Impact of Previous Cover Crops and Corn Stover Removal on Soil Organic Carbon, Aggregate Stability and Squash Yield

by

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ABSTRACT

IMPACT OF PREVIOUS COVER CROPS AND CORN STOVER REMOVAL ON SOIL ORGANIC CARBON, AGGREGATE STABILITY AND SQUASH YIELD

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Corn stover removal for biofuel production can have a negative effect on crop yield and/or soil quality. Cover crops may offer an opportunity to improve or maintain soil quality within corn stover removal systems. This research assessed the effects of cover crops systems and corn stover removal on soil aggregate stability, squash (*Cucurbita pepo* var. *pepo* cv. Autumn Delight) fruit yield, and soil carbon and nitrogen dynamics. Diffuse reflectance infrared Fourier-transform (DRIFT) spectroscopy was used to semi-quantitatively assess soil organic matter decomposition from soil amended with oat (*Avena sativa* L.), cereal rye (*Secale cereale* L.), oilseed radish (*Raphanus sativus* L. var. *oleiferus* Metzg. Stokes) (OSR), and a mixture of oilseed radish and cereal rye (OSR+rye), with and without corn stover by measuring polysaccharide-C bands regularly over a 72 d incubation study, and comparing first-order model parameters to evolved C. One year after corn stover removal in cover crop systems there were no differences in soil aggregate stability and clay dispersibility. Squash vegetable yield was also not affected by corn stover removal after one year. Results showed that all cover crop-corn stover treatment combinations had a significantly lower polysaccharide decomposition and C mineralization rates compared to the no cover treatment. Thus, although subsequent crop yield
was not impacted, this study suggests that these cover crops, in particular OSR and OSR+rye, have the potential, in the short-term, to replenish labile organic C pools and to reduce C losses when compared to the no cover control treatment.
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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1. LITERATURE REVIEW

1.1.1. Introduction

Increasing biofuel production has been suggested as a means to tackle the issues of fossil fuel dependence, climate change and energy security. Current biofuel production is dominated by ethanol made from corn grain or biodiesel made from soybean oil. Corn stover, which consists of above-ground biomass left after grain harvest, presents an opportunity as a biofuel feedstock when compared to other sources of plant biomass because of its high cellulosic content and high availability (Wilhelm et al., 2004). However, high removal rates of corn stover have shown detrimental effects on soil quality, with removal rates above 25% displaying reductions in soil organic carbon (SOC), as well as grain crop productivity (1.4 to 3.1 Mg ha\(^{-1}\) reduction in grain yield with 50, ≥75% stover removal, respectively) (Blanco-Canqui and Lal, 2009a). Therefore, there are concerns that high removal rates of corn stover may further impact agricultural soils. These concerns may also be accentuated in cropping systems with a history of intensive tillage, lack of rotational crop and reduced organic inputs (Yang and Wander, 1999; Fageria, 2012).

Alternative farming practices, which include the use of cover crops may offer a means to maintain and/or improve soil quality by providing additional organic matter inputs. Cover crops may also provide additional benefits such as nutrient recycling, increased soil organic matter (SOM), and improved soil structure (Snapp et al., 2005).
1.1.2. Soil Organic Matter

Soil organic matter is arguably one of the most important soil quality indices (SQI) as it mediates many physical, chemical and biological soil processes; these include nutrient cycling, soil structure and erosion resistance (Weil and Magdoff, 2004). The addition of organic matter to soils generally improves soil quality by decreasing bulk density, surface sealing and crust formation, and by increasing aggregate stability, cation exchange capacity, nutrient cycling, and biological activity (Andrews et al., 2004; Kay, 1990).

Soil organic matter consists of the organic fraction of the soil, and includes plant and animal residues at various stages of decomposition, cells (living and dead) and tissues of microbes, and substances synthesized by the soil population (Canadian Department of Agriculture, 1972). The quality, stability and availability of SOM vary widely due to its heterogeneous nature within the soil. The properties of organic compounds associated to SOM are also highly variable; SOM turnover time ranges from days for plant residues and root exudates (labile compounds) to >1000 yr for resistant humics (highly stable compounds) (Campbell et al., 1967a, 1967b).

The primary function of SOM is to serve as an energy substrate for plant and soil microbial communities; however it also enhance soil physical and chemical properties such as soil aggregation, water retention, cation exchange capacity and pH buffering (Baldock and Nelson, 2000). Plants are considered the primary source of organic matter (OM) to the soil, and plant-derived OM is composed of polysaccharides (e.g., cellulose, hemicellulose, chitin), proteins, lipids/aliphatic materials (e.g., waxes, cutin, suberin), and lignins (Horwath, 2007). Thus, the majority of plant inputs are C polymers and hydrocarbons that contain few essential
nutrients to facilitate decomposition (Horwath, 2007). The secondary source of OM consists of the soil fauna and microorganisms, which facilitate plant residue decomposition, leave their waste products or their bodies by death, and play a role in the transformation of plant residues (Horwath, 2007).

Soil organic matter dynamics depend largely on the balance between quantity/quality of organic inputs to the soil and SOM losses by microbial decomposition, runoff/erosion and leaching of dissolved organic and particulate C (Jenkinson and Ayanaba, 1977; Kutsch et al., 2009). Conceptual models have traditionally differentiated SOM functional pools by active, intermediate and passive based on their different turnover rates or residence times (Karlen et al., 1997).

