normality, independence and equal variance and compared between TBI and soybean sole-cropping system using an ANOVA. Statistical significance was assessed at $P<0.05$. Outliers were removed based on Lund's critical values and an analysis of variance was performed using a generalized linear model procedure. The effect of sampling distance from the tree row and depth of soil samples (main effects) within TBI systems and soybean sole-cropping system was analyzed using a factorial analysis with repeated measures (distances and depth).

2.4 Results and Discussion

2.4.1 Tree Biomass Carbon

Mean height and diameter at breast height (DBH) for the three replicates of each species that were destructively harvested are found in Table 4. Mean oven dry biomass, C concentration and biomass C of each tree component of each species are in Table 5.

Table 4. Mean height and diameter (± standard deviation) at breast height for five tree species commonly found in tree-based intercropping systems in southern Ontario, Canada (n=3 per species).

Tree Species	Mean DBH (m)	Mean Height (m)
Hybrid poplar	0.35 ± 0.01	17.17 ± 0.1
Red oak	0.22 ± 0.01	9.40 ± 1.4
Black walnut	0.26 ± 0.03	10.38 ± 1.7
Norway Spruce	0.22 ± 0.04	10.79 ± 0.3
White cedar	0.07 ± 0.02	6.06 ± 1.3

Poplar TBI systems had the highest mean biomass C per tree, both above- and belowground, followed by oak, walnut, spruce and cedar, having a total of 239, 139, 132, 117 and 49 kg C, respectively (Table 5). However, once their various densities are incorporated, the highest mean Biomass C in t per hectare is highest in poplar TBI systems followed by spruce, cedar, oak and walnut with 26.6, 15.4, 14.7, 26.0, and 16.2 t C ha^{-1}, respectively. The percentage of biomass stored aboveground was 78, 74, 82, 73 and 82 %, respectively, for poplar, oak, walnut, spruce and cedar. Percentage of belowground biomass stored in the roots was 22, 26, 18, 27 and 19 %, respectively. All trees had approximately the same C concentration aboveground; poplar, spruce and cedar all had a mean aboveground C concentration of 53% ± 0.2, 0.5, 0.4, respectively, and oak and walnut had 50 % ± 0.9 and 0.4, respectively (Table 5). In all cases, roots had less C concentration than that recorded for aboveground biomass of 50 (±4), 48 (± 1),

47 (± 2), 52 (± 1) and 47 (± 1) % for poplar, oak, walnut, spruce and cedar species, respectively (Table 5). No significant difference was observed for mean C concentration between species.

Table 5. Biomass and carbon content (mean ± standard deviation) of different tree components from five tree species in a 25-year-old intercropped system in southern Ontario, Canada (n=3 per species).

Poplar	Dry biomass (kg)	C concentration (%)	Biomass C (kg)
Trunk	196.30 ± 61.93	51 ± 1.9	99.99 ± 28.57
Primary branches	91.84 ± 63.23	53 ± 1.1	48.06 ± 32.35
Secondary branches	48.33 ± 40.83	53 ± 1.6	25.44 ± 21.34
Twigs	25.86 ± 4.59	53 ± 0.3	13.65 ± 2.46
Roots	102.63 ± 54.44	50 ± 4.0	52.33 ± 31.91
Total tree	464.95 [a]	51.90 ± 0.64 [a]	239.46 (± 61.71) [a]
Red Oak	Dry biomass (kg)	C concentration (%)	Biomass C (kg)
Trunk	102.55 ± 20.09	52 ± 1.5	53.33 ± 12.05
Primary branches	31.57 ± 11.06	49 ± 1.0	15.53 ± 5.19
Secondary branches	24.04 ± 10.70	49 ± 4.7	11.53 ± 4.73
Twigs	45.33 ± 11.98	51 ± 2.0	22.99 ± 6.05
Roots	73.94 ± 10.01	48 ± 1.5	35.79 ± 5.27
Total tree	277.43 [a]	49.82 ± 1.47 [a]	139.42 (± 22.41) [b]
Black Walnut	Dry biomass (kg)	C concentration (%)	Biomass C (kg)
Trunk	118.03 ± 17.27	52 ± 1.5	61.01 ± 10.34
Primary branches	33.98 ± 13.86	50 ± 1.9	17.19 ± 7.40
Secondary branches	18.56 ± 7.07	51 ± 1.4	9.36 ± 3.45
Twigs	41.41 ± 22.20	50 ± 0.2	20.60 ± 11.07
Roots	52.76 ± 38.47	47 ± 2.4	24.25 ± 17.54
Total tree	264.75 [abc]	49.72 ± 0.62 [a]	132.42 (± 49.65) [b]
Norway Spruce	Dry biomass (kg)	C concentration (%)	Biomass C (kg)
Trunks	67.60 ± 24.83	52 ± 0.4	35.36 ± 13.23
Branches	39.85 ± 22.87	53 ± 1.4	21.24 ± 12.68
Twigs	20.95 ± 6.00	53 ±1.4	11.01 ± 2.97
Needles	33.19 ± 17.42	53 ± 1.0	17.57 ± 9.38
Roots	61.48 ± 29.20	52 ± 1.0	32.04 ± 15.61
Total tree	223.07 [abc]	52.43 ± 0.92 [a]	117.22 (± 44.39) [bc]
White Cedar	Dry biomass (kg)	C concentration (%)	Biomass C (kg)
Trunks	36.94 ± 23.01	52 ± 2.0	19.49 ± 12.65
Branches	5.78 ± 5.97	53 ± 0.8	3.04 ± 3.11
Twigs	9.67 ± 6.28	53 ± 0.4	5.09 ± 3.34
Needles	22.89 ± 9.30	53 ± 0.3	12.14 ± 4.89
Roots	18.62 ± 3.65	47 ± 1.3	8.83 ± 1.75
Total tree	93.90 [bc]	51.67 ± 0.43 [a]	48.60 (± 24.81) [c]

[a-c] Values with different letters indicate significant difference at $p < 0.05$

Deciduous tree species (poplar, oak and walnut) have more Biomass C allocated in their trunk, than any other aboveground components, including primary and secondary branches and twigs combined. Conversely, conifer species (spruce and cedar), have more C allocated in the branches, twigs and needles combined than compared to C found in the trunk. Oak and spruce stored the most Biomass C belowground at 27 % whereas walnut stored the least with 16 %. The high variability of Biomass C of the trees is illustrated by the high standard deviations. Peichl et al. (2006) noted similar observations after destructively harvesting 13 year-old trees and attributed variation to management practices, such as pruning, and the length of cutting cycles.

Given respective tree densities, poplar, oak, walnut, spruce and cedar have 27, 15, 15, 26 and 16 t C ha^{-1}, respectively. Annually, this translates to an assimilation rate of 1.1, 0.6, 0.6, 1.0, and 0.7 t C ha^{-1} y^{-1}, respectively, over the past 25 years. Previous studies have shown that fast growing poplar trees are able to sequester more than twice as much C than a slow growing species, such as spruce (Peichl et al. 2006). However, at 25 years after establishment, poplar has reached maturity and branches have started to die-back. Therefore, this resulted in a slight decrease in the total poplar tree Biomass C from 1.17 t C ha^{-1} y^{-1} (Peichl et al., 2006) to 1.06 t C ha^{-1} y^{-1} between 13 (year 2000) and 25 (year 2012) years after establishment, respectively. Slower growing species such as spruce continue to add biomass C, from 0.49 t C ha^{-1} y^{-1} (Peichl et al., 2006) to 0.65 t C ha^{-1} y^{-1} at 111 trees ha^{-1} at 25 years after establishment. Spruce will therefore continue to sequester atmospheric CO_2 until harvest (60 years or more). Comparing these growth rates to the current rate of growth of poplars, poplars should be harvested between 13 – 15 years to prevent die-back, which usually occurs at 15 years for this particular clone (Thevathasan, pers. comm.). TBI systems designed with hybrid poplar trees should therefore allow for a second cycle of trees to be planted between years 8 and 10 of the initial planting. This

allows the initial plantings to be harvested at year 15, and the second set of trees will be sequestering atmospheric C at an exponential rate at ages 5 or 7, thus enhancing system level C sequestration on a continuous basis.

2.4.2 Soil Organic Carbon

In total, 953 samples were included in the analysis for SOC for all five tree species. To determine the mean SOC concentration (%) at each depth and distance, mean values were calculated from three subsamples. Overall SOC means at 0 – 40 cm were 1.67 ($\pm$ 0.18) % in the soybean sole-crop system. In the TBI field, SOC means were 1.66 ($\pm$ 0.10), 1.57 ($\pm$ 0.11), 1.48 ($\pm$ 0.12), 1.42 ($\pm$ 0.14), and 1.41 ($\pm$ 0.13) % for cedar, poplar, oak, spruce and walnut species, respectively, with no significant difference between species (Table 4).

When incorporating direction (east and west of the tree row), distance (0.5, 1.0, 1.5, and 2.0 m from the tree trunk) and depth (0-10, 10-20 and 20-40 cm, at each distance), a factorial analysis indicated depth as the most important factor accounting for most of the variation within the model (F value = 222.57) and the strongest relationship with species (F value = 5.36, $P < 0.0001$). Overall, SOC is shown to decrease with increasing depth across all tree species. This is expected, as it is known that SOC decrease with increasing soil depth. Mean SOC values by depth for each species are presented in Table 4.

SOC in the soybean sole-crop system did not show decreasing SOC with depth but instead were highest between 10 and 20 cm (Table 6). This is most likely due to recent management practices that have increased the amount of crop residue left behind on site. However, due to a lack of base-line data, the full extent of the soil's ability to either accumulate or deplete SOC is unknown. However, when these C concentrations are calculated into SOC

stocks with bulk density (Table 7), their C stocks show to be less than those found in TBI systems which was expected.

Table 6. Mean SOC (%) for five tree species in a 25-year-old tree-based intercropping and a soybean sole-crop system at 0 – 10, 10 – 20 and 20 – 40 cm depths (± standard error) in southern Ontario, Canada

	Soybean	Poplar	Oak	Walnut	Spruce	Cedar
0-10 cm	1.95 (± 0.03)[a]	1.91 (± 0.06)[a]	1.90 (± 0.06)[a]	1.87 (± 0.08)[a]	1.77 (± 0.13)[a]	2.04 (± 0.07)[a]
10-20 cm	2.14 (± 0.19)[ab]	1.68 (± 0.06)[b]	1.58 (± 0.04)[b]	1.49 (± 0.04)[b]	1.64 (± 0.03)[a]	1.59 (± 0.03)[b]
20-40 cm	0.91 (± 0.13)[c]	1.11 (± 0.11)[c]	0.96 (± 0.10)[c]	0.87 (± 0.03)[c]	0.85 (± 0.11)[b]	1.36 (± 0.11)[c]

[a-c] Superscripts indicating significant difference across depth, by species at $p<0.05$.

Interactions between species and distance were also significant (F value = 2.30, P = 0.0033), most likely from the influence of litter from the tree row. In all tree species, SOC concentrations were higher closer to tree rows (at 0.5 m) and decreased into the cropping alleys (2.0 m), however, they were not statistically different, as was found for poplar species in a study conducted by Bambrick et al., (2010) at the same study site 21 years after establishment. These higher SOC concentrations can be attributed to higher input of OM that accumulate closer to the tree row and have greater inputs of C and N to contribute to the SOC stock (Bambrick et al., 2010). However, as was established by Thevathasan and Gordon (2004), as trees age, their litterfall is spread in a more homogeneous pattern to the soil floor. This would impact the C input from annual litterfall and can result in SOC concentrations with spatial homogeneity within the first 2 m of the tree row in a mature TBI system. Interactions between species and direction, direction and distance and direction and depth were not significant.

With the incorporation of bulk densities, total SOC stocks for poplar, cedar, oak, spruce, and walnut species were 86.86, 83.77, 83.23, 78.29, and 76.84 t C ha^{-1}, respectively. In the

soybean sole-crop field, SOC stocks were 71.08 t C ha^{-1}, respectively, as seen in Table 5. Higher than expected SOC stock was found in cedar but is most likely due to closer spacing (1 m compared to 3 – 5 m of other species within the tree rows). To a depth of 40 cm, TBI systems show a higher SOC stock over conventional agriculture, mainly due to higher SOC concentrations in the 20 – 40 cm soil horizon. The SOC stock findings from this study are supported by findings by Peichl et al. (2006) at the same site 13 years after establishment. Peichl et al. (2006) reported barley monocrop agricultural system with SOC stocks 15.96 and 2.66 t C ha^{-1} less than poplar and spruce TBI systems, respectively. Bambrick et al. (2010) also found, at the same site, the conventional agricultural system to have 6.3 t C ha^{-1} less than poplar in a TBI system, 21 years after establishment. Given the relationship that exists between SOC and soil fertility (Bambrick et al., 2010), these higher SOC stocks found in TBI systems show the promise of sustaining soil and crop productivity in TBI systems compared to conventional agricultural systems.

Table 7. Mean bulk densities (± standard error) and soil organic carbon stocks associated with a 25-year-old intercropping and conventional agricultural systems at the University of Guelph's Agroforestry Research Station (0 – 40 cm)

Intercropping	Mean C concentration (%)	Mean bulk density (g cm^{-3})	SOC (t C ha^{-1})
Poplar	1.57 (± 0.11)	1.21 (± 0.12)	86.86
Oak	1.48 (± 0.12)	1.20 (± 0.13)	83.77
Cedar	1.66 (± 0.10)	1.14 (± 0.19)	83.23
Spruce	1.42 (± 0.14)	1.15 (± 0.18)	78.33
Walnut	1.41 (± 0.13)	1.15 (± 0.18)	76.84
Conventional Agriculture			
Soybean	1.67 (± 0.18)	1.20	71.08

Higher rates of SOC may also be attributed to recent changes in agricultural practices in the crop rows of the TBI systems. West and Post (2002) found that with the conversion of

conventional tillage to no tillage practices, SOC accumulation rates can increase and peak in 5 – 10 years of the change and reach a new equilibrium in 15 to 20 years. Reduced tillage reduces soil disturbances and soil erosion while retaining belowground roots and aboveground biomass (such as litter dropped by the trees) all of which contribute to the SOC pool (Lal, 2004). Given that this site had undergone conventional tillage until 1991, followed by no till until 1996, the 5-year span of no tillage, followed by reduced tillage practices, could have added to the CSP of the SOC stock.

2.4 Conclusions

It is evident that the CSP for each investigated tree species in this TBI system differed and was highest for fast-growing tree species (e.g. poplar) and lowest for slow-growing species (e.g. spruce). This study was the first to quantify C stocks for five commonly grown tree species in TBI systems in southern Ontario which will help to understand the long-term implications in C sequestration at the landscape level as influenced by two different land-use systems. It is also important to note that different species can be planted at different densities. For example, due to the closer in-row spacing of coniferous trees, higher densities can be used to enhance C sequestration with minimal competition with crops due their slow growth rate and cone-type canopy structure. This will also allow landowners to decide on various tree species that fit their site- and goal-specific needs. The CSP of various tree species, known from this study, may be of importance to introduce these tree species into other temperate land-use systems, such as silvopastoral systems, riparian buffers and windbreaks. This study also suggests the impact of 'relay planting' or 'staggered planting' of fast-growing trees species, such as hybrid poplar, in order to reduce die-back, and maximize the system level C sequestration in TBI systems. This management practice should be further studied as it relates to the importance if C trading, should it ever become a reality in Ontario.

This study also showed the importance of analyzing SOC stocks to a depth of 40 cm to ensure the full C stock is quantified. It was also found that even though SOC concentrations may be a higher at shallower depths in conventional agricultural systems, with the calculation of SOC stocks, a greater CSP might not be seen at the system level. It is also important to note that a slight increase in SOC between the years 13 and 25 suggest that crop rotation is an important management consideration in conventional agricultural systems in order to maintain SOC levels.

Lastly, the quantification of C sequestration in TBI systems is an important step towards making informed policy decisions associated with greenhouse gas mitigation strategies in the agricultural sector, one of the primary objectives of the Global Research Alliance (GRA) and the Canadian Agriculture Greenhouse Gas Program (AGGP). In combination with other Best Management Practices (BMPs) recommended for the conventional agricultural sector, TBI systems could contribute towards reducing the impact of Canadian agriculture on greenhouse gas emissions.

3.0 Chapter 3 – Carbon Fluxes

3.1 Introduction

Carbon fluxes within a TBI system, processes of C cycling that occur on a year-to-year basis, are an important component to consider, and include litterfall, litter decomposition, soil carbon leaching, soil respiration, assimilation by trees and crops, C input and removal from crop harvest, and C that is harvested for wood products at the end of a cutting cycle (Peichl et al., 2006). Quantifying as many fluxes as possible is important for modeling purposes to reduce estimations that can easily result in deficiencies and inadequacies (Nair, 2012). Fluxes quantified in this study include annual litterfall, litter decomposition and soil respiration.

OM inputs from litterfall are the main source of enhanced productivity and system nutrient accumulation from TBI systems. This main component, absent in conventional agricultural systems, is the largest C flux from tree components (Oelbermann et al., 2004). Lignin-rich OM is slowly decomposed and stabilized as microbial byproducts in a form that is physically protected and chemically recalcitrant (Bambrick et al., 2010; Montagnini and Nair, 2004). This OM, along with decomposition and mineralization rates aids in the accumulation and distribution of SOM and SOC (Isaac et al., 2005).

TBI systems can accumulate stable soil C from litterfall depending on its rate of decomposition. Decomposition within a TBI system is dependent on tree species, productivity, land management practices (such as pruning and tree density), site-specific soil properties and characteristics, and climate (Nair, 1993). Litter quality also influences decomposition rate and nutrient release (Kwabiah et al., 1999; Kwabiah et al., 2001), both of which are slower in temperate zones due to climatic conditions, which increase the amount of available C to enter the stable C stock (Oelbermann et al., 2004).

Soil respiration is a major component of the soil C cycle as it is an indicator of soil C storage, biological activity and soil quality (Ewel et al., 1987; Raich and Schlesinger, 1992; Tufekcioglu et al., 2001). Soil respiration is influenced by the respiration of plant and tree roots and soil microorganisms, and is dependent on the seasonal dynamics controlled by soil temperature and soil moisture (Lee and Jose, 2003; Lloyd and Taylor, 1994). Respiration rates are also impacted by the physical, chemical and biological characteristics of the soil, including soil texture, OM, root density and microbial biomass (Haynes and Gower, 1995; Kelting et al., 1998; Raich and Tufekcioglu, 2000). Measuring soil respiration, particularly in various spatial and temporal distributions, is beneficial in connecting SOM input, nutrient dynamics and C storage capacity of TBI systems (Lee and Jose, 2003).

The objective of this study was to quantify annual litterfall, litter decomposition and soil respiration within a 25-year-old TBI system. These flux data were combined with C stock sizes to estimate net CSP of each tree species in a 25 year old TBI system in southern Ontario.

3.2 Methods

3.2.1 Litterfall

To determine the annual addition of litter to the soil via the five tree species in the intercropping system, litterfall was collected every two weeks between September 11th and November 28th, 2012 using 1 m^2 wooden litter traps lined with 2 mm (nylon) fiber mesh. A 50 cm^2 quadrat was also used to collect *in situ* samples at the same time for method-comparison purposes.

Traps were placed under each tree species (3 replicates per species). As shown in Figure 2, poplar, oak and walnut trees were surrounded by six litter traps (6 traps x 3 replicates x 3 species = 54 deciduous litter traps), spruce surrounded by four litter traps and cedar surrounded

by two litter traps per replicate [(4 traps x 3 spruce replicates) + (2 traps x 3 cedar replicates) = 18 conifer litter traps]. A total of 72 litter traps were used (54 deciduous + 18 conifer litter traps = 72 litter traps total).

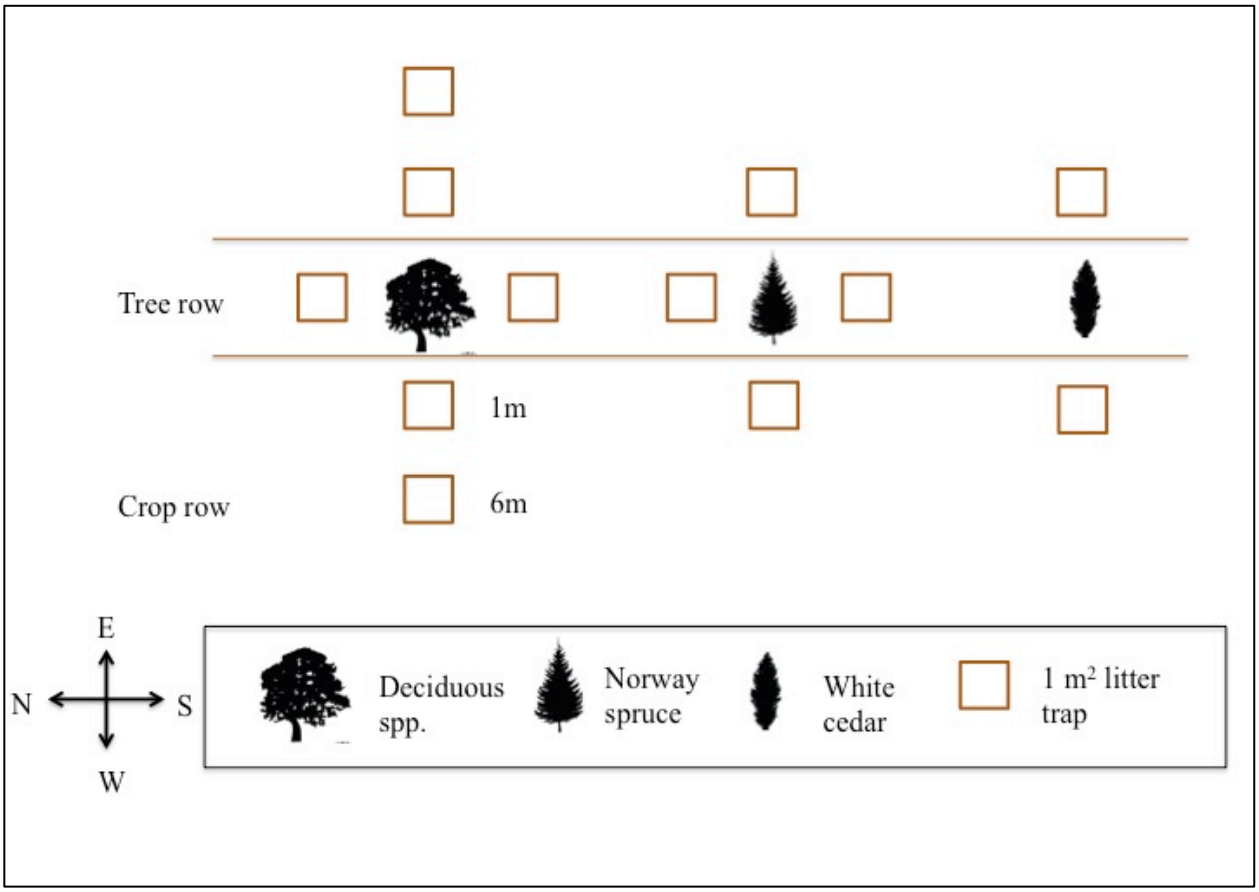

Figure 2. Distribution of litter traps for five tree species in the tree-based intercropping system. Diagram represents one replicate.