Decomposition of OM is a complex process containing many steps. The quantity and quality of plant litter are the most dominant inputs affecting microbial activity and the ability of decomposers to consume these inputs (Horwath, 2007). Temperature is also a highly influential factor for decomposition of SOM and soil organic C. The quality of SOM can be determined as the number of enzymatic steps required to release as carbon dioxide a carbon atom from an organic compound (Bosatta and Agren, 1999). The temperature-quality hypothesis stipulates that low-quality SOM involves higher activation energies and is therefore more temperature sensitive than high-quality SOM (Bosatta and Agren, 1999).

Biotic factors, such as microbial community abundance and structure are also influential determining SOM mineralisation (Strickland et al., 2009; Garcia-Pausas and Paterson, 2011). Organic matter quality (e.g. chemical recalcitrance and carbon to nitrogen (C:N) ratio) have been considered to be the exclusive drivers of SOM-mineralisation in soils; however, recent evidence
shows increasing importance of biotic factors (e.g. microbial community structure and activity) (Strickland et al., 2009; Blagodatsky et al., 2010) and their priming effects on SOM decomposition. The formation of humic substances (humification) is the result of oxidation and hydrolysis of OM, which creates materials with increased C/H and lower O content compared to the original animal, microbial and plant tissue (Horwath, 2007).

1.1.2.1 Soil Carbon

Soil organic carbon has been suggested as a globally key indicator of agricultural soil quality, and increases in SOC could contribute to maintain soil productivity and mitigate greenhouse gas emissions (Mueller et al., 2010). The benefits associated to high SOC levels in soil conservation management practices include ease of cultivation, root penetration, greater aggregate stability, reduced bulk density, and improved water holding capacity (Lal, 2002).

Carbon inputs to the soil are provided by living organisms at various stages of decomposition (Kutsch et al., 2009). The balance of SOC is governed mainly by two biological processes: the rate of OM transfer into the soil profile and the soil microbial activity (Kutsch et al., 2009). The decomposition and mineralization of SOM leads to the release of CO₂ as well as other traces gases (CH₄ and CO), some of these trace gases can be fixed by soil microbes and recommit to SOM (Kutsch et al., 2009). Carbon losses are also attributed to leaching of soluble organic C compounds, as well as particulate losses through erosion and runoff (Powlson et al., 2011). In agricultural soils, carbon inputs are mainly supplied by above-ground plant residues, plant roots, root exudates, manures and/or other organic by-products (Powlson et al., 2011). Crop residues returns increase in SOM and SOC, and contain on average 45% C on the dry weight basis (Larson et al., 1978; Lal, 1997). Soil carbon losses occur mainly from faster SOM breakdown from soil disturbances (e.g. tillage), lower plant litter input due to harvesting,
and displacement of carbon-rich surface soil by wind and water erosion (Kutsch et al., 2009). The rate of SOC sequestration also depends on tillage practices, quantity and quality of organic residues, types of crops and site-specific soil characteristics (Blanco-Canqui and Lal, 2004). Litter quality controls short-term dynamics of C decomposition and accumulation of SOC in the soil (Gentile et al., 2011). Over the long-term, the formation and stabilization of SOC is controlled by the quantity of litter input, and its interaction with the soil matrix, rather than by litter quality (Gentile et al., 2011). The ratio of carbon: nitrogen (C:N) of plant residues influences the rate of decomposition and thus the impact on soil structure; residues having higher C:N ratio persist for a long time and improve soil aggregation, whereas residues with lower C:N ratio have a reduced impact on soil structure improvement (Blanco-Canqui and Lal, 2004). Stable organic compounds, such as humic substances and phenolic compounds, decompose at slower rates than polysaccharides and amino acids, and can promote aggregation over longer periods (Martin, 1971).

1.1.2. Soil Nitrogen

Nitrogen and carbon serve as the two principal elements responsible for regulating soil biological activity, and hence nutrient cycling (Robertson and Groffman, 2007). Organic residues added to the soil surface, or incorporated into the soil, undergo decomposition by microbial biomass present in soil and/or residues. Nitrogen mineralization and immobilization are the most important processes related to N cycling (Fig. 1.1.). Mineralization is the conversion of organic N into exchangeable ammonium (NH₄⁺). If there is insufficient amounts of organic N available, soil microorganisms will consume inorganic ions in the soil to build protein for their bodies (Alexander, 1977). Immobilization is thus the conversion of inorganic N into organic N (Alexander, 1977). Both processes occur simultaneously in soil; the relative magnitude of each
process determines whether the overall effect is net N mineralization or net N immobilization. Research summarized by Whitmore (1996) clearly shows that the C:N ratio of residues is related to the amount of N released and that the break-even point between net N mineralization and N immobilization can be found between C:N ratios of 20 and 40. Nitrogen mineralization is higher under legume or grass-legume mixture primarily due to decomposability of the plant material which has a low C:N and lignin:N ratios, low(lignin+polyphenol):N ratio, and higher litter contribution compared with grasses (Fageria, 2007). In N-limited soils (e.g high C:N ratio), mineral N availability has influenced the decomposition of crop residues (Mary et al., 1996; Le Guillou et al., 2011).

1.1.3. Crop Residues and the Soil Environment

Amendments of organic residues to agricultural soils have been suggested as a means to improve soil quality and productivity (Fageria, 2007; Fageria, 2012). Crop residues are often described by their biochemical quality (decomposability) by studying the relative C to N contents (C/N ratio), concentration of structural carbohydrates, lignin and polyphenol concentrations (Probert et al., 1998; Fageria, 2012). Residue quality differs between crop species thereby affecting the cycling of C and N in plant-soil systems.

Erosion tends to preferentially remove clays and SOC because of their lighter densities (Jacinthe et al., 2002) and presents a serious threat to the long-term productivity of agricultural lands (Lu et al., 2000). In reduced and no-tillage systems, cover crops can provide large quantities of surface residues, which act as living mulches to reduce soil surface erosion. Many cover crops species also have extensive root systems (e.g rye (Secale cereale L.)) and result in superior erosion protection (Lu et al., 2000). Barley (Hordeum vulgare L.), oat and annual ryegrass are also known to benefit erosion control and N recovery (Lu et al., 2000). Tap-rooted
species such as forage radish and rapeseed are more efficient in reducing compaction compared to rye, which is composed of a fibrous root system (Chen and Weil, 2010).

In coarser texture soils, increasing water holding capacity is critical to improving soil quality and productivity (Lu et al., 2000). Long-term cover crop systems have been shown to improve water infiltration during the main crop growing season (McVay et al., 1989) as well as protecting the soil from the intensive rainfalls which destabilise surface particles and render them susceptible to erosion (Sainju et al., 1997). For growers, cover crop residues may enable them to access the fields faster after a rainfall (Lu et al., 2000).

Cover crops do present some limitations, since the benefits are often long-term, and a short-term investment for long-term gains is often a difficult concept to adopt. Growers leasing land may not plant cover crops, when the goal is to attain higher short-term economic returns (Lu et al., 2000). Similarly, cover crops are often not considered in areas with shorter growing seasons. The availability and the price of seed can also be considerable (Lu et al., 2000).

1.1.3.1. Corn Stover

Corn stover is composed primarily of unharvested leaves, stalk and cobs. Recent studies have evaluated the energy production of corn stover as a cellulose-based biofuel (direct combustion and microbial conversion of residue into ethanol) (Hess et al., 2009; Wan and Li, 2010). Karlen et al., (2011), using a Soil Management Assessment Framework (SMAF) developed by Andrews et al. (2004), evaluated six soil quality indices and showed that soils in continuous corn and crop rotation sites were functioning at an average of 93 and 83% of their inherent potential after removal of corn stover. Blanco-Canqui and Lal (2004) suggested that based on the need to maintain SOC and soil structural stability, only 25% of stover might be
available for removal. The impact of corn stover removal can be greater in sloping soils than flat soils (Blanco-Canqui and Lal, 2004). Soil organic carbon losses associated with corn stover harvest can be overcome with application of C amendments such as manure and compost (Fronning et al., 2008). Karlen et al., (2011) suggested that the sustainable harvest of corn stover must include the replacement of additional plant nutrients removed with the corn stover, the inclusion of annual or perennial cover crops, and crop rotation for enhanced benefits to succeeding crops.

1.1.3.2. Cover Crops and Crop Productivity

Apart from N mineralization from legume cover crops, the potential for either grasses and non-legume broadleaf cover crops to increase subsequent crop yields is unclear. In a 2-yr study, Ruffo et al. (2004) showed a significant correlation (r=0.64, p<0.0001) between rye N content and soil residual NO₃-N, and concluded that while rye has an ability to scavenge high amounts of soil residual N, it does not affect subsequent soybean grain yield. Maughan et al., (2009) concluded that corn grain yields under fall cover cropping systems (11.5 Mg ha⁻¹) was significantly higher than continuous corn (10.8 Mg ha⁻¹), and also resulted in improved soil quality, SOM dynamics, and crop yield. On a sandy loam soil, Morgan and Hanna (1962) found no increases in yield, N, P and K content of a succeeding sweet corn crop when rye (fertilized or not) was incorporated in the soil. On sandy soils, Andraski and Bundy (2005) reported that fall-planted cover crops such as oat, winter triticale (Triticosecale rimpauui), and cereal rye can provide significant yield benefits to the subsequent corn crop in some years. Kuo et al., (1997) showed that cereal rye and annual ryegrass were ineffective in increasing corn productivity in the short term, however they produced a greater quantity of biomass C, and resulted in a higher soil organic N than hairy vetch after long-term (9 yrs) fall cover cropping.
1.1.4. Methods of Study for Soil Carbon Dynamics

Fractionation procedures, based on density and/or size separation techniques, of organo-mineral complexes have proved useful in separating SOM pools to study stabilisation and destabilisation mechanisms, without altering any properties that might be relevant to their function in the ecosystem (Christensen, 2001; Haile-Mariam et al., 2008). With the advent of newer analytical instruments, such as Fourier transformed infrared spectroscopy (FT-IR), recent studies have attempted to identify and to follow organic constituents over time (Vasques et al., 2009). FT-IR technology offers the potential to provide new insight into the mechanisms involved in SOC dynamics and soil aggregation (Nduwamungu et al., 2009). Diffuse reflectance infrared Fourier transform (DRIFT) could represent a rapid and accurate method to follow fresh organic degradation directly in the soil matrix (Spaccini et al., 2001). In cover crop systems, Ding et al. (2006) reported organic carbon to be higher in soils under vetch/rye and rye management systems compared to no cover crop. Through spectral analysis, Ding et al. (2006) also reported that the structure and composition of humic acids were influenced by cover crop systems; rye cover crops yielding a higher aromatic contents that a mixture of rye and vetch. Spacinni et al., (2001) showed that decomposition rates derived from DRIFT results were similar to those obtained by isotopic techniques. Few studies have attempted to investigate the effect of specific cover crops on non-fractionated SOM (Spacinni et al., 2001).