In situ measurements were also used to compare litterfall between the traps and the forest floor. This was done using a 50 m^2 quadrat that was placed at a random location around the litter trap and litterfall collected in the same manner as in the traps. On *in situ* measurement was taken at each replicate in the east and west direction for each tree species (5 species x 3 replicates x 2 directions (east and west) = 30 measurements per sampling period).

Litter was collected from the traps and *in situ* locations every two weeks and included "other" non-leaf litter content that was separated by 1) leaves from other species, 2) crop residue, 3) woody debris such as twigs and 4) other items such as nuts, fruits, buds, etc. Litter was placed in paper bags, dried in an oven at 60°C until constant mass was reached, before obtaining a dry biomass weight. Biomass was expressed on a g litterfall m^{-2} y^{-1} basis.

3.2.2 Litter Decomposition

Litter for the decomposition experiment was hand picked from the soil surface of the TBI field in September 2012 after autumn litterfall had begun. Litter for all five tree species and for leaves and stalk of soybean were oven dried at 60ºC until constant mass was reached. Litter was dried in order to reduce the need of conversion of air-dry weight to oven dry weight with a moisture content subsample. Decomposition bags, prepared from 2 mm nylon mesh screen with exposed area of 30 cm^2, were filled with ~50 g oven dried litter. Three random locations were selected in the TBI field and two random locations were selected in the soybean sole-crop field to act as pseudoreplicates. At each location, quadrats were marked for each sampling period and one of each leaf species and one soybean stalk and leaf were collected from each quadrat at each location (Figure 3). Only soybean stalks and leaves were buried in the soybean sole-crop system, as litterfall from other tree species would not be present in a conventional agricultural system (Figure 4). Decomposition bags were buried to a depth of 15 – 20 cm to simulate current tillage practices in both fields. The total number of litterbags retrieved at each sampling period was 25 bags ((7 [5 species + leaves + stalks] x 3 replications/locations in TBI) + (2 [leaves and stalks] x 2 replications/locations in monocrop) = 25 bags per sampling period). The bags were retrieved in November 2012, January, April, June, August, September and October 2013 (day 36, 94, 155, 216, 277, 312, and 361, respectively) summing to 175 bags collected in total (7 sampling periods x 25 bags per sampling period = 175). The material was collected, removed from the

decomposition bag, washed with water, dried at 60ºC for 48 h or until constant mass was reached and weighed (Anderson and Ingram, 1989). Data were expressed as mass remaining (%) and fitted to a single stock exponential model, expressed as:

$$W_t = 100\ e^{-k\,t}$$

This model assumes all plant tissue components decompose at the same rate, where W_t is the percent mass remaining at time t (days) and k is the decomposition rate constant (day^{-1}). A 5% mass loss can be assumed to occur during transportation, and burying and retrieval, as determined by (Otis, 2009).

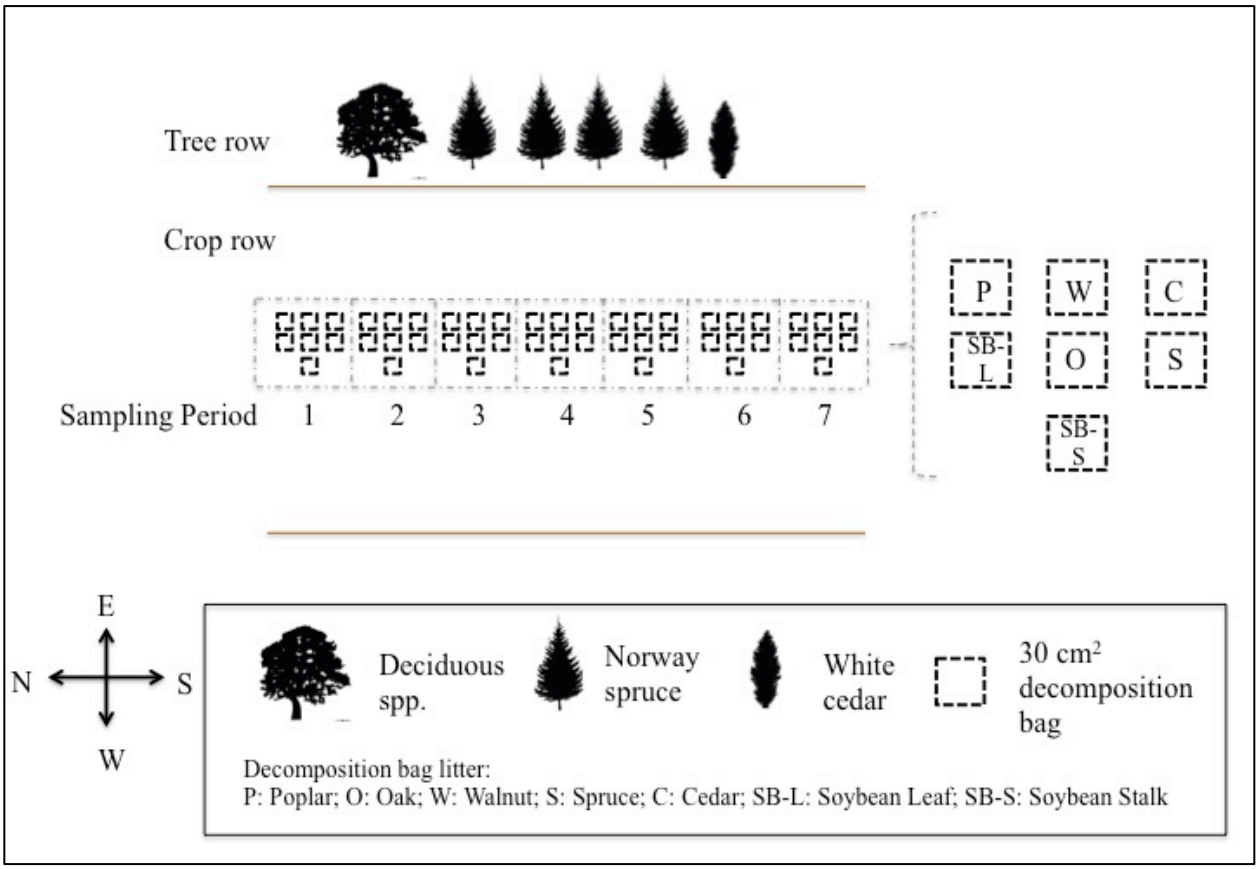

Figure 3. Distribution of 30 cm^2 litter decomposition bags containing 50 g of oven-dried leaf biomass from five tree species and soybean stalk and leaf in a tree-based intercropping system

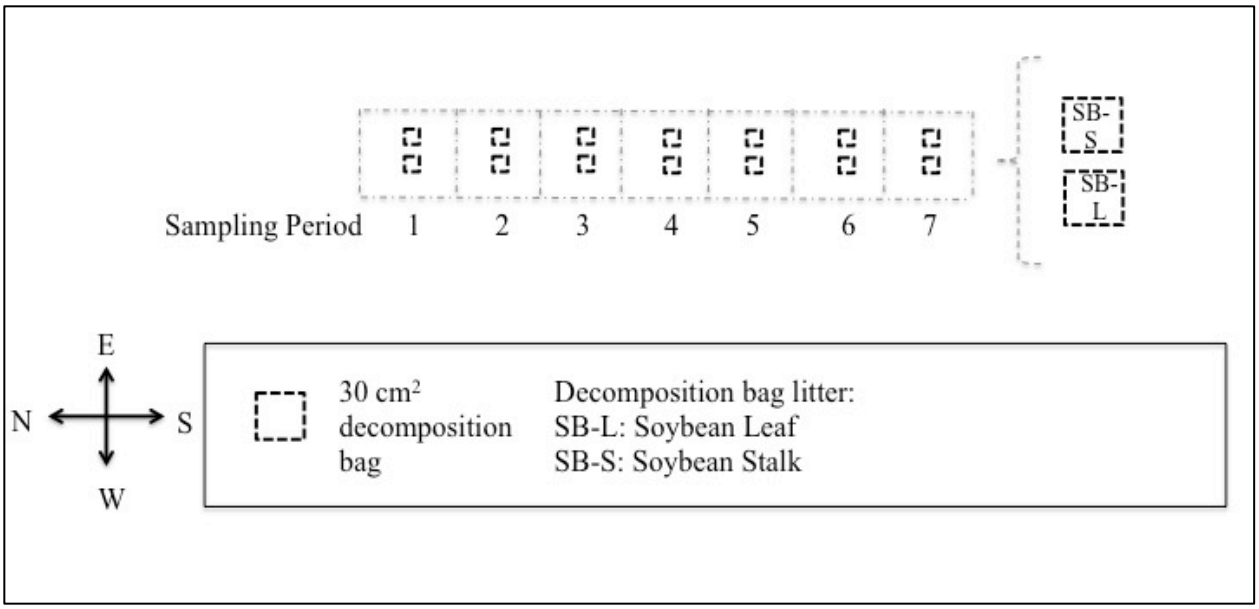

Figure 4. Distribution of 30 cm^2 litter decomposition bags containing 50 g of oven-dried biomass from soybean stalk and leaf in a soybean sole-cropping system.

3.2.3 Soil respiration

Soil respiration was measured twice a month between June 2012 and May 2013, excluding February and March 2013, as the amount of CO_2 efflux during winter months was assumed to be negligible (Peichl et al., 2006). Soil respiration was measured using the soda lime method, which is based on absorption of CO_2 by soda lime from the following chemical reactions (Edwards 1982):

$$2\ NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O \quad (1)$$

and

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3\downarrow + H_2O \quad (2)$$

Respiration chambers with an exposed soil surface area of 0.0314 m^2, were set up at 0, 2 and 6 m from the tree row into the cropping alley for poplar, walnut and spruce in the TBI system replicated 3 times (three respiration chambers per location x 3 replicates = 9 samples per

species, per sampling period) (Figure 5). Respiration chambers were made of permanently installed rings (10 cm in height, pushed 5 cm into the soil, and 20 cm in diameter) that were installed two weeks before the initial respiration measurements were conducted in June 2012. They were cleaned of any plant residue debris before each sampling period. White opaque buckets (15.75 cm in height and 20 cm in diameter) were used to cover the camber rings (using caulking to create a sealant between the bucket and ring) during the 24 h of respiration measurements. Fifty grams of granular soda lime contained in a glass mason jar (weighing approximately 200 g with a top and bottom diameter of 7 cm) was placed inside the chamber. Respiration was measured in the soybean sole-crop using three respiration chambers in the same spatial pattern as in the TBI system (3 respiration chambers x 1 location = 3 samples, per sampling period).

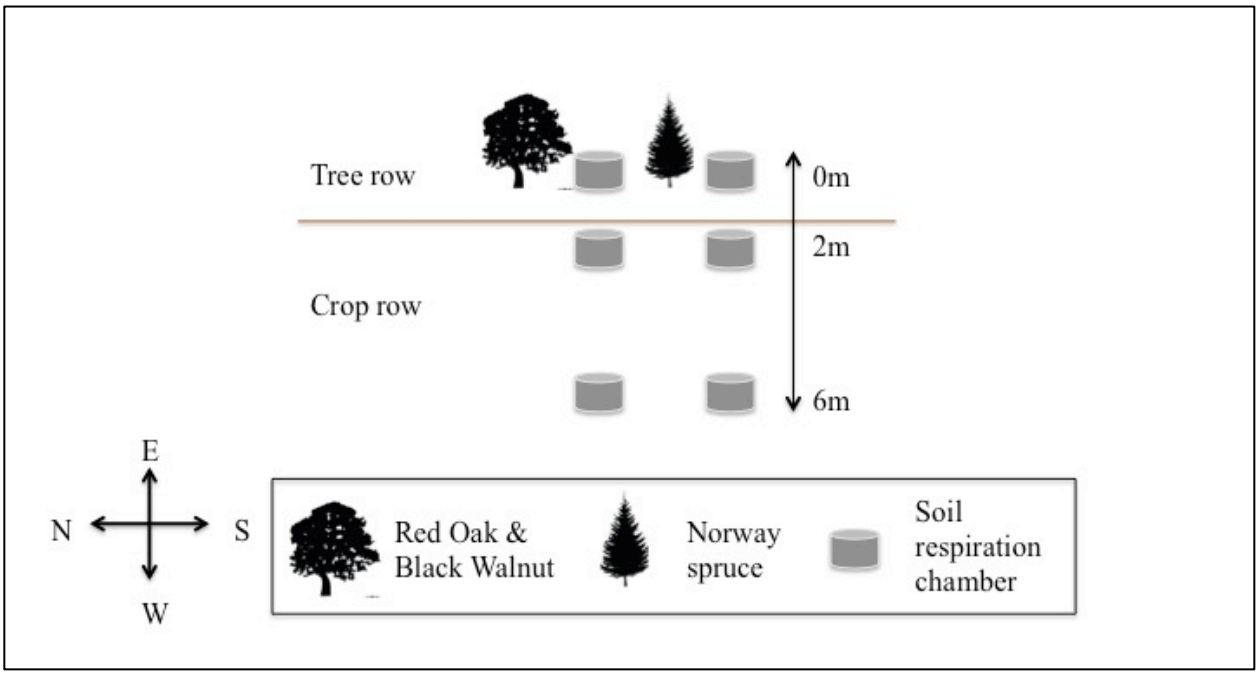

Figure 5. Distribution of soil respiration chambers for the measurement of annual CO_2 efflux using the soda lime method in a tree-based intercropping system.

For each sampling event, three "blank" soda-lime jars were used to correct for field absorption error. The difference in the CO_2 absorption measurement by the blanks were then averaged and subtracted from the CO_2 amount of each measurement to determine the true evolved CO_2 as soil respiration. A correction factor of 1.41 was used to account for water formed during chemical absorption of CO_2 by soda lime and released during drying (Edwards, 1982). Soil CO_2 efflux (g CO_2 m^{-2} day^{-1}) was calculated using equation 3. More recent literature Grogan (1998) showed that a correction factor of 1.69 should be used in place of 1.41 for the correction to account for water formed during chemical absorption of CO_2 by the soda lime; however, since our data was to be compared with previous data using the same methods, the calculations were done with the 1.41 correction factor to be consistent with previous studies.

$$\text{Soil CO2 efflux (g } CO_2\ m^{-2}\ d^{-1}) = [(\text{sample weight gain (g)} - \text{mean blank weight gain (g)} \times 1.41)) / \text{chamber area } (m^2)] \times [(24\ (h)) / \text{duration of exposure (h)})] \quad (3)$$

A distance of 6 m from the tree row was selected to be indicative of TBI systems for the purposes of C modeling over 0 and 2 m for two main reasons: 1) this area (cropping alley) occupies ~ 87% of the land area on a hectare basis, and 2) to exclude respiration by tree roots and grass roots along the tree rows.

3.3 Statistical Analysis

All treatments were tested for statistical parameters using SAS 9.3 (SAS Institute Inc. Cary, NC). Data was assessed for normality, independence and equal variance and compared between TBI and soybean sole-cropping system using an ANOVA. Statistical significance was assessed at $P<0.05$. Outliers were removed based on Lund's critical values and an analysis of variance was performed using a generalized linear model procedure. Factorial analysis was

performed to test for significant interactions between species, direction and distance (main effects) from the tree row for litterfall data and for species, month and distance (main effects) for soil respiration data. Soil respiration using repeated measures. Correlation analyses were performed between litter decomposition, soil temperature and moisture content, and between soil respiration, soil temperature and moisture content. For litter decomposition, k values were determined by performing a non-linear regression function and t-test was used to compare the different decomposition rates between species.

3.4 Results and Discussion

3.4.1 Litterfall

Mean annual litterfall flux (g m^{-2}) for the five tree species in a TBI system found in the litter traps are found in Table 1. Walnut species produced the highest mean annual litterfall, followed by poplar, spruce, oak, and cedar species with annual fluxes of 145.00 ($\pm$ 32), 70.39 ($\pm$ 30), 58.92 ($\pm$ 22), 67.98 ($\pm$ 19) and 32.67 ($\pm$ 12) g m^{-2}, respectively.

Litterfall from walnut trees was significantly different from oak (p value = 0.0081), from poplar (p value = 0.0181) and from spruce (p value = 0.0086). It was expected that deciduous trees would have higher annual litterfall compared to coniferous trees, however poplar litterfall was low likely due to die-back brought on by tree age.

Other residue collected in the litter traps was collected and quantified and also presented in Table 8. 'Other' categories were not incorporated in the litterfall contribution to CSP of each species, however they were measured as they are left on the soil surface, mineralized by soil microorganisms and contribute nutrients over longer periods of time.

Litter distribution measured using the litter traps for each species is found in Figure 6. Litterfall from walnut, poplar and spruce species was highest in the north direction. This is most

likely due to the fact that in the north and south direction the litter traps were equally spaced between two trees within the tree rows, therefore litter quantified is most likely not from a single tree. For oak and cedar species, highest litter was found in the west direction at 1 m.

Table 8. Mean annual flux of litter trap contents for five tree species in a 25-year-old tree-based intercropping system in southern Ontario, Canada (g m^{-2} y^{-1}) (± standard error)

	Walnut	Poplar	Oak	Spruce	Cedar
Annual litterfall	145.00[a] (± 20)	70.39[b] (± 19)	67.98[b] (± 21)	58.92[b] (± 19)	32.67[b] (± 8)
Leaves from other species	21.10[b] (± 30)	70.39[ab] (± 61)	35.21[ab] (± 24)	75.27[ab] (± 50)	104.23[a] (± 46)
Crop residue	2.89[b] (± 4)	19.34[b] (± 6)	28.82[b] (± 17)	23.21[b] (± 18)	67.7[a] (± 27)
Woody Debris	34.00[b] (± 45)	73.52[ab] (± 11)	133.98[ab] (± 49)	66.45[b] (± 41)	58.84[b] (± 9)
Others (fruits, seeds, buds, etc.)	244.46[a] (± 98)	37.83[b] (± 18)	80.6[b] (± 34)	37.84[b] (± 16)	12.83[b] (± 8)

[a-b] Values with different letters indicate significant difference at $p < 0.05$

For deciduous tree species, litter traps were placed at both 1 and 4 m in the east and west direction into the crop row. When oak, poplar and walnut trees were analyzed using a factorial analysis, distance was found to account for the most variability in the model, followed by species and direction (F values of 13.72, P value = 0.0010, 6.20, P value =0.0063 and 1.71, P value = 0.2024, respectively). Given the mentioned p values, species and distance were statistically significant as independent factors, however not as interactions (F value = 0.88, p value = 0.4281). This was supported by t-test showing no significant difference between litterfall accumulations at 1 m compared to 4 m, except for walnut in the west direction (P value = 0.03561). All other deciduous species showed no significant difference in traps placed at 1 m and 4 m. There was also no significant difference in annual litterfall occurring in the east direction compared to the west direction. Given the age of the TBI system, this can be expected due to mature canopy cover providing an equal distribution of litterfall over the tree and crop alley (Bambrick et al., 2010).

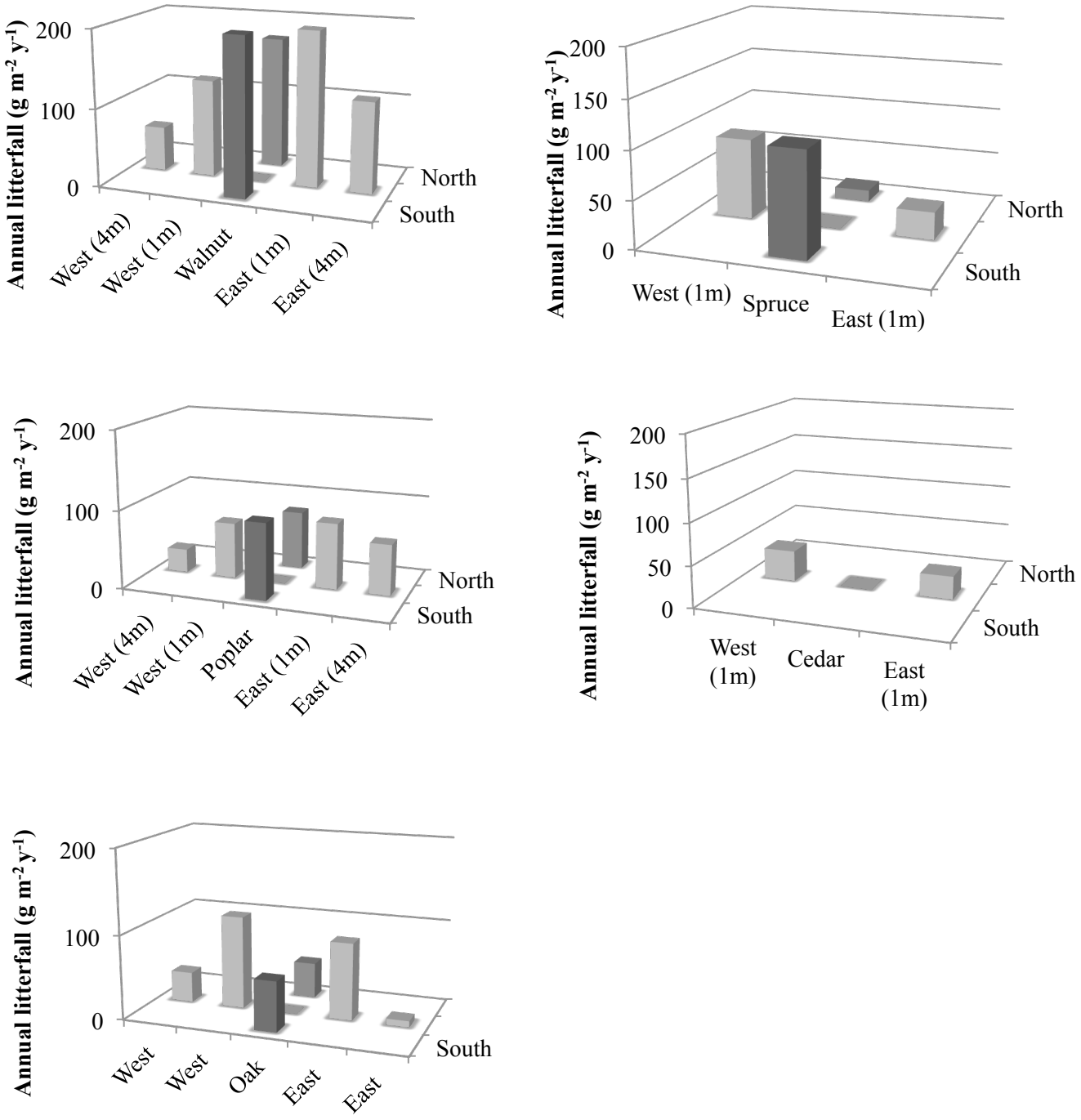

Figure 6. Spatial distribution of annual litterfall (g m^{-2} y^{-1}) for five tree species in a 25-year-old tree-based intercropping system in southern Ontario, Canada

In situ measurements were taken to compare the quantities of litterfall, as litter traps have been known to underestimate litterfall (Thevathasan, pers. comm., 2013). Annual litterfall comparison between traps and *in situ* methods are compared in Table 2. Highest litterfall was found beneath poplar, walnut, spruce, oak and cedar with 3.79, 3.48, 2.97, 2.50, and 0.45 t ha^{-2} y^{1}, respectively, with a significant difference only between poplar and cedar (p value = 0.0461). T-tests showed no significant difference between the litter trap and *in situ* method for any species except poplar (Table 9). Litterfall was always greater in the east direction (53% and greater of litterfall between east and west directions) for all tree species, although none showed significant difference. This was expected as the prevailing wind traveled from west to east throughout the year.