Many investigations on SOM pool size and turnover times have made use of fractionation techniques and solid-state $^{13}$C (Baldock and Skjemstad, 2000) or radioactive $^{14}$C with NMR spectroscopy (Buyanosky et al., 1994). Baldock and Skjemstad (2000) described two phases of decomposition. The first phase involved a decrease in the residue particle size (2-20 μm) and a rapid degradation of the labile fraction (proteins, sugars, polysaccharides) by fauna and
microorganisms over a relatively rapid time period (weeks to months). The labile fraction’s C, N, P and S (about 5-10%) was likely assimilated by the fauna and microbes, and resulted in the release of CO₂, ammonium, phosphates and sulfates (Haider, 1992). In this phase, there was also an accumulation of recalcitrant compounds, such as lignin and alkyl structures. The second phase was described as slow degradation of ligneous compounds by filamentous aerobic fungi such as white-rot fungi (Kirk and Farrell, 1987). In contrast with heterotrophic microbes in the first phase, fungi do not derive energy or assimilate C from the lignin degradation, but benefit from an exposure of labile O-alkyl C (holocellulose) buried in the lignin structures (Haider, 1992). The decomposition of lignin results in a decrease in the amount of aromatic C, where the aromatic features have significantly decreased (100-200 ppm). In the final decomposition stage, the alkyl-C fraction has become the most prominent fraction (0-50 ppm), which Baldock and Skjemstad (2000) speculated was due to its highly recalcitrant nature.

### 1.1.5 Aggregate Stability

The effectiveness of added organic materials, in increasing the stability of soil aggregates, is determined by their amount, composition, and decomposability (Albiach et al., 2000, 2001). Soil aggregation dynamics are complex and result from the interaction of many factors including the environment, climate, soil management, plant influences, soil properties (mineral composition, texture, exchangeable ions, nutrient reserves, organic carbon, pedogenic processes, moisture availability) (Table 1.1), and microbial activity (Kay, 1998). One of the most important biological processes for soil aggregation is the synthesis of organic compounds (e.g. polysaccharides) by soil microbes, roots and hyphae. These compounds act as “glues” and increase soil aggregation (Tisdall and Oades 1982). Increased soil aggregation leads to improved
water infiltration, decreases in soil surface erosion and enhanced field drainage (Abiven et al., 2009).

Monnier (1965) first described a model in which different organic inputs decomposed and influenced aggregate stability over time, and identified three periods during which the major aggressive processes occurred (Fig.1.2). Monnier suggested that the short-term effects of OM on aggregate stability were due to the turnover of microbial products while the long-term effects were due to humified compounds.

Fresh crop residues stimulate microbial activity and induce water-stable aggregation (Angers and Chenu, 1998). Easily decomposable residues (e.g. containing more polysaccharides) have been shown to increase water stable aggregates (WSA) rapidly, whereas less decomposable residues have a gradual long-term effect on WSA (Abiven et al., 2009). Plant influences have also been attributed to root and hyphae enmeshment of soil particles and the release of organic compounds, including polysaccharides, which help bind particles together (Santos et al., 1997). Grasses such as cereal rye, have extensive fibrous root systems, which further increase particle enmeshment and SOM (Bronick and Lal, 2005). Recent studies have also shown glomalin, a highly persistent glycoproteinaceous substance produced by arbuscular mycorrhizal fungal, to be associated with aggregate stability (Wright and Upadhyaya 1998; Driver et al., 2005).

Labile organic carbon pools within macroaggregates are important agents of aggregation as the microbial community consumes particulate organic matter (POM) and releases organic compounds (polysaccharides), which serves as a bridge for primary particles (Bronick and Lal, 2005). Once microbes have consumed the POM, and the C:N ratio decreases, macroaggregates
become less stable, leading to the breakdown of macroaggregates and the release of more stable microaggregates (Beare et al., 1994).

Bacterial colonies form negatively charged polysaccharide capsules, which help bind soil particles (Tisdall and Oades, 1982). Significant increases in microbial biomass and enzyme activity has been found in dryland cropping systems with a history of fall cover crops, and are reflective of changes in SOM, nutrient cycling, and C sequestration in sandy soils (Acosta-Martínez et al., 2011). Wet-dry cycles help stabilize aggregates by rearranging and linking them closer to gluing agents (e.g. glomalin and polysaccharide capsules) (Santos et al., 1997).

Compound particles consisting of clay-polyvalent metal-OM are described as binding agents involved in aggregation (Edwards and Bremner, 1967). Research in soil structure mainly rely on a conceptual model of soil aggregation in which macroaggregate (> 250µm) form and burst into more stable microaggregates (20-250µm) (Tisdall and Oades, 1982). A concentric theory has also been suggested by Santos et al (1997), in which compound particles would concentrically build on the external surface of microaggregates and form macroaggregates. Cations form the bridge between clay and SOM. Cations such as Si$^{4+}$, Fe$^{3+}$, Al$^{3+}$ and Ca$^{2+}$ also stimulate the precipitation of compounds that act as bonding agents for primary compound particle (Bronick and Lal, 2005).

Aggregates are susceptible to disruption by clay swelling, tillage and rainfall impact (Bronick and Lal, 2005). As clay particles swell they separate from other particles and decrease aggregate stability. Wet-dry cycles can disrupt aggregation by swelling clays (Singer et al., 1992). In temperate climates, freeze-thaw cycles affect soil aggregation by influencing moisture
and temperature regimes. Soil moisture can be manipulated by adopting different management practices such as irrigation and cover cropping and mulching. (Dalal and Bridge, 1996).

Wet-sieving methods have traditionally been the standard method used to assess WSA (Le Bissonnais, 1996). Pojasok and Kay (1990) proposed a method for assessing structural stability of moist aggregates using a combination of wet sieving and turbidimetry. The method was sensitive to soil and cropping factors (Pojasok and Kay, 1990; Caron et al., 1992). Pretreatment of the soil involved slow wetting (-0.1kPa for 90 minutes) in order to reduce swelling and slaking of aggregates. Soils were exposed to two pre-dominant energy inputs, the imbibition of water and the mechanical shaking in test tubes. The abrasive forces on aggregates of the sieving process were minimized because the soil is gently washed onto the sieved after being shaken in test tubes, as opposed to the classical wet sieving methods that expose the soils to abrasive forces by shaking the sieves at a specific stroke length and sieving frequency (Yoder, 1935; Kemper and Rosenau, 1986). Energy considerations are of utmost importance in wet sieving methods, as aggregates are sensitive to these external forces (Kemper and Rosenau, 1986).
1.2. HYPOTHESES AND OBJECTIVES

This study involved four related investigations. The objectives were as follows:

I. Investigate the use of DRIFT spectroscopy as a method to assess decomposition of cover crop and corn stover residues over a 72-d incubation (i) by measuring spectral regions consistent with labile and recalcitrant C pools, and (ii) by comparing first-order decomposition models obtained from DRIFT to evolved CO$_2$.

II. Assess the short-term effect of cover crop and corn stover residue on C and N dynamics.

III. Assess the short-term effect of the removal of corn stover within a cover crop-corn rotation on soil structure after one year.

IV. Assess the short-term effect of the removal of corn stover within a cover crop-corn rotation on crop productivity after one year.

The hypotheses for these investigations are as follows:

I. DRIFT spectroscopy can be used to characterize short-term soil processes, such as SOM decomposition in a cover crop-corn stover system.

II. Cover crop residue will increase soil labile organic carbon pools and help offset the impacts of corn stover removal after one year.

III. Cover crops will increase plant available N in corn stover removal systems.

IV. Cover crops will maintain soil aggregate stability in corn stover removal systems.

V. Compared to a no-cover crop, 4 yr of annual cover cropping will increase subsequent crop yield in a corn stover removal systems.
Table 1.1. Soil (0-15 cm) characteristics of Brookston sandy loam the 2012 field site which was used in the 72 d incubation study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil classification</td>
<td>Typic Hapludafts</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>6.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Organic matter, g kg(^{-1})</td>
<td>38</td>
<td>0.1</td>
</tr>
<tr>
<td>Cation exchange capacity, cmol kg(^{-1})</td>
<td>9.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Electrical conductivity, dS m(^{-1})</td>
<td>0.37</td>
<td>0.009</td>
</tr>
<tr>
<td>Total carbon, g kg(^{-1})</td>
<td>20.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Total organic carbon, g kg(^{-1})</td>
<td>18.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Total nitrogen, g kg(^{-1})</td>
<td>1.7</td>
<td>0.008</td>
</tr>
<tr>
<td>Particle size distribution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sand %</td>
<td>75</td>
<td>0.2</td>
</tr>
<tr>
<td>Silt %</td>
<td>18</td>
<td>0.2</td>
</tr>
<tr>
<td>Clay %</td>
<td>7</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Data were LSMeans of three composite cores from each plot (N= 180). Field experiment consisted of 10 treatments in quadruplicates.
Figure 1.1. Representation of the agricultural nitrogen cycle (Troeh and Thompson, 1993).
Figure 1.2. Monnier’s conceptual model; the effect of different reference products on soil aggregate stability across time (Monnier, 1965). Zone A, B, C display dominant factors influencing soil aggregate stability over time.
CHAPTER 2: DIFFUSE REFLECTANCE INFRARED FOURIER TRANSFORM (DRIFT) SPECTROSCOPY AS A METHOD TO ASSESS COVER CROP AND CORN STOVER DECOMPOSITION

2.1. INTRODUCTION

Soil organic matter (SOM) is chemically complex and dynamic in nature, yet it is one of the most important soil quality indicators as it mediates many physical, chemical and biological soil processes (Lu et al., 2000; Kutch et al. 2009). Plant residues are often used to increase SOM and to provide important ecosystem functions by reducing soil erosion, improving soil physical, chemical and biological properties, increasing agronomic production and sequestering SOC (Blanco-Canqui and Lal, 2009a). Cover crops, in particular, can play an important role in determining soil quality by maintaining or increasing SOM (Dabney et al. 2001). The quality of different plant materials is often assessed through different indices, such as the C:N ratio, the concentration of structural carbohydrates (e.g. polysaccharide), lignin or polyphenols. Carbon (C) and nitrogen (N) are, however, the two principal elements regulating soil processes related to biological activity. Therefore, determining C and N cycling in relation to plant residue quality and quantity is imperative to describing soil processes. Plant residues with large C:N ratios stimulate microbial growth and N immobilization. The break-even point between net N immobilization and mineralization of residues has been assessed in medium to long-term experiments, and has been described at a C:N ratio of 40 (Vigil and Kissel, 1991). Increases in soil C and carbohydrate concentration have been shown to result primarily from the magnitude of C inputs, from six years of continuous double cropping of silage corn with a variety of fall annual cover crops (Kuo et al, 1997). The availability of N, on the other hand, influences mineralization-immobilization processes (Stanford and Smith, 1972). The presence and amount
of lignin or recalcitrant C in plant residues also reduce the rate of N mineralization (Quemada and Cabrera, 1995). The short-term effects of residue decomposition of organic C and N pools are most often described by first order kinetics.