As annual litterfall from *in situ* measurements showed greater quantities of litterfall, it was assumed that this would be more indicative of system level C and was therefore used in the C model over the litterfall found in the litter traps. If C concentration is assumed to be 43% for deciduous leaf fall, then poplar, walnut and oak species would have litterfall C input of 1.63, 1.50, and 1.07 t C ha^{-1} y^{-1}, respectively). Assuming 50% C content for coniferous needles, spruce and cedar species would have annual litterfall C input of 1.49 and 0.23 t C ha^{-1} y^{-1}, respectively.

Table 9. Comparison of *in situ* collection methods vs. the use of litter traps to quantify annual litterfall (g m^{-2} y^{-1}) for five tree species in a 25-year-old tree-based-intercropping system in southern Ontario, Canada (n=3 per species)

	In Situ	Trap	Pr > |t| value
Hybrid poplar	379.41 (± 82)	70.39 (± 19)	0.0210
Black walnut	352.49 (± 162)	145.00 (± 20)	0.2738
Red oak	109.10 (± 55)	57.98 (± 21)	0.4376
Norway Spruce	266.98 (± 147)	58.92 (± 19)	0.2327
White cedar	33.49 (± 8)	32.67 (± 8)	0.1439

In TBI systems in temperate regions, litterfall productivity is expected to increase with system age (Oelbermann et al., 2004; Thevathasan and Gordon, 1997). This was seen at this site as C inputs increased from 107 g C m^{-2} y^{-1}, 9 years after establishment (Thevathasan and Gordon, 1997), to 117 g C m^{-2} y^{-1} at 13 years after establishment (Oelbermann et al., 2006). Assuming 43% C content of litter (Peichl et al., 2006), this study shows poplar would contribute 163 g C m^{-2} over a 25 year period (using annual litterfall found in *in situ* measurements). This shows the continual increase of C being added to the system as trees age and provide more litterfall from a mature canopy, as suggested in previous research. This value is also most likely an underestimation as poplar was experiencing die-back during this study. Assuming 43% C content of litter for walnut and oak species as well, they would contribute 152 and 47 g C m^{-2}, respectively. Assuming 50% C content of needles of spruce and cedar species (Peichl et al., 2006), they would contribute 133 and 17 g C m^{-2}, respectively.

Annual litterfall is tree-specific and dependent on tree age, stand density, management factors (such as pruning) and site- and region-specific characteristics such as edaphic and climate (Oelbermann et al., 2006). Species-specific quantification of annual litterfall in TBI systems will aid in analyzing species-specific CSP and enhanced nutrient cycling due to litterfall OM in TBI systems.

3.4.2 Litter Decomposition

Comparison of the decomposition rates of soybean leaf and stalk in the soybean sole-crop field and in the TBI system are shown in Figure 7. In the soybean sole-crop, 84 ($\pm$ 14.7) and 100 % ($\pm$ 0.0) mass of original litter was lost during 361 days of decomposition for the stalk and leaf, respectively. In the TBI field, mass loss reached 62 ($\pm$ 0.24) and 96 % ($\pm$ 5.1) for soybean stalk and leaf, respectively. In the soybean sole-crop field, there was no significant difference in the decomposition rates between soybean stalk and leaf, but there was in the TBI field (p value =

0.001). Decomposition rates of the soybean stalk differed between the soybean sole-crop field and the TBI field (p value = 0.0135) and between the soybean leaf in the monocrop field and the stalk in the TBI field (p value = 0.0005).

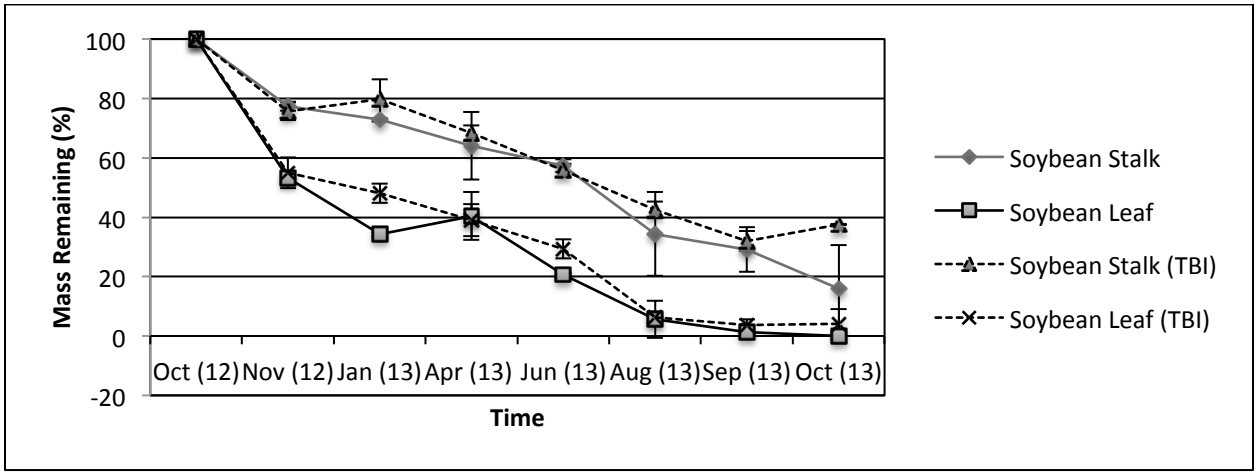

Figure 7. Leaf biomass remaining after 12 months of soybean stalk and leaf in a soybean sole-crop system and 25-year-old TBI system, respectively.

Slower rates of decomposition were found in the TBI system compared to the monocrop system, can occur for a number of reasons. Firstly, litterfall and crop residue in TBI systems are often combined with woody components dropped from trees (i.e. prunings, twigs, cones, etc.), which can increase the mean residence time of C in the system and slow decomposition (Oelbermann et al., 2004). Secondly, the presence of mature trees creates microclimates causing lower soil temperatures and moisture contents, which (Mungai and Motavalli, 2006) have related to slower decomposition rates. This slower release of nutrients from OM contributes to the accumulation of stable soil C stocks (Oelbermann et al., 2004), which were shown to be higher in the TBI field when compared to the soybean sole-crop system.

At the end of 361 days, poplar, oak, walnut, spruce and cedar had 68 ($\pm$ 0.2), 50 ($\pm$ 2.4), 86 ($\pm$ 9.2), 42 ($\pm$ 4.8), and 44 % ($\pm$ 0.4) residue remaining, respectively. Figure 8 depicts the

rates of decomposition of litter from different tree species. All species show significantly different decomposition rates except for oak, spruce and cedar (p value > 0.05). Decomposition rates of poplar are similar to those reported by Gordon and Thevathasan (2005) who found after a full year, poplar had lost 67.5 % of its mass loss in the same system.

Different rates of decomposition between species is due to varying amounts of lignin and cellulose (C:N ratios) present in the litter, as seen in the difference in decomposition rates between deciduous and coniferous tree species. Litter with lower C:N ratios, such as poplar and walnut, provide more nitrogen to the microorganisms responsible for their decomposition and provide the energy needed to breakdown C substrates (Mungai and Motavalli, 2006). Therefore, litter with higher N availability and lower C:N ratios decompose more quickly, but may not be contributing as much C and N to the long term stable SOC stock.

Based on an assumption of 43 and 50 % C content for deciduous leaves and conifer needles, respectively, annual C outputs via decomposition from tree litter for poplar, oak, walnut, spruce and cedar become 1.10, 0.53, 1.29, 0.63, and 0.30 t C ha^{-1} y^{-1}, respectively. The balance between the input of OM in the form of litter and this decomposition of dead plant matter determine the organic C stock within the soil stock (Oelbermann et al., 2004). For example, faster decomposition rates of walnut result in higher C outputs. This greater loss of C may contribute to lower SOC stocks when compared to other species with slower decomposition rates such as spruce and cedar.

Rate constants were calculated based on the percentage of residue remaining after 361 days and are presented in Table 10. Oelbermann (2002) points out that because single pool exponential equations are based solely on one stage of decomposition, they do not always allow

for the best fit. However, given the high r^2 values for the majority of the species measured in this study, the model seems suitable.

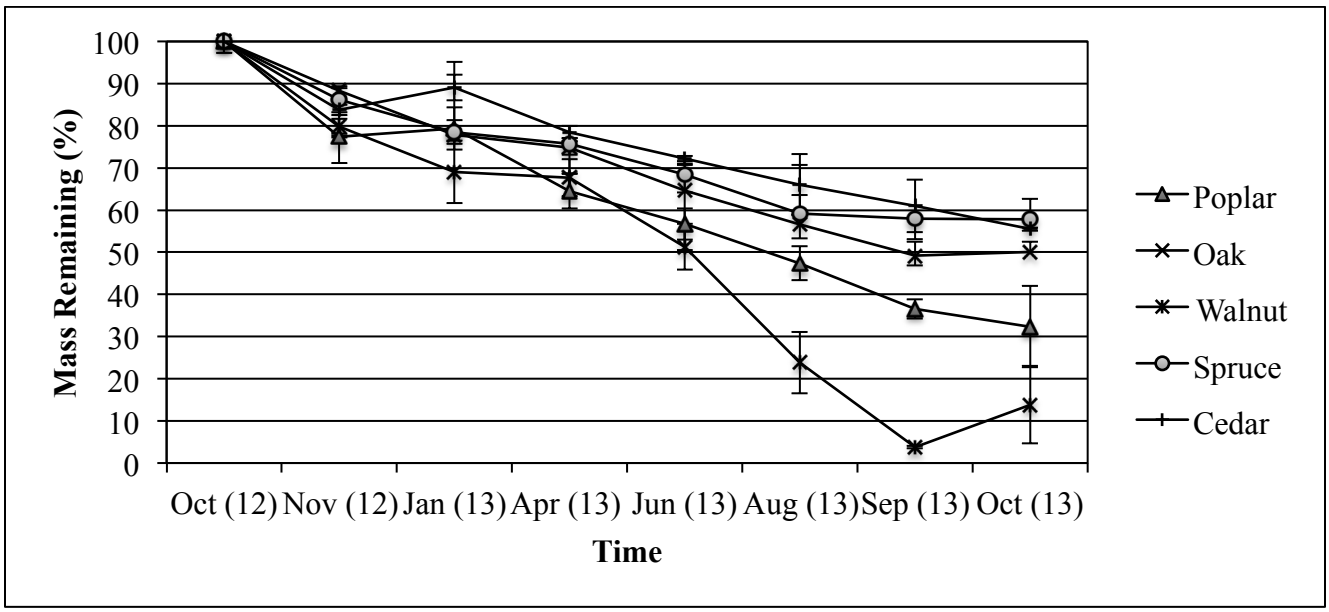

Figure 8. Leaf biomass remaining after 12 months from five different tree species in a 25-year-old tree-based intercropping system in southern Ontario, Canada.

Table 10. Single exponential models of leaf decay of five tree species and of soybean stalks and leaves over 361 days in a 25-year-old tree-based intercropping system and a conventional soybean sole-cropping system in southern Ontario, Canada

	K (day^{-1})	r^2	Half Life
Soybean Stalk	0.0058	0.92	138.04 [cde]
Soybean Leaf	0.0124	0.87	182.50 [bcde]
Soybean Stalk (TBI)	0.0027	0.92	278.80 [bc]
Soybean Leaf (TBI)	0.0112	0.91	84.91 [e]
Hybrid poplar	0.0032	0.97	238.99 [bcd]
Red oak	0.0019	0.98	281.05 [b]
Black walnut	0.0058	0.72	124.74 [de]
Norway spruce	0.0015	0.95	447.67 [a]
White cedar	0.0016	0.96	487.82[a]

[a-e] Values with different letters indicate significant difference at $p < 0.05$

3.4.3 Soil Respiration

3.4.3.1 Soil respiration in a soybean sole-crop vs. tree-based intercropping system

Higher soil respiration rates were found in the TBI field (ranging from 3.43 to 101.79 g CO_2 m^{-2} d^{-1}) compared to the soybean sole-crop field (ranging from 3.35 to 84.52 g CO_2 m^{-2} d^{-1}). Annual CO_2 efflux within the TBI system was 31.31 ($\pm$ 1.86) g CO_2 m^{-2} d^{-1} compared to 22.91 $\pm$ (5.63) g CO_2 m^{-2} d^{-1} in the soybean sole-crop system. When comparing mean soil respiration rates for each species and for soybean sole-crop (Figure 9), annual CO_2 efflux was highest under spruce, followed by poplar, walnut, and soybean sole-crop with 35.00 ($\pm$ 1.02), 29.95 ($\pm$ 0.93), 28.99 ($\pm$ 1.44), and 22.91 ($\pm$ 5.63), t CO_2 ha^{-1} y^{-1}, respectively. Annual respiration rate of spruce trees was significantly higher than walnut species (p value = 0.0418) and the soybean sole-crop field (p value = 0.0108). Gordon and Thevathasan (2005) and Peichl et al. (2006) also found higher respiration rates in intercropping fields compared to monocrop fields due to the influence of tree root and microbial respiration.

These findings can be expected due to the presence of trees in the system that provides litterfall in addition to crop residue, attracting microorganisms that promote microbial activity and therefore CO_2 evolution (Brady and Weil, 1996; Matteucci et al., 2000). In comparison, these components are minimal in conventional agricultural systems, which contain crop residue alone. Lee and Jose (2003) support this as they found soil respiration rates were positively correlated with soil microbial biomass C, SOM, and live fine root biomass, all of which would be higher in TBI systems due to higher amounts of OM. Because soil respiration was not separated into root and microbial respiration in this study, soil respiration is reflecting both components. Rates of litter decomposition, to be discussed later on, will help to measure loss of C through the system, which are not confounded by root respiration and inorganic C emissions.

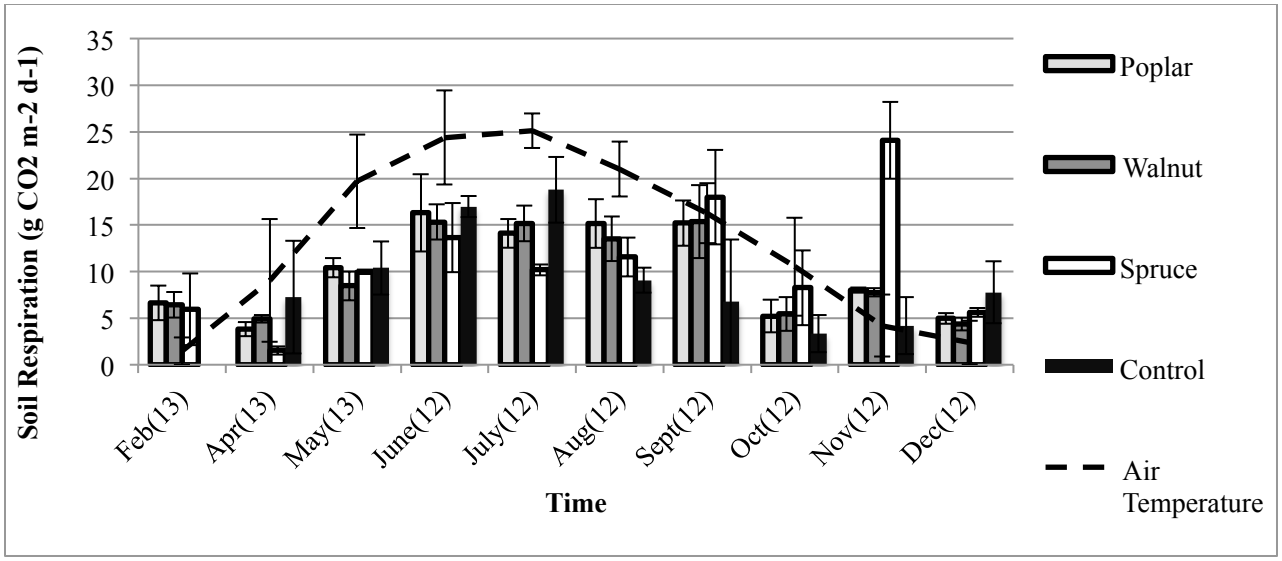

Figure 9. Soil respiration rates (g CO_2 d^{-1} m^{-2}) for three species in a 25-year-old tree-based intercropping and soybean sole-cropping system by month plus mean ambient temperature (Celsius) (error bars denote standard error).

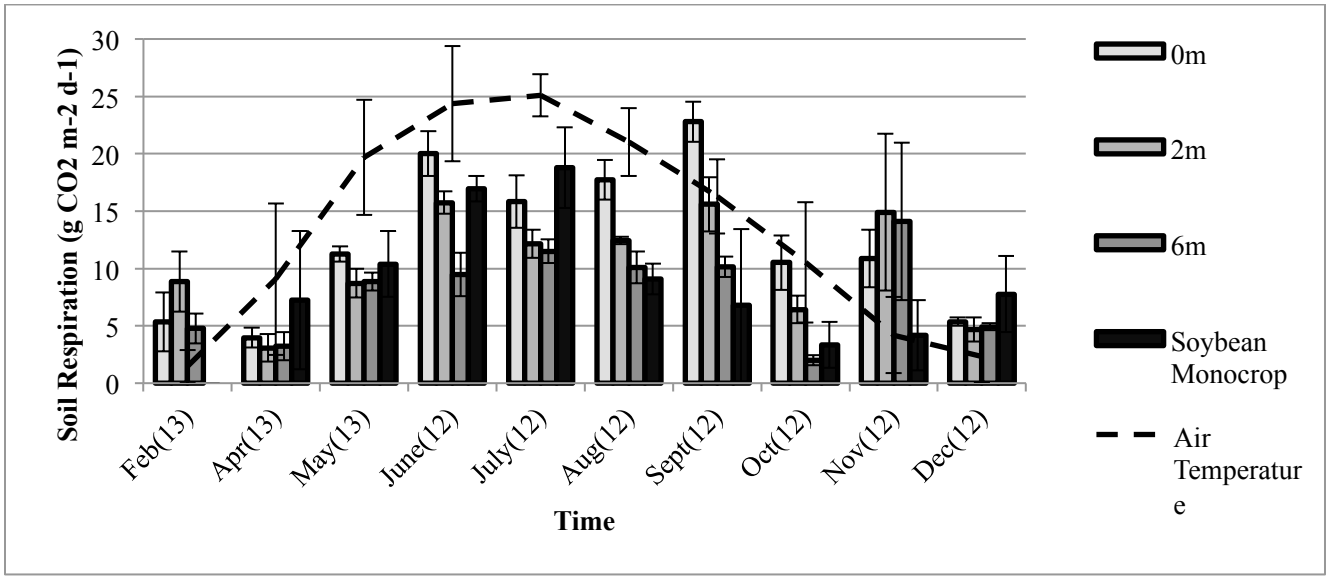

Figure 10. Mean soil respiration rates (g CO_2 d^{-1} m^{-2}) at three distances from the tree row in a 25-year-old tree-based intercropping and soybean sole-cropping system plus mean ambient temperature (Celcius) (error bars denote standard error).

3.4.3.2 Soil respiration and effects of systems, distance, month and season

Mean soil respiration rates for three tree species in relation to distance from the tree row and for soybean sole-crop are given in Figure 10. For all species in the TBI system, CO_2 efflux was always highest closest to the tree row at 0 m (ranging from 28.19 to 20.54 g CO_2 m^{-2} d^{-1}), and lowest closest in the crop row at 6 m (ranging from 12.96 to 16.94 g CO_2 m^{-2} d^{-1}), except for the month of November 2012 and February 2013. Peichl et al. (2006) found similar results indicating highest respiration within the tree row (ranging from 9.6 to 21.1 g CO_2 d^{-1} m^{-2}) and lowest being at 4 m from the tree row (ranging from 7.2 to 19.2 g CO_2 d^{-1} m^{-2}) in a 13-year-old poplar intercropping system. Significant difference between distances in different months and for different species are listed in Table 11.

Table 11. Significant differences of soil respiration (g CO_2 m^{-2} d^{-1}) at various distances from the tree row during different months for three species in a 25-year-old tree-based intercropping system in southern Ontario.

	Month	Distance	P value
Poplar	June	0 and 2 m	0.0034
		0 and 6 m	< 0.0001
		2 and 6 m	0.0261
	August	0 and 2 m	0.0228
		0 and 6 m	0.0004
Spruce	August	0 and 6 m	0.0002
		2 and 6 m	00028
	September	0 and 6 m	<0.0001
		2 and 6 m	0.0049
	October	0 and 6 m	0.0009
	November	0 and 2 m	0.0149
		0 and 6 m	0.0096
Walnut	June	0 and 2 m	0.0129
		0 and 6 m	0.0017
	July	0 and 2 m	0.0061
		0 and 6 m	0.0021
	August	0 and 2 m	<0.0001
		0 and 6 m	<0.0001
	September	0 and 2 m	<0.0001
		0 and 6 m	< 0.0001
	October	0 and 2 m	0.0176
		0 and 6 m	0.0003

When observing mean soil respiration between species CO_2 fluxes is highest between June and September 2012. Thevathasan (1998) and Lee and Jose (2003) also found higher values observed in summer months and attributed lower respiration rates (from October to May in this study) to lower temperatures during fall, winter and spring, as compared to summer, as can be seen in and 5. High values during the month of November were unexpected and most likely due to a random weather event. Previous research by Lee and Jose (2003) found that November had the lowest soil respiration in a pecan-cotton alley intercropping system, which would be more typical given normal temperatures.

Soil respiration data were also separated by season; seasonal soil respiration in the TBI system and the soybean sole-crop system are presented in Table 12. Soil respiration rates were highest during the summer in both the TBI and soybean sole-crop field ranging from 8.47 to 16.20 and 9.06 to 18.77 g CO_2 m^{-2} d^{-1}, respectively. Lowest respiration occurred in fall for both systems, ranging from 5.22 to 18.00 and 3.35 to 6.79 g CO_2 m^{-2} d^{-1} for TBI and soybean sole-crop, respectively. Winter values ranged from 1.52 to 24.08 g CO_2 m^{-2} d^{-1} in the TBI system and from 4.17 to 7.77 g CO_2 m^{-2} d^{-1} in the soybean sole-crop system. Abohassan (2004) reported similar findings, except that his estimation of 10 % of total annual soil respiration for the winter months was lower, at 9 % of total respiration, whereas in our study winter respiration accounted for 28 and 23% in the TBI and soybean sole-crop, respectively. This is most likely an overestimation in the TBI systems as respiration rates for November were unexpectedly high.

3.4.3.3. Influence of temperature and moisture content on soil respiration.

Higher soil respiration rates were almost always consistently higher at closer distances to the tree row, most likely due to influence from root respiration. By using the soda lime method, respiration of soil, roots, and grass roots, cannot be distinguished; therefore all are accounted for

with CO_2 efflux values presented in Figure 10 at a distance of 0 and 2 m. These closer distances and the influence of root respiration are higher in TBI systems due to inputs from litterfall which create an environment promoting heterotrophic microorganisms resulting in an increased release of CO_2 (Abohassan, 2004). Because of these confounding effects, respiration rates at 6 m were considered most likely to be indicative of true TBI respiration rates because tree-root and grass-root respiration in the tree row will be minimal, but crop-root respiration will still be accounted for. If respiration rates at 0 and 2 m were excluded to reduce the influence of root and microbial respiration, as in the C model, overall annual CO_2 efflux become highest under poplar species at 22.91 ($\pm$ 4.01) t CO_2 ha^{-1} y^{-1}, followed by walnut, spruce, and soybean sole-crop with 21.74 ($\pm$ 4.03), 21.49 ($\pm$ 4.50), and 16.77 ($\pm$ 0.86) t CO_2 ha^{-1} y^{-1}, showing no significant difference between any of the species within the TBI system between the soybean sole-crop system. When converted to C efflux, C output for poplar, walnut, spruce, and soybean sole-crop were 6.25 ($\pm$ 1.09), 5.93 ($\pm$ 1.10), 5.86 ($\pm$ 1.22) and 4.57 ($\pm$ 0.23) t C ha^{-1} y^{-1}, respectively.

High respiration during warmer months and vice versa, as seen in Figure 9, can be attributed to a positive correlation between respiration and air and soil temperature, as temperature is said to be the best predictor of soil respiration at a specific location (Raich and Schlesinger, 1992). Warmer temperatures allow for higher rates of microbial activity involved in the decomposition process of organic matter and therefore contribute to higher rates of CO_2 evolution. On the contrary, in colder winter months, microbial communities are slowed (Love, 2005) and therefore are not as active in the decomposition of organic matter and root turnover, as can be seen in the colder months of this study. This is also supported by Love (2005) who found winter soil respiration rates (January – March) of 0.01 g CO_2 d^{-1} m^{-2}.

Air temperature, soil temperature and moisture content of all species within the TBI systems and the soybean sole-crop system are presented in Appendix III. In this study,

correlations between soil respiration and air and soil temperature and soil moisture were not the strongest. Correlation between soil respiration and air temperature was higher (r^2=0.3489) than correlation between soil respiration and air temperature. Correlation between air and soil temperature was low in this study (r^2=0.2594), however past studies by Abohassan (2004) found higher correlation of r^2 = 0.885 between air and soil temperature showing both will positive influence soil respiration. In addition to air and soil temperature, this study found low correlation between soil temperature and moisture content (r^2 = 0.1193), however literature shows that moisture content is also an important component of soil respiration, as moisture contents control organic matter decomposition and thus the evolution of CO_2 from the microbial community responsible (Moyano et al., 2012).

3.4 Conclusions

C fluxes vary from year to year and are species- and system age-specific. Therefore, annual quantification of as many C fluxes as possible is an important asset to C modeling to ensure the most accurate estimations of CSP of each species.

Annual litterfall and decomposition were both highest for walnut species, followed by poplar and lowest for oak. Spruce had higher litterfall compared to cedar but with lower decomposition rates, although decomposition rates were not statistically different. Slower decomposition of crop residue (soybean stalk and leaves) was observed in the TBI field compared to the soybean sole-crop system (although not statistically different). No significant difference was found between soil respiration rates of the TBI system and soybean sole-crop system when comparing respiration rates at 6 m from the tree row. Patterns of C fluxes from this study show that although species such as poplar and walnut provide higher amounts of C input in the form of litterfall, it may be too quickly lost through decomposition to contribute to long term C sequestration within the soil as compared to spruce and cedar. This can be seen in lower SOC

stock of walnut, which had high litterfall with fast decomposition compared to spruce that had high litterfall but slow decomposition. Further analysis of net C balance between C fluxes and pools will be discussed in the next chapter.

Table 12. Annual soil respiration (g CO_2 d^{-1} m^{-2}) for summer, fall and winter for a 25-year-old tree-based intercropping system and a soybean sole-crop system in southern Ontario, Canada

	TBI		Soybean Sole-Crop		
	Total soil respiration (g CO_2 m^{-2} d^{-1})	Soil respiration (% of total)	Total soil respiration (g CO_2 m^{-2} d^{-1})	Soil respiration (% of total)	Pr > |t|
Summer (May-August)	51.27 [a] (± 3.11)	51	55.19[a]	65	0.355
Fall (September-October)	22.00 [a] (± 5.58)	22	10.15[b]	12	0.0147
Winter (November-April)	28.02 [b] (± 4.57)	28	19.18 [a]	23	0.9155
Total	101.29		84.52		

[a-c] Letters indicate significant difference between seasons within systems (down columns)

Further research should be conducted on the associated C:N ratios of leaves from various species and at stages throughout the decomposition process to investigate a step-by-step process of nutrient release into the soil. This study was one of the first studies that examined decomposition of buried litter, simulating current tillage practices. Therefore these rates of decomposition may be more representative of a managed TBI system where litter is being brought down into the soil through tillage as oppose to the use of decomposition estimates from natural forest systems where leaves are left on the soil surface or are blown away by wind.

C fluxes quantified in this study can now be applied to C modeling to reduce estimations and assumptions that may cause under- or over-estimations of net C values for different tree species. These C fluxes and their use in C modeling can aid in supporting the use of TBI systems to sequester atmospheric CO_2, mitigate climate change and promote agroforestry land use practices over conventional agricultural.

4.0 Chapter 4 – Comparison of carbon pools and fluxes between tree-based intercropping and soybean sole-cropping systems

In order to estimate the CSP of each species within this 25-year-old temperate TBI, all the data from the C pools and fluxes quantified in this study were compiled along with data from the literature gathered from the same site, and presented in Appendix II. For comparison purposes, similar assumptions and estimations were made following Peichl et al. (2006). Sample calculations can be found in Appendix III.

4.1 Carbon Pools

Summing the C pools, including the above- and belowground biomass and SOC accumulated over the past 25 years, C pool sequestration for poplar, oak, walnut, spruce, cedar TBI systems and a soybean sole-crop system are 113, 99, 92, 104, 99 and 71 t C ha^{-1}. In TBI systems, faster-growing tree species show potential to sequester more atmospheric CO_2 within their above and belowground biomass, and with the contribution of annual litterfall and fine root turnover to the SOC stock. The SOC stock is the largest contributor to C pools and is responsible for 93 – 97 % of carbon belowground and 86 – 97 % of total C in this system. Conventional agricultural containing only crops rely solely on the soil reserves and the small amount of crop residue left over after harvest to contribute to SOC and sustain its C pools. Annual input of C from crop residue input will be slightly greater in conventional agricultural system compared to TBI systems due to the greater percent of land covered by crops, but still lack the major contributing factor of litterfall found in TBI systems. Because of this, nutrients from soil reserves are lost at a faster rate from conventional agricultural systems compared to TBIs which lead to land degradation and the need to clear more natural forest land for agriculture.

The last C model for this site was examined at 13 years after establishment (Peichl et al., 2006), and since then has increased by 16. 9, 16.0, and 2.6 t C ha^{-1} for a poplar and spruce TBI

system and conventional agricultural system, respectively. Twenty five years after establishment, all tree species in the TBI systems show high levels of C sequestration; poplar, oak, walnut, spruce and cedar species have sequestered 60, 40, 29, 29 and 40 % more C at the system level than the soybean sole-crop field, respectively.

Table 13. Carbon sequestration (t C ha^{-1} y^{-1}) potentials of five tree species commonly grown in tree-based intercropping systems in comparison to conventional agricultural systems in southern Ontario, Canada

Inputs	Poplar	Oak	Walnut	Spruce	Cedar	Soybean Sole-Crop
Aboveground tree C assimilation	0.83	0.46	0.48	0.76	0.53	
Belowground tree C assimilation	0.23	0.16	0.11	0.28	0.12	
Litterfall C inputs	1.63	1.07	1.50	1.49	0.68	
Fine root turnover	0.82	0.54	0.75	0.45	0.20	
Above and belowground Crop C input	1.22	1.22	1.22	1.22	1.22	1.40
Outputs (via decomposition)						
Litterfall C outputs	1.10	0.53	1.29	0.63	0.30	0
Root output	0.55	0.27	0.65	0.19	0.09	1.42
Crop C outputs	0.96	0.96	0.96	0.96	0.96	1.29
C leachate	0.05	0.05	0.05	0.04	0.04	0.05
Net						
Net C balance	+ 2.07	+ 1.63	+ 1.10	+ 2.37	+ 1.35	- 1.36

For comparative purposes between 13 and 25 year old system level TBI and conventional agricultural field, the same assumptions were followed as those outlined by Peichl et al. (2006).

4.2 Carbon Fluxes

Annual C fluxes were calculated as the difference from annual C outputs subtracted from annual C inputs. Total annual C inputs consist of annual fluxes from tree and crop assimilation, litterfall C inputs, and root turnover and for poplar, oak, walnut, spruce, cedar and a soybean sole-crop sum to 4.73, 3.45, 4.06, 4.19, 2.75, and 1.40 t C ha-^{1} y-^{1}, respectively (Table 13).

Total annul C outputs consist of C lost through decomposition of OM, C lost through fine root turnover and C outputs for poplar, oak, walnut, spruce, cedar and a soybean sole-crop sum to 2.67, 1.81, 2.95, 1.82, 1.39 and 2.76 t C ha^{-1} y^{-1}, respectively. By subtracting outputs from inputs,

net C flux for poplar, oak, walnut, spruce, cedar species sum to 2.07, 1.63, 1.10, 2.37, and 1.35 t C ha^{-1} y^{-1}, respectively. Net C flux for the soybean sole-crop system summed to -1.36 t C ha^{-1} y^{-1} (Table 13). These net C sequestration values over the period of 25 years for poplar, oak, walnut, spruce, cedar and a soybean sole-crop system sum to 51.75, 40.75, 27.50, 59.25, 33.75, and -34.00 t C ha^{-1}, respectively.

Net C sequestration for poplar is most likely an underestimation due to approximately 40% die-back (Thevathasan, pers. comm., 2013); since year 13, the annual net flux of C at the system level has decreased from 13.2 t C ha^{-1} y^{-1} to 2.07 C t ha^{-1} y^{-1}. From these net C values, it would suggest that two cutting cycles of 12 years would have been more beneficial for C sequestration. On the contrary, spruce has increase its net C flux from 1.1 t C ha^{-1} y^{-1} to 2.37 t C ha^{-1} y^{-1} over the past 12 years, indicating the long term CSP for slower growing tree species.

Net C loss from conventional agricultural system is also assuming a constant soybean sole-crop system over the past 25 years. Given the actual corn-soybean-winter wheat/barley rotation, various amounts of crop residue are left behind and does not allow the system to complete deplete its resources and would not be a net C loss under all crops within the rotation. SOC has actually been slightly increased by 2.6 t C ha^{-1} since year 13 to 25 which indicate the additions of C back to the system from crop rotation.

Net C values of various tree species from this study show the ability to sequester C in magnitudes greater than conventional agriculture. With a tree density of 111 trees ha^{-1}, poplar, oak and walnut are able to sequester 3.4, 3.0 and 2.5 t C ha^{-1} y^{-1} more C, respectively. At a tree density of 222 trees ha^{-1}, spruce trees sequester 3.7 t C ha^{-1} y-1 and at 333 trees ha^{-1}, cedars sequester 2.7 t C ha^{-1} y^{-1} more C than a soybean sole-crop system.

4.3 Conclusions

Many factors influence the net C flux in both the TBI and the conventional agricultural system including crop species, crop rotation, crop residue left over after harvest, tree density and species, tree rowing spacing, percentage of land covered by trees, climate, management practices and soil type. With the CSP of five tree species of a 25-year-old TBI system presented in this C model, Peichl et al. (2006) point out that the benefits of TBI systems extend beyond that of C sequestration and can also help in the production of wood for fossil fuel, reducing pressure on natural forests for timber products, and reducing the need for chemical fertilizer on crops (Dixon, 1995b; Kürsten and Burschel, 1993; Montagnini and Nair, 2004; Schroeder, 1994). With the addition of those benefits, Kürsten and Burschel (1993) suggest that CSP could increase by 2 – 15 times. With all the benefits combined, TBI systems show great potential for the reduction of GHGs, atmospheric CO_2 sequestration and a sustainable land-use system for climate change mitigation.

5.0 Summary and Final Thoughts

By re-introducing trees with croplands, TBI systems have the ability to mitigate climate change by sequestering atmospheric CO_2. In this study, CSP of poplar, oak, walnut, spruce and cedar were 3.4, 3.0, 2.5, 3.7 and 2.7 t C ha^{-1} $year^{-1}$, respectively, greater than a soybean sole-crop system that had a net C flux of -1.4 t C ha^{-1} $year^{-1}$. This is due 1) to the sequestration of C within above- and belowground biomass, 2) the contribution of OM in the form of litter and needles that provide a natural source of C into the system and 3) the decomposition of this litter that adds to the long term stable SOC stock.

The trees' ability to store C in above- and belowground biomass in the TBI system gives them a greater CSP over conventional agricultural field. Highest Biomass C was found in the poplar species, followed by oak, walnut, spruce and cedar trees. The onset of die-back found in older poplar trees showed the importance of cutting cycles so that trees are able to be continually replanted and store greater amounts of C over longer periods of time. C stored in the SOC stocks showed mean SOC concentrations were highest in the soybean sole-crop system, followed by cedar, poplar, walnut, oak and spruce species and were always highest in the top 0 – 10 cm of the soil and decreased with soil depth, except for the soybean sole-crop. Higher concentrations in the soybean sole-crop field were most likely due to higher C input to the soil surface due to minimum tillage practices (Abohassan, 2004), whereas higher concentrations found beneath cedar species is most likely due to higher density of planting and contributing greater amounts of annual litterfall. However, with the incorporation of depth and bulk density to convert SOC concentrations to stocks, the highest SOC stock was found for poplar species, followed by cedar, oak, spruce and walnut with 86.86, 83.77, 83.23, 78.29, and 76.84 t C ha^{-1}, respectively.

Litterfall distribution with the use of litter traps showed 145 (± 20), 70 (± 19), 59 (± 19), 58 (± 21) and 33 (± 8) g m^{-2} for walnut, poplar, spruce, oak and cedar trees, respectively. *In situ* measurements were able to account for a higher quantity of litterfall (379 (± 82), 352 (± 162), 267 (± 147), 109 (± 55) and 33 (± 8) g m^{-2} for poplar, walnut, spruce, oak and cedar trees, respectively) therefore these values were used to show a more accurate quantification of litterfall in the C model. When this litter was left to decompose for 361 days, walnut litter decomposed the fastest in the TBI field followed by poplar, oak, spruce and cedar, due to the varying C:N ratios found within the litter of each respective species. The decomposition of soybean leaves and stalk was found to be slower in the TBI system over the monocrop field (although no statistically significant), due to presence of trees, microorganisms and microclimates.

Trees, microorganisns and microclimates were also found to be responsible for higher rates of soil respiration closer to the tree row (0 and 2 m) when compared with within the crop alley (6 m). Lower respiration rates were seen during winter months that corresponded to lower temperatures and higher respiration in the summer and fall. Total soil respiration in TBI field was significantly higher from those found in the soybean sole-crop field.

In conclusion, we can reject the null hypothesis that tree-based intercropping systems and sole cropping systems sequester the same amount of carbon per year. All species quantified in this study sequester greater C in the above- and belowground carbon pools. Poplar, oak, walnut, spruce and cedar stored net C of 51.5, 40.7, 27.6, 59.2, 33.7 t C ha^{-1}, respectively over the past 25 years, compared to -34.0 t C ha^{-1} in the soybean sole-crop system. Therefore, the alternative hypothesis is accepted that TBI systems can sequester more C when compared to conventional agriculture.

This study was the first to examine a variety of tree species, in addition to the few fast and slow growing species that have been previously examined in the literature. Previously, poplar and hybrid and Norway spruce had been studied for their potential to sequester atmospheric C in tree-based intercropping systems (Peichl et al., 2006), silovpasture (Gordon and Thevathasan, 2005) and shelterbelts (Kort and Turnock, 1999). Now with the C pools and fluxes quantified for red oak, black walnut and white cedar, these systems can be examined with a variety of tree species that may have additional ecological or monetary gain or for their use in specific agroforestry systems. For example, other deciduous species such as oak and walnut can be used for long-term valuable wood timber or for short-term money gain from nut production, respectively and cedars are commonly used in windbreak systems for their low porosity in high-density plantings. With data obtained from this study, CSP of agroforestry systems using these species can be more easily estimated.

This study was also one of the first to measure the CSP of these species in a 25-year-old system, so now the long term benefits of agroforestry can be reiterated including 25 years of environmental services, but also as a long term sink for atmospheric CO_2. This was found to be promising for most tree species in this study, except for poplar species which was experiencing approximately 40% die-back at 25 years of age. This shows the importance of not overestimating the CSP of fast-growing species over longer periods of time, as they may be better suited for multiple short rotations, such as two cycles of 12 years instead of one cycle of 25 years. The net C flux values quantified from each tree species in this study also shows the potential for TBI systems as a tool for climate change mitigation and to act as a long term C sink to sequester atmospheric CO_2. By adopting this land use practice, southern Ontario can contribute towards climate change mitigation through the sequestration of atmospheric CO_2 into above- and

belowground biomass, the increase of SOC, and the reduction of GHGs caused by deforestation. Given the net C flux values for each tree species found in this study, TBI systems planted with Norway spruce, hybrid poplar, red oak, white cedar and black walnut would help to mitigate 16, 14, 11, 9 and 7 % of Canada's required CO_2 reduction level by 2020.

With these CSP of various tree species in a 25-year-old TBI system, there are still areas of further research. As mentioned, fast growing tree species that had experienced dieback by the 25th year should be further investigated as to their ability to sustain multiple cutting cycles. This would help ensure continuous litterfall, fine root turnover and therefore contributions to the SOC stock but also to the sequestration of atmospheric CO_2. The fate of trees and wood products after harvest is also an important area that has yet to be studied, as the CSP of each system is dependent on its lifespan and the fate of products. If wood harvested from these systems is put it into long-term products, it ensures the continual sequestration of C and not its release back into the atmosphere (Kort and Turnock, 1999). Given that greater CSP can be achieved with higher tree densities within TBI systems, further research should be conducted on tree densities to optimize the sequestration of atmospheric CO_2 while ensuring crop production; this research will also vary with the tree species being studied, as this study found that due to different growth patterns, densities are species-dependent.

Once CSP of TBI systems are realized, further emphasis should be placed on their adoption into temperate regions to promote their environmental services as well as their ability to mitigate climate change through C sequestration. Their implementation would also help to remediate marginal/degraded land and therefore reduce the need to remove natural forest land for the development of agriculture. This form of afforestation and reduction of deforestation are

other ways that TBI systems can reduce CO_2 emissions and support a carbon market should programs come to Ontario and therefore meet national climate change mitigation goals.

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APPENDIX I

Comparison of three methods for measurement of soil organic carbon[1]

[1] Manuscript by Wotherspoon et al. currently submitted and pending approval for publication in *Communications in Soil Science and Plant Analysis*. Submitted June 2013.

COMPARISON OF THREE METHODS FOR MEASUREMENT OF SOIL ORGANIC CARBON

Wotherspoon, A*., I. Mohammed, N. V. Thevathasan, A.M. Gordon, and R.P Voroney

Abstract

The quantification of soil organic carbon (SOC) has become an important component for the assessment of soil quality, carbon sequestration potential and soil remediation for tree-based intercropping (TBI) systems. The SOC concentration of TBI systems often differ when compared to conventional agricultural systems, due to the presence of additional litterfall and soil characteristics only present when in conjunction with trees. The presence of carbonates in the soil can confound measurements of SOC. This study compares three methods for measuring SOC: (i) measurement of the total soil C in one subsample and, after treatment in a muffle furnace (575ºC), measurement of soil inorganic C (SIC) in another subsample, (ii) SOC measured after treatment with 12 M HCl, and (iii) SOC measured after treatment with 0.73 M H_2SO_3. SOC and SIC concentrations (%) were determined with a LECO CR-12 Carbon Analyzer (LECO Corporation, MI, USA). A correction factor was applied to express measured SIC and SOC concentrations on an original, untreated soil basis. SOC concentrations for soils measured with a muffle furnace had the highest overall SOC concentrations but were only significant at shallow (0 – 10 cm) and deep (20 – 40 cm) depths. A stronger relationship and correlation was found between soils pre-treated with HCl and H_2SO_3. Mean values for correction factors increased with depth and had the most impact on SOC in soils pre-treated with H_2SO_3. These results have implications for the method of measuring SOC concentrations in calcareous soils surrounding specific tree-species and at various depths within the soil profile.