Many studies that have assessed SOM pool size and turnover time have made use of isotopic techniques, where plant residues are labelled with either radioactive $^{14}$C (Buyanosky et al., 1994) or stable $^{13}$C (Rochette et al., 1999; Helfrich et al., 2006). Fractionation procedures based on density and/or size separation techniques have also proved useful in separating SOM pools (Christensen, 2001; Haile-Mariam et al., 2008). However, an accurate assessment of chemical or physical fractions may be difficult to attain through known fractionation procedures, particularly for active C pools as they are more susceptible to change over a short period of time (McLauchlan and Hobbie, 2004; Oyonarte et al., 2007). Analytical procedures are also often cost-intensive, time-consuming, and thus impractical for large sample sets (Cambardella and Elliot, 1992; Petisco et al., 2006; Reeves et al., 2006; Viscarra Rossel et al., 2006).

Diffuse reflectance infrared Fourier-Transform (DRIFT) spectroscopy offers the opportunity for in situ, non-destructive, highly reproducible, rapid, quantitative or semi-quantitative measure of organic compounds in the soil matrix (Capriel, 1997; Kaiser et al., 1997). Recent studies have suggested the use of DRIFT spectroscopy for examining changes in SOM composition over time by investigating band intensities resulting from bending and stretching vibrations of functional groups associated with organic compounds in the mid-infrared range from 4000 to 400 cm$^{-1}$ (Spaccini et al., 2001; Zaccheo et al., 2002). Quantitative measurements have successfully predicted different SOM size and/or density fractions using multivariate prediction models (Janik et al., 2007; Zimmermann et al 2007). Quantitative spectral analysis of SOM fractions typically requires large sample sets (>70 samples) for calibration and validation.
procedures (Nduwamungu et al. 2009). Semi-quantitative measurements have included the use of spectral peak heights (Niemeyer et al., 1992; Haberhauer et al., 1998; Ding et al., 2006) as well as spectral peak area (Tseng et al., 1996; Spaccini et al., 2001; Demyan et al., 2012). However, due to the variability in soil mineralogy and potential overlap of inorganic and organic functional groups in the mid-infrared range, peak selection is imperative for an accurate assessment of SOM composition in different soils (i.e., different types and/or textures) (Wander and Traina, 1996). The spectral region of 1050 to 1200 cm$^{-1}$, for example, is consistent with mineral interferences caused by Si-O of silicate impurities and clay minerals possibly in complex with humic acids (Filip and Bielek, 2002; Senesi et al., 2003). As such, an accurate assessment of SOM composition through spectroscopic methods has to take into consideration mineralogical interferences that might obscure or alter the extent of peaks in different soils (i.e., different types and/or textures) (Demyan et al., 2012). Semi-quantitative characterization of SOM through DRIFT spectroscopy should be compared to commonly used wet-chemistry extraction procedures, such as CO$_2$ evolved, microbial biomass C (MBC) and soil mineral nitrogen (SMN).

Our objectives were to; 1) investigate the use of DRIFT spectroscopy as a method to assess organic matter (OM) decomposition of cover crop and corn stover residues in an incubation time-course study by (i) measuring spectral regions consistent with labile and recalcitrant C pools over a 72 d incubation period, and by (ii) comparing first-order decomposition curves from DRIFT to cumulative evolved CO$_2$, and 2) Evaluate the effect of cover crops on C and N dynamics (polysaccharide decomposition, evolved C, SMN and MBC with and without the addition of corn stover.
2.2. MATERIALS AND METHODS

2.2.1. Experimental Design

A field experiment was established in 2012-2013 to determine the effects of cover crop systems on soil C and N dynamics at the University of Guelph Ridgetown Campus in southwestern Ontario (42°46’N, 81°96’W, elevation 200m). The cover crop trial was conducted on a Brookston sandy loam (Orthic Humic Gleysol) soil with 3.5 to 3.8% OM and pH of 6.3 to 7.0 (Table 1.1). Soil characteristics were determined from samples taken at a depth of 0-15 cm in spring 2012 at Agri-Food Laboratory Ltd. (Guelph, Ontario, Canada), according to Carter and Gregorich (2008).

The experimental design consisted of a randomized split-plot with four replications, where cover crops treatments planted in 2007-2010 served as the main plot factor. Treatments included a control with no cover, oat, cereal rye (rye), oilseed radish (Raphanus sativus L. var. oleoferus Metzg. Stokes) (OSR), and a mixture of oilseed radish and cereal rye (OSR&rye) planted at 81, 67, 16, and 9+34 kg seed ha⁻¹, respectively. Grain corn was grown in 2011, with the retention or removal of corn stover at harvest as the split-plot factor. After mechanical grain harvest, corn stalks were chopped and either retained on the soil surface or removed. Corn residues were incorporated the following spring by disking in late May 2012, 2013. The trial was planted with acorn squash in May 2012, 2013 (Cucurbita pepo var. pepo cv. Autumn Delight) at 10759 seeds ha⁻¹.

Soil characteristics were determined for non-amended soils in each split plot (Carter and Gregorich, 2008). Particle size distribution was determined by the hydrometer method (Day, 1965). Soil pH was determined using a 1:1 H₂O: soil ratio (v v⁻¹) (McLean, 1982) and electrical
conductivity (EC$_{1:1}$) was measured with a Hanna DiST®3 portable EC Tester (Hanna Instruments, Smithfield, RI, USA). Soil C was determined by dry combustion using a LECO carbon analyzer (Leco Corporation, St. Joseph, MI). Soil inorganic C was measured using LECO carbon analyzer on samples that had been placed in a muffle furnace at 420°C for a 24-hour period. Soil organic C was the difference between soil C and inorganic C.

### 2.2.2. Soil Sampling and Incubation

In September 2012, at squash harvest, sixty 3.5-cm diameter soil cores were sampled at a depth of 0-15 cm from each split-plot. Soil samples were homogenized in the field, sealed into plastic bags and stored at 4°C until processed. Soil sampling was conducted after a rain event to have soil moisture as close to field capacity as possible (Villamil et al., 2006). Gravimetric water content at sampling was 65% to 80% (13-24 g g$^{-1}$) of field capacity. Composite soil samples were sieved through 4.75 mm to remove roots and rocks, 3500 g weighed based on gravimetric water content and stored at 4°C before plant biomass amendment.