Keywords: Tree-based intercropping, soil organic carbon, dry combustion, hydrochloric acid, sulphurous acid

1.0 Introduction

Clearing land for agriculture not only results in losses of 40 – 60 % of soil organic carbon (SOC) from native land but also the global release of more than 150 Pentagrams of CO_2 into the atmosphere (Sanderman et al. 2009). For cultivated farmland, the question remains of how to slow SOC losses and to sequester the carbon back into the soil. A potential solution lies in sustainable land management practices that reduce tillage and through cropping systems that return more organic carbon (OC) to the soil. This can include tree-based intercropping (TBI), a form of agroforestry in which agriculture crops are planted between widely spaced tree rows. This form of management returns organic material to the soil in the form of litterfall, a component absent in conventional agricultural systems that has the highest available SOC sequestration potential (Sanderman et al. 2009). Even a slight increase in carbon (C) stocks in agricultural soils would result in high levels of greenhouse gas (GHG) mitigation (Sanderman et al. 2009).

GHG mitigation and sequestration potential is often gauged by measuring SOC is formed of soil organic matter (SOM), which includes all the various organic compounds within the soil. Within SOM, carbon can be present in 40 – 60 % of material by mass (Baldock and Skjemstad 1999). The top meter of the soil alone, stores ~1500 Pg as OC globally and exchanges 60 Pg C/year with the atmosphere, of which ~750 Pg C is CO_2 (Eswaran et al. 1993; Schlesinger 1997). Clearing land for agriculture has caused the initial loss of 78 Pg C of global soil – 26 Pg due to soil erosion and 52 Pg to mineralization (Lal 2004). Clearing native forests and pasture for agricultural use has reduced SOC stocks by 42% and 59%, respectively (Guo and Gifford 2002). Documenting these changes in SOC stocks of carbon to historical land clearing generates the need for quantification of agricultural soils to show the potential for sequestering atmospheric carbon and mitigating GHG (Sanderman et al. 2009).

In this study, three common methods for measuring SOC concentrations ([SOC]) in calcareous soils in a 25-year-old TBI system in southwestern Ontario were evaluated. The goal of this study was to

identify the method(s) providing precise measures of [SOC] in TBI soils. The three methods were evaluated in soils sampled at various soil depths and distances from two different tree species.

1.1 Measuring SOC

SOC stocks are determined from measurements of [SOC] and soil bulk density. In this study, SOC was determined using a dry combustion method (i.e. Wang and Anderson 1998), as wet oxidation is known to underestimate the amount of OC in most soils. It also requires a correction factor (Walkley and Black 1934), which has been known to lead to over- or underestimations (Nair 2011), and has environmental disposal concerns, due to the use of potassium dichromate (Kimble et al. 2001). Dry combustion methods allow for larger sample sizes (0.3000 g) that ensure sample homogeneity (Caughey et al. 1995) and short analysis time (a few minutes per sample), and the precision of automated systems over manually operated systems (Schumacher 2002). Comparison of these three methods involved selecting one that would remove all carbonates with minimal undesirable effects on samples and lab equipment and personnel, and could minimize total analysis time and labour. (Caughey et al., 1995). Table 1, outlines the various uses and problems associated with possible acids used for carbonate removal prior to SOC analysis.

The first method used a muffle oven to heat soil samples to 575°C for 24 h (adapted from Nelson and Sommers 1982) to burn off all organic C. For each soil sample two C analyses were done (LECO CR-12 carbon analyzer (LECO Corporation, MI, USA)), on an original (unheated) soil sample for total C (following methods by Page et al. 1982), and on a treated (muffle oven) soil sample for inorganic C (SIC). The SOC concentration is calculated based on the following equation from Tabatabi and Bremner 1970 with the incorporation of a correction factor (CF):

$$\% \text{ Soil organic C} = \% \text{ total } C_{(measured)} - (\% \text{ inorganic } C_{(measured)} \text{ X } CF_1) \qquad (1)$$

The second method fumigated soil samples with 12 M hydrochloric acid (HCl) to remove SIC

prior to SOC analysis (LECO CR-12 carbon analyzer). Exposure time to HCl fumigation and time required to remove all carbonates from samples is a function of inorganic carbon (IC) content of the soil and amount of sample being fumigated (Ramnarine et al. 2011).

The third method used aqueous 0.73 M sulphurous acid (H_2SO_3) to remove SIC prior to SOC analysis. Nelson and Sommers (1996) support this method and Bremner (1949) claimed it is "the only reagent suitable for preliminary destruction of carbonates" because it is easily removed by evaporation, and it's reducing properties minimizes the oxidation of SOC during treatment (Piper 1944). Furthermore, its vapours are less corrosive for metal objects, such as lab equipment (Caughey et al. 1995). However, due to the low concentration of H_2SO_3 in commercially available supplies (Caughey et al. 1995), Schmidt et al. (2012) suggest that it may not completely remove soil IC

The SOC concentration in soils pre-treated to remove carbonates is calculated as follows (Ramnarine et al. 2011):

$$\% \text{ soil organic C} = \% \text{ organic } C_{measured} \times CF_2 \quad (2)$$

2.0 Experimental Design

2.1 Site and soil characteristics

Soil samples were collected from the University of Guelph Agroforestry Research Station (lat. 43°32'28"N, long. 80°12'32"W), in Guelph, Ontario, Canada. This research station, established in 1987, is a tree-based intercropping system located on sandy loam (Albic Luvisols) soil [(560 g sand kg^{-1}, 340 g silt kg^{-1}, 100 g clay kg^{-1}, (Bambrick et al. 2010)] with pH 7.4 and calcareous parent material. A variety of deciduous and coniferous tree species are planted in rows along a north/south orientation over 30 ha in a randomized complete block design at a density of 111 trees/ha.

2.2 Sample collection and preparation

Three replicates were collected at 0-10 cm, 10-20 cm, and 20-40 cm depths, at 0.5, 1.0, 1.5 and 2.0 m from the tree row, using a metal soil core (250 cm^3). Samples were collected in the East and West direction (72 samples per tree) under *Populus* sp. (poplar hybrid) and *Picea abies* (Norway spruce), tree species commonly used in TBI systems. After collection, soil samples were stored at -20°C until ready for analysis. In preparation for analysis, samples were thawed, air dried for 48 h and passed through a 2-mm sieve to exclude gravels and allow fine root removal by hand. A ~15 g subsample was taken for air-dry gravimetric moisture content. Subsamples to be treated by HCl fumigation and H_2SO_3 digestion were further ground to <0.125 mm to ensure a homogenized sample for SOC analysis (Caughey et al. 1995).

3.0 Materials and Methods

3.1 Organic carbon removal by muffle oven

An air-dried soil sample (~0.3000 g) was analyzed for total soil carbon (LECO CR-12, combusted at 1300°C). A soil subsample of ~5 g was placed in a muffle oven (Lindberg, MI, USA) for 24 h at 575°C to remove SOC. A sample of this treated soil (~0.3000 g) was analyzed for SIC.

3.2 Inorganic carbon removal with 12 M HCl

Fumigation with 12 M HCl follows methods adapted from Ramnarine et al. (2011). Soil (~1.000 g) which had been ground to <0.125 mm, was transferred to a 20mL glass vial. Vial weights, initial soil mass and final soil mass were recorded for determination of soil mass change due to the fumigation treatment. Soils were moistened with 500 µL of nanopure water and then placed in a glass vacuum desiccator (7.5 L) fitted with a porcelain plate and containing 100 mL of 12 M HCl. The desiccator was vacuum-sealed using a water-jet vacuum system and soils were left exposed to HCl vapour within the

desiccator for seven days.

After the fumigation period, the acid was removed; the desiccator was resealed and evacuated for 1 h to remove HCl vapour. This step is critical in preventing degradation and corrosion of lab equipment in later steps (Ramnarine et al. 2011). The samples were dried at 50°C for 24 h (or until constant mass was reached), cooled and weighed for final soil mass measurement. A sample (~0.3000 g) sample was analyzed for SOC content.

3.3 Inorganic carbon removal with 0.73 M H_2SO_3

H_2SO_3 digestion follows methods adapted from Shaw (1959), Skjemstad and Baldock (2008), and Ramnarine et al. (2011). The same steps were carried out as were for HCl fumigation up to and including the addition of nanopure water. After moistening soils with water, vials were placed on a hot plate set to 65°C. H_2SO_3 was added, 1 mL at a time by micropipette, until effervescence had stopped (to a minimum of 6 mL and maximum of 8 mL). Once samples had stopped reacting, they were removed from the hot plate and placed in a glass vacuum desiccator (7.5 L) together with a beaker containing sodium hydroxide pellets as a desiccating agent (adapted from Shaw, 1959). The desiccator was vacuum-sealed using a water-jet vacuum system and samples were left overnight. The following day the hydroxide pellets were removed and discarded and the samples were dried at 50°C for approximately 48 h (or until constant mass was reached). Soil was cooled, weighed for final soil mass and (~0.3000 g) sample was measured for SOC content.

3.4 Use of a correction factor

The following chemical reaction occurs during fumigation of soil with 12 M HCl:

- $- CO_3 (s) + 2HCl (g) \; CO_2 (g) + — Cl_2(s) + H_2O$ (3)

The change in mass of — Cl_2 formed (70.91g mol^{-1}) is of greater mass than the original — CO_3

(60.01 g mol^{-1}). The remaining OC becomes diluted within the acid-fumigated soil sample and weighs more than the original soil sample (Ramnarine et al. 2011, Harris et al. 2001). For this mass change, the initial and final soil weights were recorded during treatments. From this, a correction factor was obtained to express SOC content on a pre-treated soil mass basis. This correction factor converts the mass of the acid fumigated soil analyzed in the LECO to an equivalent mass of untreated soil. Ramnarine et al. (2011) noted that a correction factor would not be required if weighed soil samples are acid-treated directly and combusted in the same container with the SOC content expressed on the original pre-acidified soil weight. The same approach was followed for obtaining a correction factor for soil pre-treated with H_2SO_3. To account for the mass change of soil treated with the muffle furnace, crucible weights, initial soil mass and final soil mass were recorded before and after SOC combustion in the muffle furnace.

3.5 Statistical Analysis

Variance analysis, using a general linear model and least square means were analyzed using SAS, version 9.3 (SAS Institute, Inc., Cary, NC). Independence, random of residuals were confirmed along with Shapiro-Wilkes test to test normality of residuals. Highest data points were compared with Lund's critical value to test for outliers. If data points were not explainable, they were removed from the data set. The remaining data points were analyzed using simple linear regression with log transformations along with tests of assumptions of constant variance and normality of data distribution. Type I error rate (α) was set at 0.05 for all statistical tests.

4.0 Results

4.1 SOC concentrations by depths, species and treatment

In total, 842 samples were included in the analysis of [SOC] with muffle furnace, HCl, and H_2SO_3, and

ranged from 0.2 to 3.6, 0.3 to 3.7, and 0.3 to 3.3 %, respectively. Mean [SOC] for soil treated with muffle furnace, HCl and H_2SO_3 were 1.6, 1.5, and 1.6, respectively, with concentrations broken down by species and depths shown in Table 2. A significant difference was found between [SOC] and method at shallower (0 – 10 cm) and deeper (20 – 40 cm) between the muffle furnace and H_2SO_3 (p value < 0.05) and at deeper depths (20 – 40 cm) between the muffle furnace and HCl (p value < 0.05) (Figure 1). HCl and H_2SO_3 methods were consistent when measuring between species, although the use of the muffle furnace showed high variability between poplar and spruce (Figure 2).

4.2 Regression analysis of the three methods for SOC measurement

When testing the strength of relationships between methodologies, it was important to separate treatments by species and by depth to account for the soil heterogeneity and variation occurring in the field at various depths under different tree species. When testing the relationships of methods between species and depth, regression analysis and log transformations were tested to determine strongest relationship as determined by R^2. The strongest relationships between treatments and depths for poplar and spruce are shown in Figure 3. Log transformations increased strength and relationship for poplar species at 0 – 10 cm (R^2=0.39), and 20 – 40 cm (R^2=0.53), and for spruce species at 20 – 40 cm (R^2=0.88) (Figure 3). In all but one case, [SOC] following HCl and H_2SO_3 treatments had the strongest relationship for both species. At 20 – 40 cm for spruce, the strongest relationship was seen between SOC following muffle furnace and H_2SO_3 treatment. Treatments showed strongest relationships at all depths for spruce (R^2 ranging from 0.88 – 0.95) over poplar (R^2 ranging from 0.30 to 0.54). For poplar, the strongest relationship was found at 20 – 40 cm (R^2=0.54), and for spruce was at 0 – 10 9 (R^2=0.95). When methods were compared using Proc Corr correlation tests, poplar showed strongest correlations between HCl and H_2SO_3 of 0.61, 0.55, and 0.70 at 0 – 10, 10 – 20, 20 – 40 cm depths respectively. Spruce showed strongest correlations between HCl and H_2SO_3 of 0.98, and 0.94 at 0 – 10 and 10 – 20

cm depths and between muffle and H_2SO_3 of 0.93 at 20 – 40 cm.

4.3 Correction factor

The incorporation of the correction factor resulted in an average overall increase of [SOC] for pre-treated soils. [SOC] increased an average of 0.3, 0.2 and 0.1% for soil pre-treated with muffle furnace, HCl, and H_2SO_3, respectively. Mean correction factors calculated by treatment method and by depth are found in Table 3. The lack of variation in correction factor values across depths for soils pre-treated with the muffle furnace is due to a small sample size that was used for the estimation and that was then applied across all replicates. When correction factors were used to convert measured OC concentrations to SOC in soil sample pre-treated with HCl and H_2SO_3, there was a higher influence at deeper depths reflecting increasing soil carbonates. This increase was particularly noticeable for soils pre-treated with H_2SO_3 which had a mean correction factor of 1.63 at 20 – 40 cm was significantly higher than correction factors of 1.11 and 1.12 at 0 – 10 cm and 10 – 20 cm depths, respectively.

5.0 Discussion

Although there was less variability in overall [SOC] across three methods, more variation was observed when they were compared at various depths. Lower [SOC] at deeper depths were observed across all methods, as can be expected as deeper soil depths contain increasing carbonate concentrations. Significant differences between methods were shown in the shallowest depths (0 – 10 cm) and the deepest depths of the soil (20 – 40 cm). Explanation of variation in shallower depths can most likely be attributed to the amounts of SOC and soil characteristics that are most prevalent at this depth. Although this study did not measure such effects, it would be beneficial to do so in the future. At deeper depths, carbonates are present in both lithogenic and pedogenic forms of varying distribution in the landscape.

At this field site, total C found in surface soil (0 – 20 cm) is comprised mostly of soil organic

matter and minimal SIC. While there is a concern that SOC may be lost during soil pre-treatment with acids, Midwood and Boutton (1998) found the $\delta^{13}C$ signature of SOM C is unaffected by high acid concentrations. Given the sensitivity and [SOC] to pre-treatments at shallower depths with acids, there would be other factors to explain why [SOC] when pre-treated with acids is less than those pre-treated with muffle furnace. The muffle furnace showed significantly higher [SOC] for poplar over spruce when compared to the consistency across species of the other methods (Figure 2). Higher than expected [SOC] values in soils pre-treated with the muffle furnace may be due to incomplete combustion during heating. Use of the correction factor with soils combusted in the muffle furnace also had the lowest variation and caused the highest increase of calculated [SOC] and could have resulted in an over-estimation.

On the contrary, [SOC] in soils pre-treated with H_2SO_3 may have been underestimated due to the difficulties in knowing when all the carbonates have been removed. Incomplete removal of carbonates may also be due to the formation of insoluble products formed from the reaction of calcium carbonate and H_2SO_3 as found by Schmidt et al. (2012) and Fernandes and Krull (2008). The use of the correction factor from Ramnarine et al. (2010), and Harris et al. (2001) may have compensated for some of this underestimation by increasing [SOC] compared to pre-treated soils without such correction (Table 2).

With the use of regression analysis to compare the three methods, it would seem as though methods may be species- and depth-specific. For poplar species, there was much lower correlation and less of a relationship between the methods. This could be attributed to lack of spatial variation in SOC due to litterfall and other uniform processes affecting soil C (Bambrick et al. 2010). In all cases, the strongest correlation was found between pre-treatment with HCl or H_2SO_3, suggesting either method is preferred over the use of muffle furnace. For spruce species, there was much higher correlations and relationships found between HCl and H_2SO_3 at shallower depths, indicating that either are preferable for measuring SOC at depths between 0 – 20 cm. At deeper depths (0 – 20 cm) the strongest relationship

was shown in soil pre-treated with H_2SO_3. This is most likely correlated with the significantly higher correction factor found in Table 3.

5.1 Correction factor

The correction factor, originally intended for fumigation of soils with 12 M HCl, adapted by Ramnarine et al. (2011) and Harris et al. (2001), had a most consistent influence across all depths for soils pre-treated with HCl. For use of correction factor for soils pre-treated with muffle furnace, initial and final soil weights of all individual samples may have shown more influence across depths. Significantly higher correction factors at deeper depths (20 – 40 cm) for soils pre-treated with H_2SO_3 should be further investigated.

6.0 Conclusions

Quantifying [SOC] is an important part of site characteristics, especially for TBI systems when determining their ability to sequester atmospheric carbon and for land remediation. While there are many methods available to do so, from the results presented in this paper, soil pre-treatment with either acid is recommended. Stronger relationships between soils pre-treated with HCl or H_2SO_3 indicate their values are more precise than the overestimated values of [SOC] obtained using the muffle furnace method. When assessing time and safety requirements, soils pre-treated with muffle furnace require more processing time due to the two rounds of samples with the LECO. Fumigation with HCl requires the least amount of sample preparation time and digestion, although fumigation in the desiccator requires more time. H_2SO_3 is a safer chemical with less harmful vapours as compared with 12 M HCl. Further analysis of the correction factor for soils pre-treated with H_2SO_3 is required given the high variation across depths. However, the correction factor application to all methods shows a significant difference is [SOC] among different species and soil depths and should be accounted for in all future [SOC] quantification.

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8.0 Tables and Figures

Table 1. Various acids and their uses and associated problems that were evaluated when choosing acids for carbonate removal

Acid	Use	Problem	Source
H_3PO_4	Acidification of geological carbonates	Combustion of residual phosphate salts interferes with quartz furnace tubes	Caughey *et al.* (1995)
$HClO_4$	Converts carbonates quantitatively to CO2	May react with other sample components to form perchlorates which are hazardous when heated. May oxidize labile organic compounds	Schilt (1979), Caughey et al. (1995)
HCl, H_2SO_4, HNO3	Carbonate removal before high temperature combustion	OC losses between 10-80% when using stronger mineral acids	Gibbs (1977)
H_2SO_3	Carbonate removal before high temperature combustion	OC losses > 2%	Gibbs (1977)

Table 2. Mean SOC concentration at three depths for treatments with muffle furnace, HCl and H_2SO_3 for hybrid poplar and Norway spruce at a 27-year-old TBI system in Guelph, Ontario, Canada. $\pm$ SE in brackets. Values with different letters across treatments (within depths) indicate a significant difference.

	Poplar		
	Muffle	HCl	H_2SO_3
0-10cm	2.3^{A} ($\pm$ 0.06)	1.9^{BC} ($\pm$ 0.07)	1.9^{BC} ($\pm$ 0.12)
10-20cm	2.0^{A} ($\pm$ 0.05)	1.7^{BC} ($\pm$ 0.06)	1.8^{BC} ($\pm$ 0.07)
20-40cm	1.5^{A} ($\pm$ 0.07)	1.1^{BC} ($\pm$ 0.07)	1.0^{BC} ($\pm$ 0.07)
	Spruce		
	Muffle	HCl	H_2SO_3
0-10cm	1.6^{A} ($\pm$ 0.05)	1.8^{BC} ($\pm$ 0.07)	1.8^{BC} ($\pm$ 0.07)
10-20cm	1.4^{A} ($\pm$ 0.02)	1.6^{BC} ($\pm$ 0.03)	1.6^{BC} ($\pm$ 0.03)
20-40cm	0.8^{A} ($\pm$ 0.05)	0.9^{A} ($\pm$ 0.05)	0.9^{A} ($\pm$ 0.06)

Table 3. Mean correction factor at three depths for treatments with muffle furnace, HCl an
H_2SO_3. $\pm$ SE in brackets. Values with different letters across treatments (within depths) in
a significant difference.

	Muffle	HCl	H_2SO_3
0-10cm	0.95^{A} ($\pm$ 0.01)	1.07^{B} ($\pm$ 0.02)	1.11^{C} ($\pm$ 0.02)
10-20cm	0.96^{A} ($\pm$ 0.01)	1.08^{B} ($\pm$ 0.01)	1.12^{C} ($\pm$ 0.02)
20-40cm	0.95^{A} ($\pm$ 0.01)	1.08^{B} ($\pm$ 0.01)	1.63^{C} ($\pm$ 0.27)

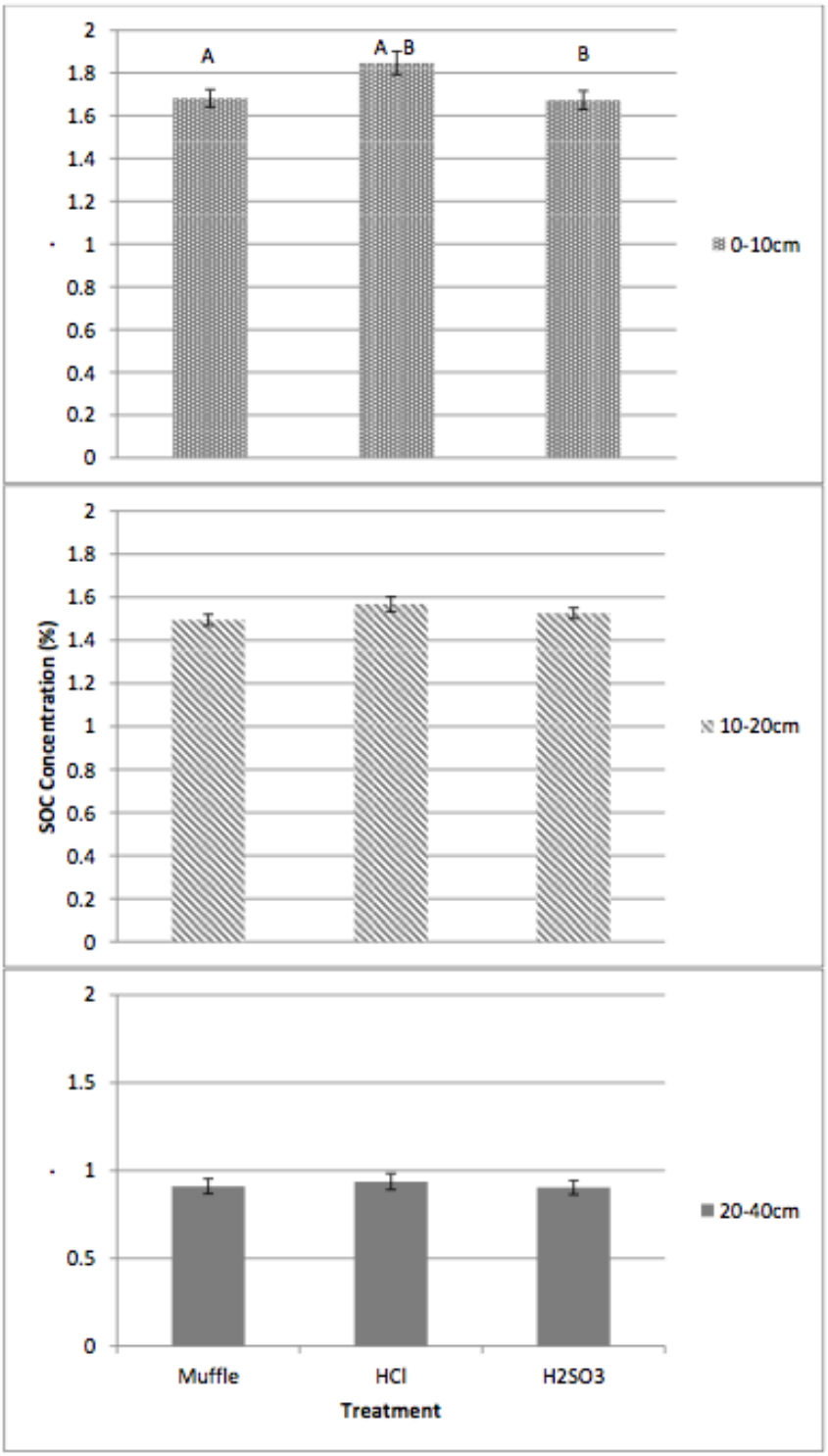

Figure 1. Mean SOC concentration (%) at depths 0 - 10, 10 - 20, and 20 - 40cm using a muffle furnace to remove SOC and using either 12 M HCl 0.73 M H_2SO_3 to remove soil carbonates

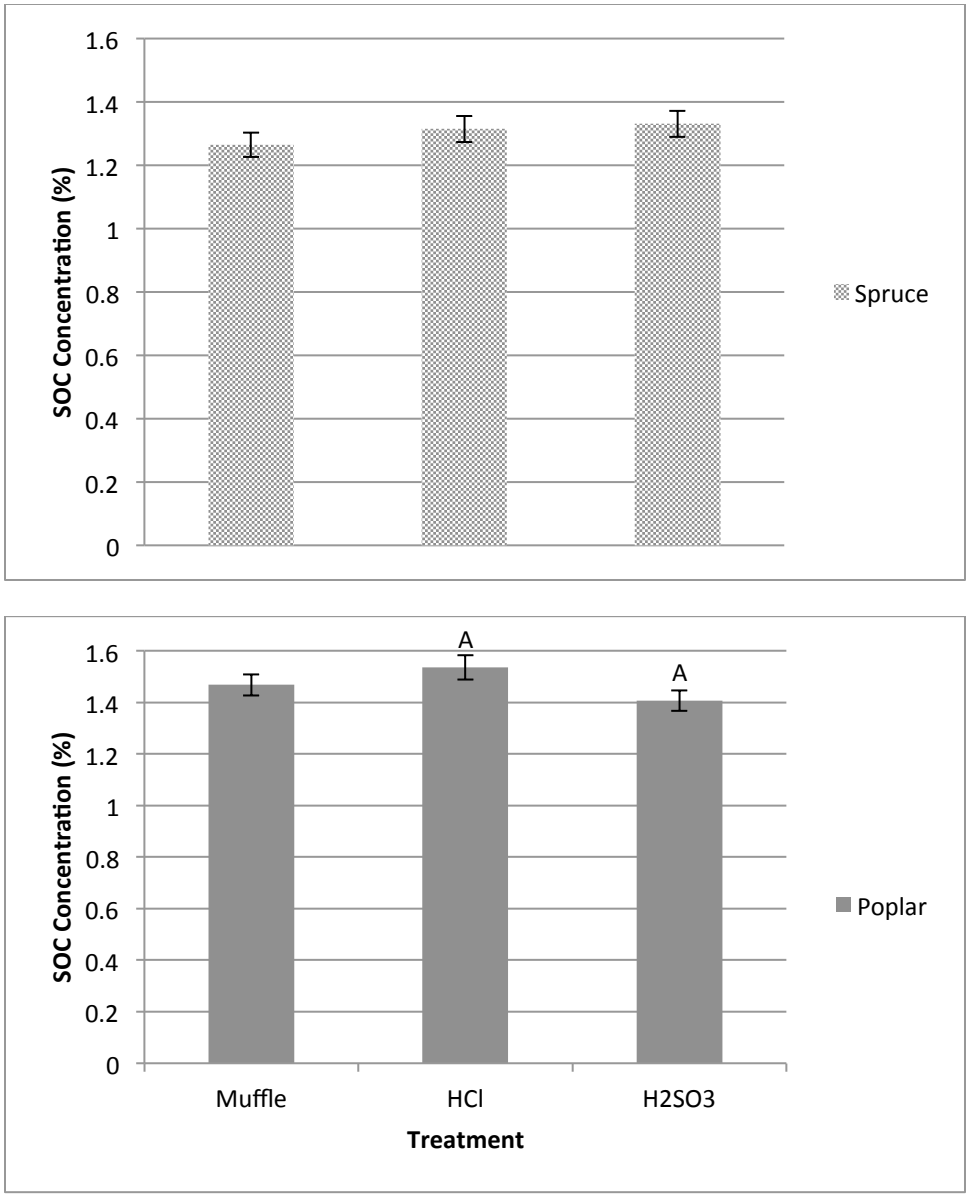

Figure 2. Mean SOC Concentration (%) for 25-year-old a) Norway Spruce *(Picae apies)* and b) Poplar hybrid (*Populus* spp.) across all depths and distances (± SE bars) for all treatments. A shows significant difference using T-Test (α0.05)

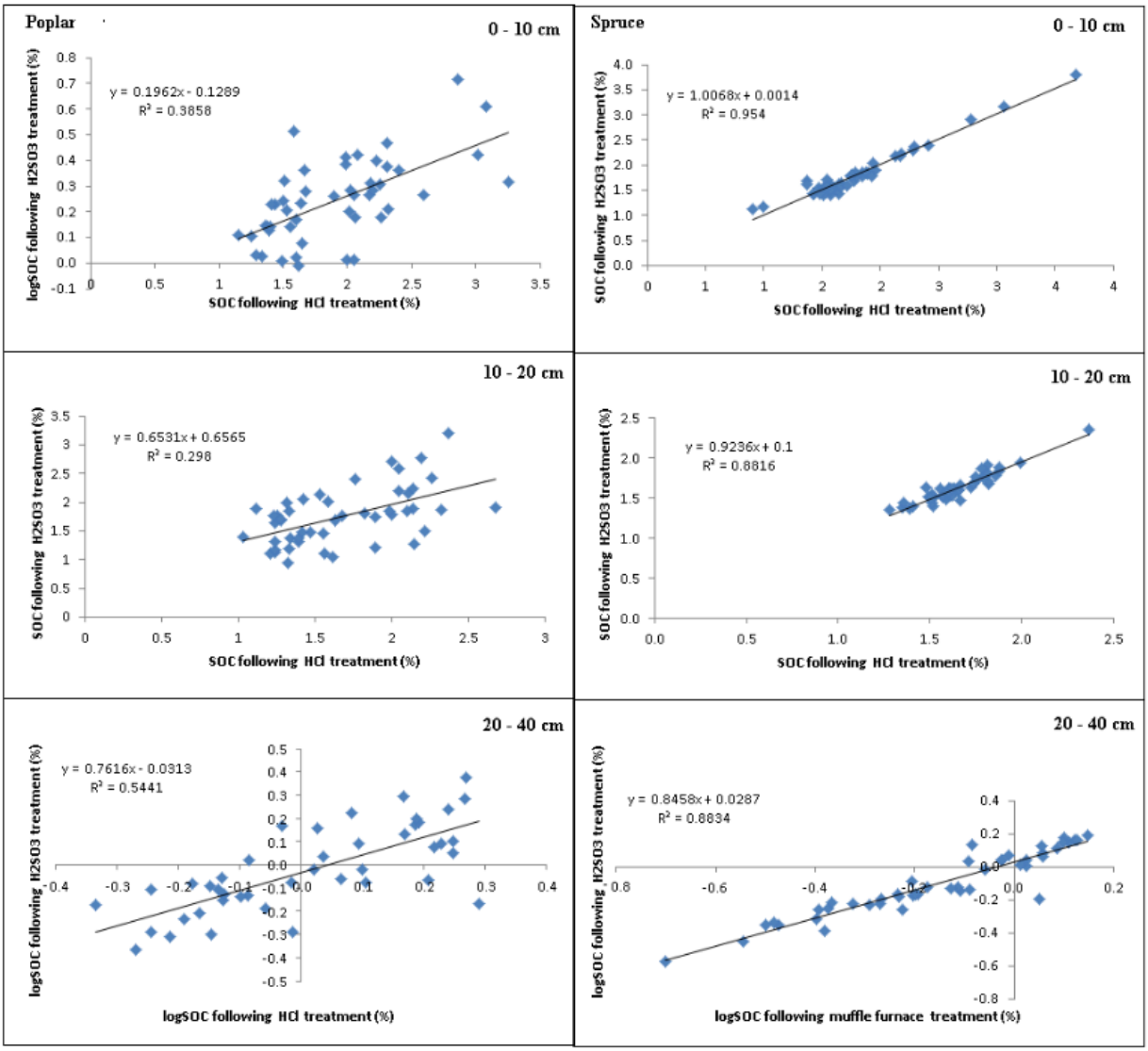

Figure 3. Plot of SOC concentration (%) of soils following treatment with muffle furnace, 12 M HCl and 0.73 M H_2SO_3. The best fit linear relationship is shown.

APPENDIX II

Carbon sequestration potential of five tree species in a 25-year-old temperate tree-based intercropping system in southern Ontario, Canada[2]

[2] Manuscript by Wotherspoon et al. currently approved pending edits for publication in *Agroforestry Systems*. Submitted December 2013.

CARBON SEQUESTRATION POTENTIAL OF FIVE TREE SPECIES IN A 25-YEAR-OLD TEMPERATE TREE-BASED INTERCROPPING SYSTEM IN SOUTHERN ONTARIO, CANADA

Amy Wotherspoon*, Naresh V. Thevathasan, Andrew M. Gordon and R. Paul Voroney

Keywords: C pools and fluxes, C sequestration, greenhouse gases, agroforestry, intercropping

Abstract

Carbon (C) sequestration potential was quantified for five tree species, commonly used in tree-based intercropping (TBI) and for conventional agricultural systems in southern Ontario, Canada. In the 25-year-old TBI system, hybrid poplar (*Populus deltoids* x *Populus nigra* clone DN-177), Norway spruce (*Picae abies),* red oak (*Quercus rubra*), black walnut (*Juglans nigra)*, and white cedar (*Thuja occidentalis*) were intercropped with soybean (*Glycine max* L.). In the conventional agricultural system, soybean was grown as a sole crop. Above- and belowground tree C Content, soil organic C, soil respiration, litterfall and litter decomposition were quantified for each tree species in each system. Total C pools for poplar, cedar, oak, walnut, spruce and soybean were 113.4, 99.4, 99.2, 91.5, 91.3, and 71.1 t C ha^{-1}, respectively at a tree density of 111 trees ha^{-1}, including mean tree C content and soil organic C pools. Net C flux for poplar, spruce, oak, walnut, cedar and soybean monocrop were + 2.1, + 1.6, + 0.8, + 1.8, +1.4 and – 1.2 t C ha^{-1}, y^{-1}, respectively. Results presented suggest greater atmospheric CO_2 sequestration potential for all five tree species when compared to conventional agricultural systems.

1.0 Introduction

A 30% increase in atmospheric carbon dioxide (CO_2), since 1750, has been recorded and is predicted that this trend will continue and give rise to another 50% increase towards the latter part of this century (IPCC, 2001). The conversion of agricultural land into agroforestry systems (IPCC, 2001), a land-use practice where trees are planted among agricultural crops in the hopes of sequestering atmospheric CO_2, is one of the many recommendations put out by the International Panel on Climate Change (IPCC) in order to reverse this trend. Tree-based intercropping (TBI) is one of the temperate agroforestry systems, where trees are considered to be long-term carbon (C) sinks through sequestration of atmospheric CO_2 into permanent tree components and in soil (Bambrick et al., 2010; Dixon, 1995a; Montagnini and Nair, 2004; Peichl et al., 2006; Sampson, 2001; Thevathasan and Gordon, 2004). In TBI systems, various tree species and planting densities are incorporated in order to test their potentials to sequester C and for environmental benefits over conventional agricultural systems.

These systems not only sequester atmospheric CO_2 but also provide nutrient-rich organic matter in the form of litterfall, which is absent in conventional agricultural systems. Litterfall adds to the soil organic matter (SOM) and thereby enhances soil C sequestration (Sanderman et al., 2009); a greenhouse gas (GHG) mitigation strategy not realized in conventional agricultural systems. Litterfall is also the most important pathway by which nutrients are returned to the system promoting soil productivity.

In recent years, studies have shown the *in situ* potential of TBI systems to sequester C in the temperate region (Bambrick et al., 2010; Oelbermann et al., 2004; Peichl et al., 2006; Thevathasan and Gordon, 2004). However, these studies have only examined the C sequestration potentials of one or two tree species. An extensive study to test many temperate tree species

suitable for TBI systems and to document their potential to sequester C at the system level is lacking for the temperate region. Therefore, this study was designed to test C sequestration potential of five tree species that are commonly integrated into a 25-year-old TBI system in southern, Ontario, Canada.

The objectives of this study are, a) to quantify above- and belowground C pools and fluxes within a 25-year-old TBI system and b) to compare its C sequestration potential to an adjacent conventional agricultural system. The results from this study will help to quantify levels of atmospheric CO_2 that could be sequestered by TBI systems for this climatic region of Canada. It may also contribute towards national climate change mitigation goals and the development of C models for TBI systems.

2.0 Materials and Methods

2.1 Site description and experimental design

This study was conducted in 2012 - 2013 at the University of Guelph Agroforestry Research Station; 30 ha of agricultural land established in 1987 in southwestern Ontario (43°32'28" N, 80°12' 32" W). Mean annual temperature is 7.2°C with 136 mean annual frost-free days and mean precipitation of 833 mm (344 mm of which fall during the growing season) (Simpson 1999; Oelbermann 2002). Site has a Canadian Land Index of 3 and soil is Albic Luvisols with a sandy loam texture (65% sand, 25% silt, 10% clay) and pH of 7.4 (Thevathasan 1998; Oelbermann 2002). Field is tilled annually to 15 cm each autumn and is tile drained.

In 1987, hybrid poplar (*Populus deltoids* x *Populus nigra* clone DN-177), Norway spruce (*Picae abies),* red oak (*Quercus rubra*), black walnut (*Juglans nigra)*, and white cedar (*Thuja occidentalis*) were planted at a density of 111 trees ha^{-1} in groups of eight within a row.

Within-row and between-row spacing are 6 and 15 m, respectively (Simpson 1999; Peichl et al. 2006). All deciduous trees were pruned to 3 m height from the ground during 2000 – 2001, and spruce trees were pruned to 2 m height during 2004/2005.

Trees are intercropped with corn (*Zea mays* L.), soybean (*Glycine max* L.), and winter wheat (*Triticum aestivum)* or barley (*Hordeum vulgare*L) in a corn-soybean-winter wheat/barley rotation. At the time of this study, the annual crop was soybean (2012) and barley (2013). An adjacent conventional agricultural field was also planted with the same crop in both years. Data from the conventional agricultural field, referred to as 'soybean monocrop' from this point on, were used for comparison with data collected in TBI system.

2.2 Tree carbon content

To determine the above- and belowground C content of five tree species, three replicates of each species were destructively harvested and weighed. Belowground roots were excavated to a depth of 1.5 m using a 580 Robert Tire Backhoe. All roots, having a diameter greater than that of pencil thickness, from a soil volume of 24 m^3 [4 m along the tree row (2 m north and 2 m south from the tree trunk) and 4 m into the crop alleys (2 m east and 2 m west from the tree trunk) and a depth of 1.5m], were handpicked. Distances from the tree trunk were determined from a previous study by (Gray, 2000), who determined that 90% of the roots on this site were within the above given distances from the trunk. The tree components were classified as primary and secondary branches, twigs (plus needles for spruce and cedar) and roots. Subsamples from these components were taken to determine moisture content, gravimetrically. Moisture content obtained from the sub-samples was then used to convert fresh weights to oven dry biomass, which was then multiplied by C concentration (%) to determine C content. Percentage C in

respective tree components was determined by analyzing the sub-samples in a LECO CR-12 dry combustion Carbon Analyzer (LECO Corporation, MI, USA).

2.3 Soil carbon

Soil samples were collected for each tree in both east and west directions, at 0.5, 1.0, 1.5 and 2.0 m distances from the tree trunk and at 0 - 10, 10 - 20 and 20 - 40 cm depths ((4 East locations + 4 West locations) x 3 depths x 3 replicates = 72 samples per tree in total x 3 trees = 216 samples per species)) in the TBI system. Samples were collected in the same spatial pattern in the soybean monocrop (36 samples, without sampling from two directions as soil C was assumed to be homogeneous in all directions for 1 replication). Soil samples were stored at -20°C until ready for analysis at which point they were thawed, air dried for 48 h and passed through a 2-mm sieve to exclude gravels and fine roots. A ~15 g subsample was measured from each air-dried soil sample to determine moisture content, gravimetrically at 105ºC. Another ~15 g sample was further ground to 0.125 mm adopting the ball-mill method to ensure a homogenized sample for SOC analysis. After being ball-milled, ~1.000g samples were fumigated with 12 M hydrochloric (HCl) acid for 7 d in order to remove the inorganic C fraction following methods adapted from (Ramnarine et al., 2011), including the use of a correction factor. After fumigation, samples were dried at 50°C for 24 h and a ~0.3000 g sample was analyzed for SOC content using the LECO CR-12 dry combustion Carbon Analyzer (LECO Corporation, MI, USA).

2.4 Soil respiration

Soil respiration was measured, at the least, once a month, between June and October 2012 and May 2013. Winter months were excluding as the amount of CO_2 efflux was assumed to be negligible (Peichl et al., 2006). Mean monthly soil respiration was measured using the soda lime

method, based on absorption of CO_2 (Edwards 1982) at 0, 2 and 6 m from the tree row into the cropping alley for poplar, walnut and spruce in the TBI system replicated 3 times (three respiration chambers per location x 3 replicates = 9 samples per species, per sampling period). The distance 6 m from the tree row was selected to be indicative of TBI systems over 0 and 2 m for two main reasons: 1) this area (cropping alley) occupies ~ 87% of the land area on a hectare basis, and 2) to exclude respiration by tree roots and grass roots along the tree rows. Respiration was measured in the soybean monocrop using three respiration chambers in the same spatial pattern as in the TBI system (3 respiration chambers x 1 location = 3 samples, per sampling period). Soil CO_2 efflux (g CO_2 m^{-2} day^{-1}) was calculated using the following equation:

$$Soil\ CO_2\ efflux\ (gCO_2m^{-2}d^{-1})$$
$$= \frac{sample\ weight\ gain\ (g) - mean\ blank\ weight\ gain\ (g) x\ 1.41)}{chamber\ area\ (m^2)}\ x\ \frac{24\ (h)}{duration\ of\ exposure\ (h)}$$

2.5 Litterfall and decomposition

Litterfall was collected every two weeks between September 11 and November 28, 2013 from a 0.5 m^2 quadrat at three replicates per tree species. Two random locations were selected every week, one in the east direction and one in the west, within a 4 m distance into the crop alley and a 1 m distance into the tree row in either direction. Litter was collected, oven-dried at 65ºC for 48 to 72 hours until constant weight was observed and weighed.

The decomposition of litter, using litter bags, 30 cm^2 in size with 2-mm mesh, were used for all five tree species and for leaves and stalk of soybean. The decomposition bags were buried in the cropping alley at 15 to 20 cm depth to simulate current tillage practices in the TBI field. Each decomposition bag was filled with ~50 g of oven dried litter from each respective tree species. In the soybean monocrop field, only soybean leaves and stalks were buried in two

locations. The total number of litterbags that were retrieved at each sampling period was 25 bags ((7 [5 species + leaves + stalks] x 3 replications/locations in TBI) + (2 [leaves and stalks] x 2 replications/locations in monocrop) = 25 bags per sampling period). The bags were retrieved in November 2012, January, April, June, August and September 2013 (day 36, 94, 155, 216, 277 and 312, respectively (6 sampling periods x 25 bags per sampling period = 150 bags collected in total). The material was collected, removed from the decomposition bag, cleaned, dried and weighed as described by (Anderson and Ingram, 1989). Mass remaining or loss (%) was calculated by dividing the amount of mass remaining over the original mass (time zero, original mass) and multiplied by 100%.

2.6 Statistical analysis

All treatments were tested for statistical parameters using SAS 9.3 (SAS Institute Inc. Cary, NC). Outliers were removed based on Lund's critical values and an analysis of variance was performed using a generalized linear model procedure. Statistical significance was assessed at $P<0.05$. A factorial analysis was performed to test for the significance of various factors and their interactions where applicable.