Plant biomass amendments originated from cover crop and corn stover biomass harvested in 2007-2010, and consisted of dried above-ground biomass, oven-dried at 60°C, ground in a Wiley mill through a 2-mm diameter opening mesh screen, and stored in plastic vials. Field-moist soils were weighed based on 250 g of calculated (gravimetric moisture content) dry weight, placed in 500 mL jars and adjusted to 60% water holding capacity with distilled water. Sieved soil from each split-plot was mixed with ground plant biomass (Table 2.1): i) cover crop ground biomass quantity was based on mean above-ground biomass yields from 2009, 2010 and 2011 from the same cover crop trial, ii) corn stover biomass quantity was based on mean above-ground biomass yield from typical Ontario production. Microcosms were incubated at 22±3°C in the dark for 72 d. Soil moisture was maintained constant throughout the incubation period by
weekly weighing and addition of distilled-water when required. To further reduce soil moisture loss, a humidifier was installed in the room and incubation units were covered with perforated aluminum paper. Destructive sampling was conducted at 0.5, 2, 4, 7, 10, 15, 22, 29, 36, 43, 50, 64, and 72 d for the following measurements: SMN, microbial biomass C and DRIFT analysis.

2.2.3. DRIFT Spectroscopy

A 5 g subsample of soil was air-dried (40°C) for 7 d, freeze-dried for 12 h, ball milled for 2 min (75 rpm) and homogenized with ground KBr (2.5/100, w w⁻¹) using a pestle and mortar (Wander and Traina, 1996), and stored in a P₂O₅ desiccator prior to sample measurement.

Soil DRIFT spectral data were recorded with a Varian Cary 660 FT-IR Spectroscopy (Agilent Inc., Santa Clara, CA) equipped with a EasiDiff Diffuse Reflectance accessory, where 100 co-added scans per spectra were collected at a resolution of 4 cm⁻¹. The instrument operated under the following settings: a nominal resolution of 2 cm⁻¹ and a mid-infrared spectrum from 4000 to 400 cm⁻¹. The spectrometer was equipped with a liquid N-cooled mercury cadmium telluride (MLT) detector and a KBr beamsplitter. Absorption spectra were converted to a Kubelka-Munk (KM) function using Varian Resolutions Pro™ 5.0 software (Agilent Inc., Santa Clara, CA), which determined sample absorptance (a), concentration (c) and scattering coefficient (s). The ratio of diffuse reflectance from the KBr-soil sample to diffuse reflectance from a non-absorbing powder was calculated in Eq.1. (Kubelka and Munk, 1931).

\[
KM = \left(\frac{2.303ac}{s}\right)
\]

[1]

Background KBr spectra were performed with ground KBr, scanned under the same environmental conditions as the soil-KBr mixtures, and subtracted from each soil-KBr spectra.
Baseline correction, second derivative, curve fitting and peak integration was performed using Varian Resolutions Pro™ 5.0 software. An average spectral curve was calculated and smoothed across the spectral bands (wavelengths) using a second derivative smoothing algorithm (Savitzky and Golay, 1964). Spectral peak resolution was further enhanced through deconvolution of smoothed spectral curves.

Polysaccharide-C (950-1050 cm\(^{-1}\)) (Tseng et al., 1996) summed peak area was used to construct a decomposition curve of crop residues in incubated soils by plotting summed peak areas versus incubation days. The spectral peaks ascribed to the polysaccharide region were 1159, 1106, 1056, 1036 cm\(^{-1}\) (Tseng et al. 1996) (Table 2.2). Peak region of 3010-2800 for aliphatic C-H stretching, 1660-1580 for aromatic C=C and/or -COO\(^{-}\) stretching, and 1546-1520 for aromatic C=C stretching were collected, with hypothesised stability of labile, and intermediate respectively, for bulk soil (Demyan et al., 2012). Aliphatic-C (2930 cm\(^{-1}\)) and aromatic-C (1620, 1530 and 1159 cm\(^{-1}\)) were recorded and measurement expressed as relative peak area (corrected area from each peak divided by the sum of the area of the four peaks and multiplied by 100).

2.2.4. Microbial Biomass C

Soil MBC was determined by chloroform-extraction method (Vance et al., 1987), where one 30 g soil sample was fumigated (ethanol-free CHCl\(_3\) for 24h), while another 30 g soil sample was not fumigated. Total organic C was extracted from both samples with 0.5 M K\(_2\)SO\(_4\), and analyzed using a Shimadzu TOC-5000 carbon analyzer (Shimadzu Scientific Instruments, Columbia, MD). Microbial biomass C was calculated as the difference between total organic C from fumigated (C\(_F\)) and unfumigated (C\(_UF\)) samples with k\(_EC\) set to 0.45 (Joergensen, 1996) according to Eq. 2.
2.2.5. Soil Mineral N

At each sampling time, 15 g of incubated soil was frozen until SMN analysis according to Maynard et al., (2008). Frozen soil samples were thawed overnight. A 5 g subsample was oven-dried for gravimetric moisture content. Another 5 g of soil was extracted with 25 ml of 2 M KCl, shaken for 30 minutes, filtered and analyzed colorimetrically on an Autoanalyzer (SEAL Analytical Inc., Mequon, WI, USA) equipped with a high resolution digital colorimeter to quantify NH$_4^+$-N (method G-102) and NO$_3^-$-N (method G-200) using the cadmium reduction and phenate methods, respectively. Ammonium and nitrate in KCl extracts were not analysed within the first 24 hr of extraction; KCl extracts were frozen until further analysis (Keeney and Nelson, 1982). Nitrogen concentration was expressed as mg N kg$^{-1}$ soil based on bulk density.