3.0 Results

3.1 Tree carbon content

Poplar had the highest mean C content per tree, both above- and belowground, followed by oak, walnut, spruce and cedar, having a total of 239 ($\pm$ 63), 139 ($\pm$ 22), 132 ($\pm$ 50), 114 ($\pm$ 43) and 146 ($\pm$ 24) kg C, respectively. For poplar, oak, walnut, spruce and cedar the percentage of biomass stored aboveground was 78 ($\pm$ 4), 74 ($\pm$ 4), 82 ($\pm$ 5), 72 ($\pm$ 4) and 93 ($\pm$ 2) %, respectively. Percentage of belowground biomass stored in the roots was 22 ($\pm$ 4), 26 ($\pm$ 4), 18 ($\pm$

5), 28 (± 4) and 7 (± 2) %, respectively. All trees had approximately the same C concentration aboveground; poplar, spruce and cedar all had a mean aboveground C concentration of 53% ± 0.2, 0.5, 0.4, respectively, and oak and walnut had 50 % ± 0.9 and 0.4, respectively. In all cases, roots had less C concentration than that recorded for aboveground biomass, having a % C of 50 % (±4), 48 %(± 1), 47% (± 2), 52 %(± 1) and 47 % (± 1) for poplar, oak, walnut, spruce and cedar, respectively.

At a tree density of 111 trees ha^{-1}, poplar, oak, walnut, spruce and cedar sequestered 27, 16, 15, 13 and 16 t C ha^{-1} respectively, over the past 25 years. As an annual flux, this translates to a carbon sequestration potential of 1.06, 0.62, 0.59, 0.52, and 0.65 t C ha^{-1} $year^{-1}$, respectively. It appears that fast growing hybrid poplar has the greatest potential to sequester C than the slow growing coniferous tree species such as, spruce and cedar.

3.2 Soil carbon

In total, 953 samples were included in the analysis for SOC across all five tree species. To determine the mean SOC concentration (%) at each depth and distance, mean values were calculated from three subsamples. Overall SOC means at 0 – 40 cm were 1.67% (± 0.18), 1.66% (± 0.10), 1.57% (± 0.11), 1.48% (± 0.12), 1.42% (± 0.14), and 1.41% (± 0.13), for soybean, cedar, poplar, oak, spruce and walnut, respectively, with no significant difference between species.

When incorporating direction (east and west of the tree row), distance (0.5, 1.0, 1.5, and 2.0 m from the tree trunk) and depth (0-10, 10-20 and 20-40 cm, at each distance), a factorial analysis indicated depth as the most important factor accounting for most of the variation within the model (F value = 222.57) and the strongest relationship with species (F value = 5.36, P <

0.0001). Overall, SOC is shown to decrease with increasing depth across all tree species. This is expected, as it is known that carbonates increase with increasing soil depth, especially in calcareous soils. Mean SOC values by depth for each species is presented in Table 1. The soybean monocrop field did not follow such patterns but instead had highest SOC between 10-20 cm (Table 1).

Table 1. Mean SOC (%) for five tree species and soybean monocrop at 0 – 10, 10 – 20 and 20 – 40 cm depths (± SE)

	Soybean	Poplar	Oak	Walnut	Spruce	Cedar
0-10 cm	1.95 (± 0.03)^{A}	1.91 (± 0.06)^{A}	1.90 (± 0.06)^{A}	1.87 (± 0.08)^{A}	1.77 (± 0.13)^{A}	2.04 (± 0.07)^{A}
10-20 cm	2.14 (± 0.19)^{AB}	1.68 (± 0.06)^{B}	1.58 (± 0.04)^{B}	1.49 (± 0.04)^{B}	1.64 (± 0.03)^{A}	1.59 (± 0.03)^{B}
20-40 cm	0.91 (± 0.13)^{C}	1.11 (± 0.11)^{C}	0.96 (± 0.10)^{C}	0.87 (± 0.03)^{C}	0.85 (± 0.11)^{B}	1.36 (± 0.11)^{C}

Superscripts indicating significant difference amongst depths of species (down columns) at $p<0.05$.

Interactions between species and distance were also significant (F value = 2.30, P = 0.0033), most likely from the influence of litter from the tree row. In all tree species, SOC were higher closer to tree rows (at 0.5 m) and decreased into the cropping alleys (2.0 m), however, were not statistically different. The difference in SOC between 0.5 and 2.0 m distance was 0.26, 0.32, 0.23 0.52 and 0.29 for poplar, oak, walnut, spruce and cedar, respectively. Interactions between species and direction, direction and distance and direction and depth were not significant.

SOC pools (t C ha^{-1} y^{-1}) were calculated to quantify total pools associated with tree species. Soil bulk densities for each depth were obtained from a parallel study conducted at the same site by Borden (2013, pers. comm.) for 0-10, 10-20 and 20-40 cm (Table 2). Species- and depth-specific bulk densities and corresponding SOC stock are found in Table 2. This allowed

for the calculation of tree-specific SOC stock to 40 cm soil depth. Total SOC pools for poplar, cedar, oak, spruce, walnut and soybean monocrop were 86.86, 83.77, 83.23, 78.29, 76.84, and 71.08 t C ha^{-1}, respectively, as seen in Table 2. Cedar had higher SOC stock than expected but was most likely due to closer spacing (1 m compared to 3 – 5 m of other species within the tree rows).

Table 2. Mean bulk densities (± SE) and SOC pools associated with intercropping and conventional agricultural systems (0 – 40 cm)

Intercropping	Mean bulk density (g cm^{-3})	SOC (t C ha^{-1})
Poplar	1.21 (± 0.12)	86.86
Oak	1.20 (± 0.13)	83.77
Cedar	1.14 (± 0.19)	83.23
Spruce	1.15 (± 0.18)	78.33
Walnut	1.15 (± 0.18)	76.84
Conventional Agriculture		
Soybean	1.20	71.08

3.3 Litterfall

Highest litterfall was found beneath poplar, walnut, spruce, oak and cedar with 379, 348, 297, 250, and 45 g m^{-2} y^{-1}, respectively. Significant difference lies between cedar and poplar (P=0.0071), cedar and walnut (P=0.0153), poplar and spruce (P=0.0045) and spruce and walnut (P=0.0098). Litterfall was always greater in the east direction (53% and greater of litterfall between east and west directions) for all tree species, although none showed significant difference. If C concentration is assumed to be 43% for deciduous leaf fall, then poplar, walnut and oak input 1.63, 1.50, and 1.07 t C ha^{-1} y^{-1}, respectively). Assuming 50% C content for coniferous needles, spruce and cedar input 1.49 and 0.23 t C ha^{-1} y^{-1}, respectively (Table 3).

3.4 Litter decomposition

To measure the rate of decomposition over time for litter from five tree species and soybean leaf and stalk, mass loss method was adopted. The decomposition rates of soybean leaf and stalk in the soybean monocrop field and in the TBI system are shown in Figure 1a and 1b. In the soybean monocrop, 71 (± 7.5) and 97 % (± 1.0) mass was lost during the 312 days of decomposition for the stalk and leaf, respectively. In the TBI field, mass loss reached 68 (± 3.4) and 96 % (± 1.9) for soybean stalk and leaf, respectively. At the end of 312 days, poplar, oak, walnut, spruce and cedar had 37 (± 2.3), 49.2 (± 3.2), 3.8 (± 0.3), 57.9 (± 0.4), and 61 % (± 6.2) residue remaining, respectively (Figure 2).

Figure 2 depicts the rates of decomposition of litter from different tree species. It can be observed that due to the presence of varying amounts of lignin and cellulose present in the litter, the decomposition rates vary significantly between tree species, especially as seen in spruce needles and cedar litter. For each species, annual C outputs via decomposition from tree litter for poplar, oak, walnut, spruce and cedar become 1.0, 0.54, 1.44, 0.63, and 0.26 t C ha^{-1} y^{-1}, respectively (Table 3).

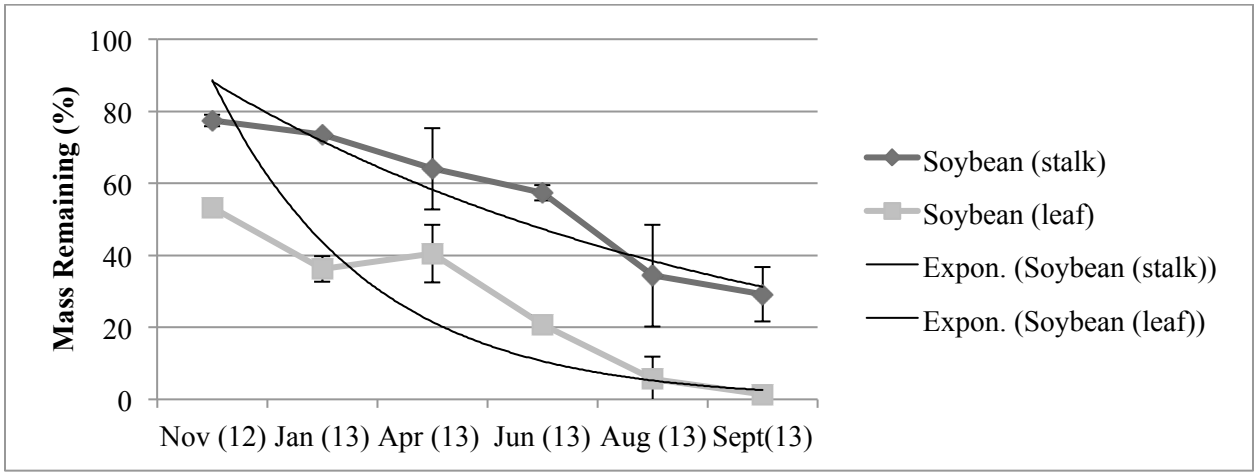

Figure 1. Mass remaining of soybean stalk and leaf after 312 days in a soybean monocrop system

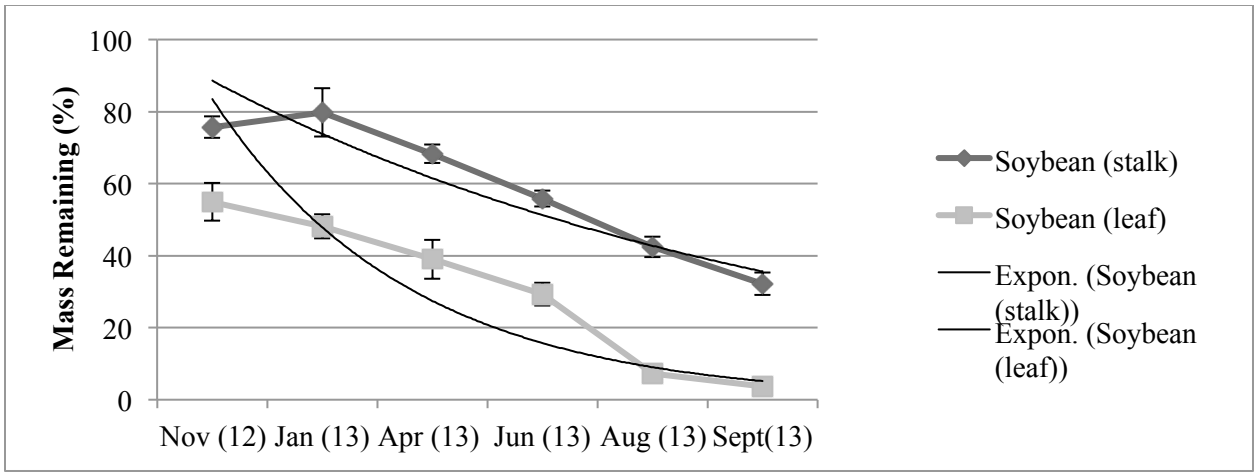

Fig 1b Mass remaining of soybean stalk and leaf after 312 days in a 25-year-old TBI system

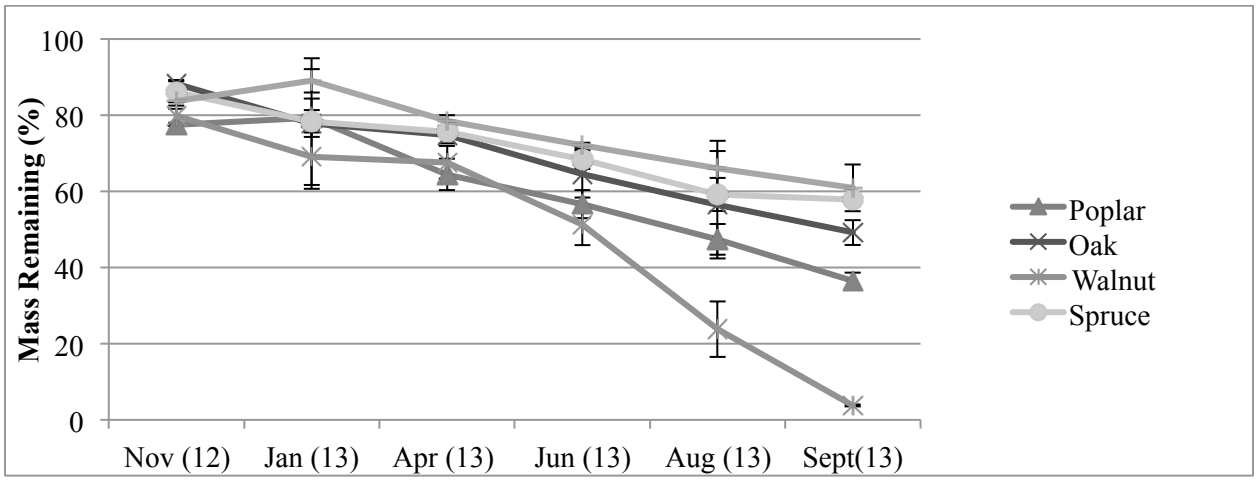

Figure 2. Mass remaining of litter from five tree species in a 25-year-old TBI system

Soil respiration

Overall means of CO_2 efflux between species was found to be highest for poplar at 22.91 ($\pm$ 4.01) t CO_2 ha^{-1} y^{-1}, followed by walnut, spruce, and soybean monocrop with 21.74 ($\pm$ 4.03), 21.49 ($\pm$ 4.50), and 16.77 ($\pm$ 0.86) t CO_2 ha^{-1} y^{-1}, showing no significant difference between species or between TBI and conventional agricultural systems.

Mean soil respiration rates for the TBI at various distances from the tree row and in comparison to the soybean monocrop are presented in Figure 3. For all species in the TBI

system, CO_2 efflux was always highest closest to the tree row at 0 m (ranging from 28.19 to 20.54 g CO_2 m^{-2} d^{-1}), and lowest closest in the crop row at 6 m (ranging from 12.96 to 16.94 g CO_2 m^{-2} d^{-1}), most likely due to root respiration. Respiration rates at 6 m were considered most likely to be indicative of true TBI respiration rate as at 6 m into the crop alley, tree-root and grass-root respiration in the tree row will be minimal but crop-root respiration will still be accounted for. When converted to C efflux, poplar had the highest output of C with 6.25 ($\pm$ 1.09) t C ha^{-1} y^{-1}. Following poplar was walnut, spruce and soybean monocrop with 5.93 ($\pm$ 1.10), 5.86 ($\pm$ 1.22) and 4.57 ($\pm$ 0.23) t C ha^{-1} y^{-1}, respectively.

4.0 Discussion

4.1 Tree Carbon Content

Above- and belowground biomass and the addition of litterfall and fine root turnover provide greater C inputs to TBI systems and therefore have a greater ability to sequester atmospheric CO_2 compared to conventional agricultural systems. The quantification of these C inputs and outputs helps to determine which species may be best suited for TBI systems in southern Ontario, Canada. Previous studies have shown that fast growing poplar trees are able to sequester more than twice as much C than a slow growing species, such as spruce (Peichl et al. 2006). However, at 25 years after establishment, poplar has reached its maturity and branches have started to disintegrate. Therefore, this resulted in only a slight increase in the total poplar C pools from 96.5 (Peichl et al., 2006) to 113.4 t C ha^{-1} between 13 (year 2000) and 25 (year 2012) years after establishment, respectively. Slower growing species such as spruce are continuing to add significantly higher amounts of C from 75.3 t C ha^{-1} at 13 (Peichl et al., 2006) to 91.3 t C ha^{-1} at

25 years after establishment. Spruce will therefore continue to sequester atmospheric CO_2 until harvest (60 years or more).

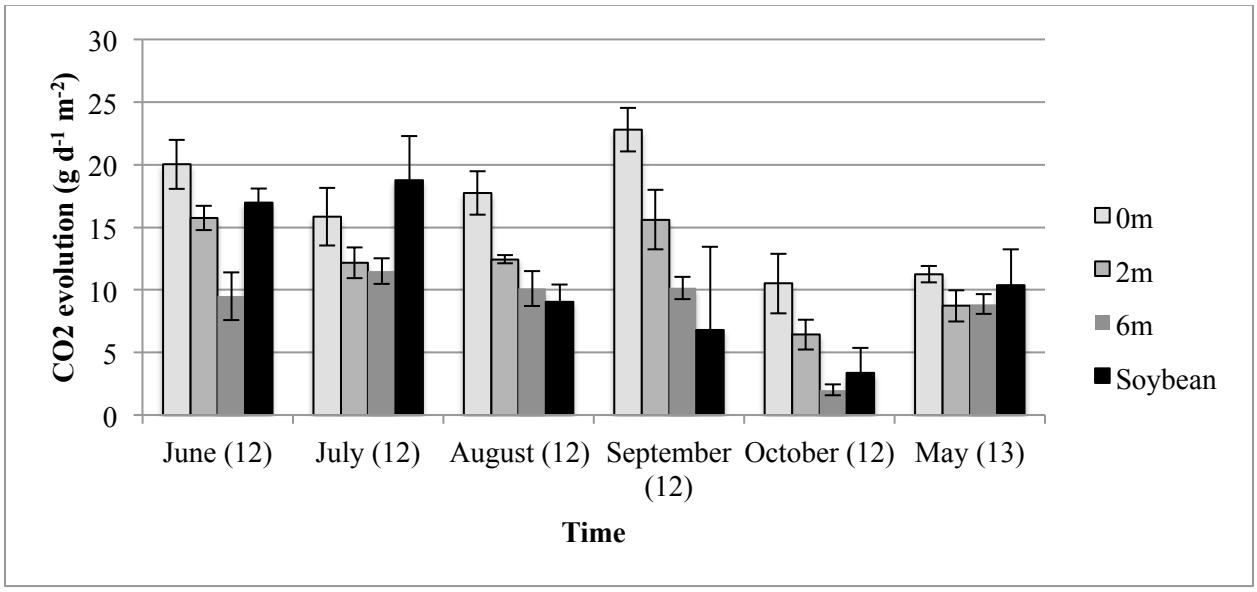

Figure 3. Soil respiration rates (g CO_2 d^{-1} m^{-2}) for the 25-year-old tree-based intercropping and conventional agricultural system (planted with soybean) at each distance from the tree row, by month (error bars denote standard error)

In order to calculate the total C sequestered by each tree species, the tree density was kept at 111 trees ha^{-1}; common density for hardwood tree species grown in TBI systems in the temperate region (Thevathasan and Gordon 2004). Based on the above tree density, the C sequestered in above and belowground components of trees over 25 years was 27, 16.25, 15.5, 15 and 13 t C ha^{-1} for poplar, cedar, oak, walnut and for spruce, respectively. It should be noted that even though cedar is a coniferous tree species, it ranked second to poplar, a fast-growing tree species. This was unexpected as the cedar tree growth on the research site was very low, a maximum mean tree height of only 5 m compared to over 20 m for poplar. The main reason was the pruning practices adopted in the past at this research site. As cedar tree branches were not

hindering with cropping practices (vertical branches instead of horizontal), the branches were never pruned, whereas, branches from all other tree species were pruned to a height of 4 to 5 m in deciduous species and to 2 m in spruce. Therefore, C sequestration results presented in this study for the rest of the tree species is an under estimation and thus conservative values. This also demonstrates the benefit of integrating a slow-growing tree species, such as cedar, for long term C sequestration, which can also be established at higher densities with less competitive interactions with adjacent agricultural crops due to its characteristic vertical branching ability.

4.2 Soil Carbon

Results in this study suggest that SOC concentration (%) is higher in the top soil layers (0-20 cm) in the soybean monocrop field. However, when converted to SOC content (pool) (t C ha^{-1}) to a depth of 0-40cm, TBI systems are showing a higher SOC content over conventional agriculture, mainly due to higher SOC concentrations in the 20-40cm soil horizon (Table 1) Walnut and spruce TBI systems had more or less similar SOC values at 20-40 cm when compared to the agricultural system. The SOC finding from this study is in support of results presented for this site by Peichl et al. (2006). They reported that barley monocrop agricultural system had 15.96 and 2.66 t C ha^{-1} less than poplar and spruce TBI systems, respectively, 13 years after establishment. Bambrick et al. (2010) also found, at the same site, conventional agricultural system to have 6.3 t C ha^{-1} less than poplar in a TBI system, 21 years after establishment.

4.3 Litterfall

The largest C flux contributor in a TBI system is litterfall (Oelbermann et al., 2004) therefore its quantity and decomposition rates were an important quantification of this study. Litterfall

quantities of poplar were expected to be lower due to die-back, therefore it should be noted that C inputs vial litterfall would be significantly higher than what is reported in this study; please refer to Thevathasan and Gordon 1995, 2004 and Peichl et al. 2006. For other tree species such as oak, walnut, spruce and cedar, it is expected that C inputs will continue to increase as the trees age (Oelbermann et al., 2004). With tree age and the increase in canopy size, it is assumed that litterfall input will be equally distributed amongst the crop alley as trees drop their leaves more uniformly (Oelbermann et al., 2004; Peichl et al., 2006). This uniform distribution of litter may have contributed to the lack of significant difference in levels of SOC across the cropping alley. Bambrick et al. (2010) have also suggested that uniform litter distribution would result in lack of spatial variation, this phenomenon has been observed at this site since 1993 (Thevathasan and Gordon, 1997). Previous studies from the same field site also show lack of spatial variation in SOC under poplar and Norway spruce at 13 years (Peichl et al., 2006) and 21 years (Bambrick et al., 2010).

4.4 Litter decomposition

SOC is not only influenced by the quantity of input from litterfall but also by the loss through decomposition. Decomposition and the return of organic matter from litterfall also add to the accumulation of stable soil C pools (Oelbermann et al., 2004). Slower rates of decomposition (Figure 1a and b) found in the TBI system compared to the monocrop system can occur for a number of reasons. Firstly, litterfall and crop residue in TBI systems are often combined with woody components dropped from trees (i.e. prunings, twigs, cones, etc.) that can increase the mean residence time of C in the system and slow decomposition (Oelbermann et al., 2004). Secondly, the presence of varying amounts of lignin and cellulose present in the litter, the decomposition rates vary significantly between tree species, as seen in Figure 2, between

deciduous and coniferous litter types. This is because microorganisms responsible for decomposition use nitrogen to breakdown C substrates, therefore litter with higher N availability and lower C:N ratio decompose more quickly (Mungai and Motavalli, 2006). Thirdly, the presence of mature trees also creates the presence of microclimates influencing soil temperature and moisture content. (Mungai and Motavalli, 2006) found lower mass loss at the middle of the crop alley in a temperate 21-year-old pecan-bluegrass alley cropping system, which corresponded to lower soil temperature. Lee and Jose (2003) also have reported that under these microclimates soil temperatures were 5 to 6% lower in a 47-year-old pecan-cotton alley cropping system than compared to a cotton monocrop system. In a study conducted on the same site(Clinch et al., 2009) has also reported lower soil temperature and higher soil moisture in the cropping alleys of a walnut-based TBI system.

4.5 Soil respiration

Litter decomposition rates also influence the varying rates of soil respiration between TBI and conventional agricultural systems. Higher soil respiration rates in the TBI field compared to the soybean monocrop field were expected due to the presence of trees in the system. Higher respiration rates seen closer to the tree row, also observed by (Peichl et al., 2006), can be attributed to higher tree root respiration and microbial respiration (Peichl et al., 2006). Because soil respiration was not separated into root and microbial respiration in this study, rates of decomposition, as previously discussed, can help to measure loss of C through the system and not being confounded by root respiration and inorganic C emissions.

Higher soil respiration rates found in TBI systems in this study may also be due to microbial respiration associated with litterfall, which attracts microorganisms that promote

microbial activity and therefore CO_2 evolution (Brady and Weil, 1996; Matteucci et al., 2000). In support of the above, (Lee and Jose, 2003) found that soil microbial biomass C was highest in a 47-year-old pecan-cotton alley cropping system than compared to 3-year-old system. In the same study, soil respiration rates were also positively correlated with soil microbial biomass C, SOM, and live fine root biomass.

5.0 Carbon modeling of pools and fluxes between five tree species in a tree-based intercropping system and a soybean monocrop system

5.1 Carbon pools

In order to determine total C pool at the system level, all C pools were combined (SOC, above- and belowground C content for trees and soybean crop). Tree density was maintained at 111 trees ha^{-1} for all tree species; a recommended spacing TBI systems in southern Ontario for deciduous trees. , System level total C pools were 113.4, 99.4, 99.2, 91.5, 91.3, and 71.1 t C ha^{-1} for poplar, cedar, oak, walnut, spruce, and soybean monocrop, respectively. When combining belowground tree C content with SOC stock, the ratio of aboveground C pools to belowground C pools for poplar, cedar, oak, walnut and spruce are 1:4, 1:6, 1:7, 1:6, and 1:8, respectively. The largest C pool is SOC, which contributed 93 – 97 % of the belowground pool and 86 – 97 % of the total C pool. The last study that examined C pools at this study site, 13 years after establishment, found C pools of 96.5, 75.3 and 68.5 t C ha^{-1} for poplar, spruce, and barley monocrop, respectively (Peichl et al., 2006). C pools for TBI and monoculture agricultural systems, since year 13, has increased by 16.9, 16.0 and 2.6 t C ha^{-1} for poplar, spruce and monocrop respectively. 25 years after establishment, all tree species in the TBI systems show a

significant increase in carbon sequestration. Poplar, oak, walnut, spruce and cedar have sequestered 60, 40, 29, 29 and 40 % more C at the system level than the soybean monocrop field, respectively, as seen in Figure 4.

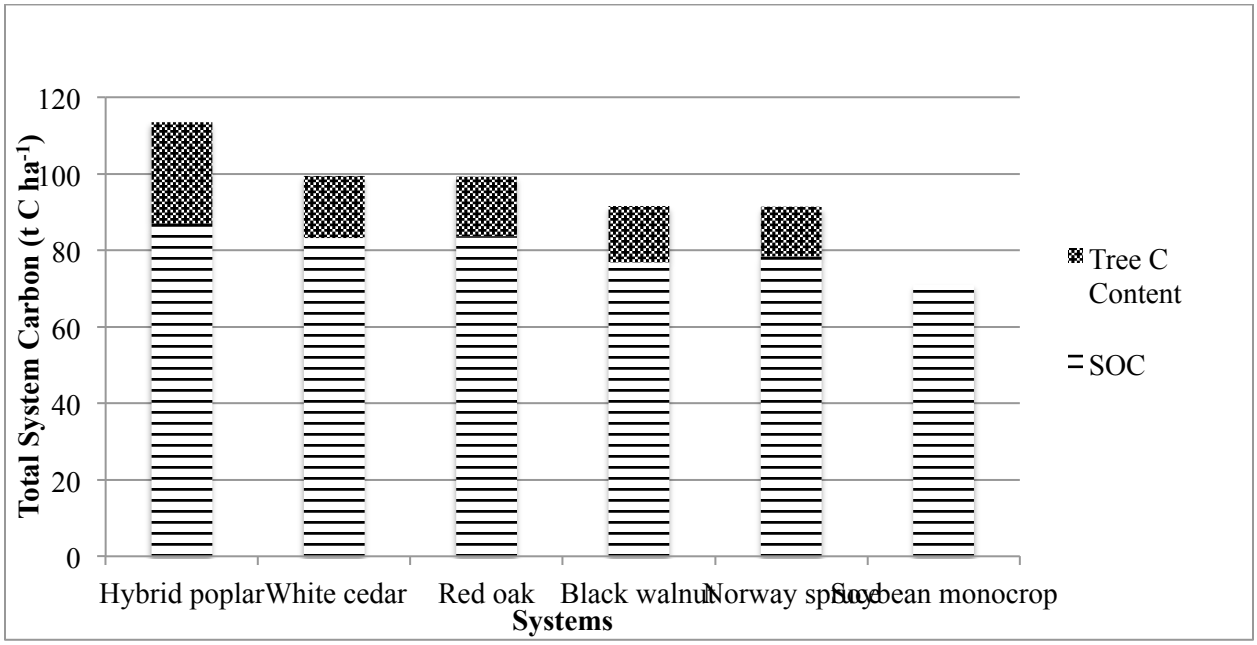

Figure 4. System level greenhouse gas emission reduction potential of five tree species commonly found in tree-based intercropping systems over the past 25 years in comparison with a conventional agricultural system in southern Ontario, Canada

Higher quantities of C stored in the TBI systems compared to the conventional agricultural system are expected due to the addition of organic matter in the form of litterfall in the TBI system (Peichl et al., 2006) and of course the presence of trees.. Fast growing hybrid trees, such as poplar, and deciduous trees are expected to have high C sequestration potential in TBI systems due to their large volume of above and belowground biomass. This was true for all tree species investigated in this study. However, poplar, which has the potential to grow fast and sequester C comparatively in a shorter time period (less than 15 years), should be harvested

within 15 years as their lifespan is short and does not sequester significant amounts of C beyond 15 years. In this study, poplar has shown a decline in above- and belowground tree C content from 35.9 t C ha^{-1} 13 years after establishment (Peichl et al., 2006) to 26.6 t C ha^{-1}, 25 years after establishment due to dieback. At the current rate of growth, poplars should be harvested between 13 – 15 years to prevent die back (usually occurs at 15 years, for this particular clone). TBI systems designed with hybrid poplar trees should therefore allow for a second cycle of trees to be planted between years 8 and 10 of the initial planting. This allows the initial plantings to be harvested at year 15, and the second set of trees will be sequestering atmospheric C at an exponential rate at ages 5 or 7, thus enhancing system level C sequestration on a continuous basis.

5.2 Carbon fluxes

Total fluxes were calculated based on assimilation of trees, litterfall C inputs and outputs, root turnover, crop C inputs and outputs, and C leachate in 2012. The net C fluxes are shown in Table 3. For poplar, oak, walnut, spruce and cedar the net fluxes were + 2.1, + 1.6, + 0.8, + 1.8 and + 1.4 t C ha^{-1} y^{-1}, respectively. Net C flux for soybean in the conventional agricultural field was – 1.2 t C ha^{-1} y^{-1}. It should be mentioned that the negative C flux observed in the agricultural system in this study was based on numbers derived for soybean cultivation. But, because the monocrop agricultural field goes through a corn-bean-wheat rotation, it should not be considered as a net C loss under all crops. Given an increase in SOC in the agricultural field, 2.6 t C ha^{-1} since year 13 to 25, this indicates that the crop rotation has contributed, at least slightly, to an increase in SOC pool. Die-back of poplar in the current study has also resulted in a decline in the annual net flux of C at the system level from + 13.2 t C ha^{-1} y^{-1} (year 13) to 2.12 C ha^{-1} y^{-1}

(year 25). However, the net C flux in spruce increased from + 1.1 t C ha^{-1} y^{-1} to 1.8 t C ha^{-1} y^{-1} in the past 12 years.

Numerous factors will influence the net C flux in both TBI and conventional agricultural systems. These include, but are not limited to, crop species, crop rotation, amount of residue left on the soil surface after crop harvest, tree density and species, tree row spacing, percentage of land covered by trees, climate, management practices and soil type.

Table 3. Carbon sequestration (t C ha^{-1} y^{-1}) potentials of five tree species commonly grown in tree-based intercropping systems in comparison to conventional agricultural systems in southern Ontario, Canada

	Poplar	Oak	Walnut	Spruce	Cedar	Soybean Monocrop
Inputs						
Aboveground tree C assimilation	0.83	0.46	0.48	0.38	0.53	
Belowground tree C assimilation	0.23	0.16	0.11	0.14	0.12	
Litterfall C inputs	1.63	1.07	1.50	1.49	0.68	
Fine root turnover	0.82	0.54	0.75	0.45	0.20	
Above and belowground Crop C input	1.22	1.22	1.22	1.22	1.22	1.40
Outputs (via decomposition)						
Litterfall C outputs	1.04	0.54	1.44	0.63	0.26	0
Root output	0.52	0.27	0.72	0.19	0.08	1.31
Crop C outputs	1.00	1.00	1.00	1.00	1.00	1.19
C leachate	0.05	0.05	0.05	0.04	0.04	0.05
Net						
Net C balance	+ 2.12	+ 1.58	+ 0.84	+ 1.81	+ 1.36	- 1.15

For comparative purposes between 13 and 25 year old system level TBI and conventional agricultural field, the same assumptions were followed as those outlined by Peichl et al. (2006).

6.0 Conclusions

It is evident from this study that the net C flux for each investigated tree species in TBI systems differed, highest being for fast-growing tree species and lowest for slow-growing tree species, such as spruce. However, C sequestration in slow-growing trees can be enhanced by doubling or tripling their tree density to 222 or 333 trees ha^{-1} as they compete less for light with adjacent agricultural crops due to their vertical branching habit and slow growth. In the case of spruce or cedar, they also can be marketed as Christmas trees and the land-owner could derive an income in the short-term. This will also help thinning the stand and thereby reduce competition with adjacent agricultural crops, when trees are 7 to 10 years old.

This study was the first to develop extensive C models for five commonly grown tree species in TBI systems in southern Ontario and was also the first study to investigate the change in C dynamics at the "system level" between ages 13 and 25 and compare the same to an adjacent conventional agricultural system. . Therefore, species-specific quantifications reported in this study should help to understand the long-term implications in C sequestration at the landscape level as influenced by two different land-use systems. This study also showed the importance of 'relay planting' or 'staggered planting' of fast-growing trees species, such as hybrid poplar, in order to maximize and enhance the system level C sequestration in TBI system as compared to conventional agricultural systems. This management practice will become crucial when C trading is enforced in Ontario. This will also allow landowners to decide on various tree species that fit their site- and goal-specific needs. The C sequestration potential of various tree species, known from this study, may be of importance to introduce these tree species into other temperate land-use systems, such as, silvopastoral systems, riparian buffers, windbreaks, and forest farming.

In conventional agricultural systems, it appears that all agricultural crops and management practices do not always contribute to a net C flux, as seen for soybeans in this study. However, even a slight increase in SOC between the years 13 and 25, suggest that crop rotation is an important management consideration in conventional agricultural systems in order to maintain SOC levels. Lastly, the quantification of C sequestration in TBI systems is an important step towards making informed policy decisions associated with greenhouse gas mitigation strategies in the agricultural sector, one of the primary objectives of the Global Research Alliance (GRA) and the Canadian Agriculture Greenhouse Gas Program (AGGP). In combination with other Best Management Practices (BMPs) recommended for the conventional agricultural sector, TBI systems could contribute towards reducing the impact of Canadian agriculture on greenhouse gas emissions.

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APPENDIX III

Sample Calculations for Carbon Model

Soil Temperature, Soil Moisture Content (± standard deviation) of three species in a 25-year-old tree-based intercropping system and a soybean sole-cropping system in southern Ontario.

	Hybrid Poplar		Black walnut	
	Soil Temperature	**Soil Moisture Content**	**Soil Temperature**	**Soil Moisture Content**
February	-0.8 (± 0.3)	0.37	0.4 (± 2.1)	0.29 (± 0.02)
April	2.5 (± 0.3)	0.41 (± 0.19)	2.6 (± 3.1)	0.39 (± 0.16)
May	14.8 (± 1.1)	0.27 (± 0.05)	14.6 (± 2.4)	0.05 (± 0.53)
June	19.3 (± 4.4)	0.17 (± 0.06)	20.4 (± 6.0)	0.19 (± 0.05)
July	23.5 (± 2.1)	0.07 (± 0.02)	42.7 (± 6.1)	0.09 (± 0.03)
August	18.7 (± 0.4)	0.16 (± 0.03)	18.3 (± 06).	0.20 (± 0.04)
September	15.3 (± 4.0)	0.15 (± 0.02)	14.8 (± 3.1)	0.18 (± 0.04)
October		0.15 (± 0.01		0.42 (± 0.47)
November	2.8 (± 0.8)	0.20 (± 0.03)	3.8 (± 0.4)	0.24 (± 0.06)
December	0.0 (± 0.7)	0.21 (± 0.05)	0.2 (± 2.4)	

	Norway Spruce		Soybean Sole-Crop	
	Soil Temperature	**Soil Moisture Content**	**Soil Temperature**	**Soil Moisture Content**
February	37.1 (± 6.9)	0.25 (± 0.03)		0.38 (± 0.03)
April	4.6	0.45 (± 0.12)		0.24 (± 0.01)
May	13.0 (± 27.0)	0.26 (± 0.07)		0.22 (± 0.01
June	5.5 (± 30.5)	0.19 (± 0.16)		
July	9.6 (± 26.5)	0.08 (± 0.03)		0.07 (± 0.02)
August	18.8 (± 14.5)	0.18 (± 0.03)		0.14 (± 0.03)
September	23.9 (± 24.0)	0.17 (± 0.02)		0.16 (± 0.60)
October		0.17 (± 0.01)		0.18 (± 0.02)
November	3.3 (± 30.4)	0.20 (± 0.04)		0.18 (± 0.01)
December	0.5 (± 30.2)	0.26 (± 0.06)		0.22 (± 0.06)

1. Crop Input

	Intercropping	Monocropping
Aboveground biomass (t ha^{-1})	3.869	3.869
Belowground biomass (t ha^{-1})	1.548	1.548
Total crop yield (t ha^{-1})	2.16	2.16
Aboveground biomass remaining after harvest (t ha^{-1})	1.709	1.709
Belowground biomass remaining after harvest (t ha^{-1})	1.548	1.548
Biomass C (t C ha^{-1})	1.218 (87% of the land)	1.400 (100% of the land)

2. Trees and Soil Organic Carbon Sequestration

	Poplar	Oak	Walnut	Spruce	Cedar
Mean aboveground biomass (kg)	186.38	75.49	123.00	53.33	22.28
Mean belowground biomass (kg)	30.05	38.11	33.53	14.51	6.84
Mean biomass per tree (kg)	464.95	277.43	264.75	223.07	93.90
Mean Biomass C per tree (kg C)	239.46	139.17	132.42	117.22	5.39
Density (trees ha^{-1})	111	111	111	222	333
Biomass C (t C ha^{-1})	26.58	15.45	14.69	26.02	16.18

2a. Soil organic carbon for all depths and distances

SOYBEAN	O.5	1	1.5	2	Mean SOC (%)	O.5	1	1.5	2	SOC Stock
0-10cm	2.02	1.91	1.97	1.93	1.67 (± 0.18)	24.25	22.89	23.60	23.10	23.46
10-20cm	1.66	2.41	2.03	2.46	Mean BD	19.97	28.86	24.36	29.52	25.68
20-40cm	0.78	0.61	1.14	1.13	1.20	18.81	14.63	27.29	27.03	21.94
										71.08 t C ha^{-1}
POPLAR	O.5	1.00	1.50	2.00	Mean SOC (%)	O.5	1.00	1.50	2.00	SOC Stock
0-10cm	2.14	1.92	1.81	1.76	1.57 (± 0.11)	25.94	23.27	21.94	21.33	23.12
10-20cm	1.81	1.66	1.69	1.55	Mean BD	21.97	20.06	20.42	18.80	20.31
20-40cm	1.23	1.08	1.05	1.10	1.21	29.73	26.29	25.35	26.68	43.43
										86.86 t C ha^{-1}
OAK	O.5	1.00	1.50	2.00	Mean S OC (%)	O.5	1.00	1.50	2.00	SOC Stock
0-10cm	2.11	1.93	1.84	1.73	1.48 (± 0.12)	25.37	23.22	22.14	20.81	22.88
10-20cm	1.64	1.60	1.64	1.43	Mean BD	19.70	19.32	19.77	17.23	19.00
20-40cm	1.18	0.97	0.89	0.79	1.20	28.31	23.42	21.46	19.14	41.89
										83.77 t C ha^{-1}
WALNUT	O.5	1.00	1.50	2.00	Mean SOC (%)	O.5	1.00	1.50	2.00	SOC Stock
0-10cm	2.11	1.96	1.79	1.62	1.41 (± 0.13)	24.17	22.44	20.52	18.49	21.41
10-20cm	1.52	1.53	1.55	1.35	Mean BD	17.40	17.53	17.68	15.45	17.02
20-40cm	0.83	1.02	0.82	0.82	1.14	19.06	23.23	18.80	18.71	38.42
										76.84 t C ha^{-1}
SPRUCE	O.5	1.00	1.50	2.00	Mean SOC (%)	O.5	1.00	1.50	2.00	SOC Stock
0-10cm	2.27	1.51	1.76	1.53	1.42 (± 0.14)	26.08	17.31	20.24	17.58	20.30
10-20cm	1.74	1.65	1.66	1.53	Mean BD	19.91	18.94	19.04	17.55	18.86
20-40cm	1.26	0.93	0.58	0.62	1.15	28.86	21.24	13.35	14.30	39.16
										78.33 t C ha^{-1}
CEDAR	O.5	1.00	1.50	2.00	Mean SOC (%)	O.5	1.00	1.50	2.00	SOC Stock
0-10cm	2.27	2.13	1.91	1.85	1.66 (± 0.10)	26.07	24.47	21.87	21.25	23.41
10-20cm	1.60	1.62	1.58	1.55	Mean BD	18.36	18.55	18.11	17.79	18.20
20-40cm	1.62	1.44	1.18	1.22	1.15	37.23	33.00	27.04	27.90	41.62
										83.23 t C ha^{-1}

3. Intercropping System Annual C Input

	Poplar	Oak	Walnut	Spruce	Cedar	Soybean
Annual Litterfall (t ha^{-1} y^{-1})	3.79	2.49	3.49	2.97	1.36	0.00
Annual litter C input (t C ha^{-1} y^{-1})	1.63	1.07	1.50	1.49	0.68	0.00
Soil respiration (t C ha^{-1} y^{-1})	4.96	4.80	4.80	3.65	3.65	4.71
Annual roots turnover (t ha^{-1} y^{-1})	0.82	0.54	0.75	0.45	0.20	0.00
Annual Crop C input (t ha^{-1} y^{-1})	1.22	1.22	1.22	1.22	1.22	1.40
Annual C input via tree assimilation	1.06	0.62	0.59	1.04	0.65	
Total system input	**4.73**	**3.44**	**4.05**	**4.19**	**2.75**	**1.40**
Assumes 43% C content of litter for deciduous species and 50% C content for conifer species *Root respiration is assumed to be 25% of soil respiration* *Assume 50% root turnover for deciduous species and 30% for conifer species*						

4. Water Carbon Leachate

Assumed leaching rate = 200 mm y^{-1}
1 l = 1000 g; 1 % C of 1 leaching soil solution = 10 g C
C leachate at 200 mm y^{-1} leaching rate = 2 x 10^6 l

	Poplar	Oak	Walnut	Spruce	Cedar	Soybean
C from species (%)	0.083	0.083	0.083	0.072	0.072	0.082
C leachate at 200 mm y^{-1} (t C)	1.66	1.66	1.66	1.44	1.44	1.64

5. System Carbon Output

	Poplar	Oak	Walnut	Spruce	Cedar	Soybean
Annual litterfall (t ha^{-1} y^{-1})	3.79	2.49	3.49	2.97	1.36	0.00
% lost as decomposition	67.67	49.89	86.18	42.21	44.49	
Annual litter C output (t C ha^{-1} y^{-1})	1.10	0.53	1.29	0.63	0.30	0.00
Annual root output (t C ha^{-1} y^{-1})	0.55	0.27	0.65	0.19	0.09	1.42
Annual crop input (t C ha^{-1} y^{-1})	1.22	1.22	1.22	1.22	1.22	1.40
Annual crop C output (t C ha^{-1} y^{-1})	0.96	0.96	0.96	0.96	0.96	1.29
Annual C leachate (t C ha^{-1} y^{-1})	0.05	0.05	0.05	0.04	0.04	0.05
Total System Output (t C ha^{-1} y^{-1})	**2.67**	**1.82**	**2.95**	**1.82**	**1.40**	**2.76**
Annual C leachate is 3% of total leaching						

6. Net C Flux

	Poplar	Oak	Walnut	Spruce	Cedar	Soybean
Annual System Input (t C ha^{-1} y^{-1})	4.73	3.44	4.05	4.19	2.75	1.40
Annual System Output (t C ha^{-1} y^{-1})	2.67	1.82	2.95	1.82	1.40	2.76
Net C Flux (t C ha^{-1} y^{-1})	**2.06**	**1.63**	**1.10**	**2.37**	**1.35**	**-1.36**