2.2.6. Carbon Mineralization

Evolved C was measured by closed chamber incubation with the alkali CO$_2$ traps method (Carter and Gregorich, 2008). Experimental design consisted of a randomized complete block with four replicates of cover crop-stover biomass combinations. Soil (100 g dry weight basis), was placed in 250 mL volume mesh (1 mm$^2$) screen aluminium containers, and sealed inside a 2 L glass jar (Congreves et al., 2013). A plastic vial containing 20 mL of 1 M NaOH was added to each jar to trap evolved CO$_2$. Soil moisture inside the jar was maintained with a 20 mL vial of distilled water. Background CO$_2$ levels were quantified with two 2 L jars containing only NaOH and water vials. Microcosms were incubated in the dark at 22°C±3°C, and non-destructive sampling occurred on 0.5, 2, 4, 7, 10, 15, 22, 29, 36, 43, 50, 64, and 72 d. Carbon evolution was determined by adding 2 mL of 1 M BaCl$_2$ to C traps and back-titrating NaOH with 0.5 M HCl to a phenolphthalein endpoint. At each sampling day, NaOH traps were replaced with fresh NaOH.
and the jars opened for 30 to 60 min before sealing. Carbon mineralization was expressed as a cumulative value in mg C kg\(^{-1}\) soil, as well as a rate of mg C kg\(^{-1}\) soil d\(^{-1}\). Net carbon mineralization was estimated from the difference in evolved CO\(_2\) in amended with crop residue and non-amended (no cover, no corn stover) treatment.

2.2.7. Statistical Analysis

Soil mineral nitrogen and MBC values for the ten cropping systems, within all sampling times, were analyzed by analysis of variance (ANOVA) with a MIXED procedure from SAS (SAS Institute, Inc., version 9.3, Cary, NC, USA). ANOVA assumptions met were: i) model effects were additive, ii) normality was assessed using Shapiro-Wilk test, iii) homogeneity was tested by plotting residuals of predicted by fixed effects (Bowley, 2008), iv) errors were random, independent, with a mean of zero. An outlier test was performed using Lund’s table (Bowley, 2008) of critical values, and comparing to studentized residuals; data point having a higher absolute studentized residuals than Lund’s critical value, were removed. Significance was determined with a type 1 error rate of 0.05 probability.

NLIN procedure in SAS was used to determine a non-linear regression for evolved CO\(_2\). Peak C mineralization was at day 0.5, thus a first order exponential model \(y = A(1-\exp^{-kt})\), where \(y\) = C mineralized by time t, \(A\) = amount of mineralizable C at day 0.5, \(k\) = rate constant, and \(t\) = incubation day. As well, PROC NLIN was used to determine a non-linear regression for polysaccharide-C decomposition curve \(y = C_1 + C_0(\exp^{-kt})\), where \(y\) = polysaccharide spectral peak area at time t, \(C_1\) = summed spectral peak area of rapidly decomposable polysaccharide-C pool, \(C_0\) = summed peak area at 0.5 d of total C, \(k\) = rate constant, and \(t\) = incubation day (Jones, 1984). Significance was determined with a type 1 error rate of 0.05.
2.3. RESULTS AND DISCUSSION

2.3.1. Polysaccharides

All soil samples amended with crop residues displayed sharp and intense bands in the 1050 to 1170 cm\(^{-1}\) peak region, which indicated the presence of polysaccharides (Inbar et al., 1989; Tseng et al. 1996; Calderón et al. 2013) (Table 2.2). Polysaccharide content in the incubated soil was mainly attributed to cellulose and residual hemicellulose, as they are the main inputs to the soil system (Inbar et al, 1989). Although field soil inherently contained polysaccharide or polysaccharide-like substances, previous research by Cheshire and Mundie (1981) has suggested that plant-derived polysaccharides are preferentially degraded compared to indigenous soil polysaccharides or organic C. Total polysaccharide content in soil does not differentiate between active and inactive carbohydrate binding agents. Robertson et al. (1991) suggested that the heavy fraction of carbohydrate, which is composed of microbial extracellular polysaccharides, can increase in response to C inputs from cover crops. In addition to spectral peaks associated with polysaccharide substances, other peaks may also be responsive to crop residue amendments. Calderón et al. (2013) reported mid-Infrared incremental response of spectral bands at 3100 to 3500, 2930 to 2870, 1377 to 1310, and 1220 to 1070 cm\(^{-1}\) to incremental additions of cellulose. The polysaccharide bands studied (Table 2.2) were, however, within the spectral region (900 and 1200 cm\(^{-1}\)) where inversion is caused by specular reflection of silica in soils. Spectral bands were, thus, likely to be affected by mineral interferences caused by Si-O of silicate impurities, and clay minerals complexed with humic acids (Filip and Bielek, 2002; Senesi et al. 2003). Given that mineral interferences can occur, the proposed polysaccharide spectral region may not be suited for the analysis of different soils (i.e., different types and/or textures) without spectral subtraction.
Spectral peaks in the 1223-975 cm\(^{-1}\) region were used to derive polysaccharide decay curves for crop residues containing different C inputs in a 72 d incubation (Fig. 2.1; Fig. 2.2; Fig. 2.3). Spectral peak area was fitted to a first order decay model commonly used for studying carbon dynamics \(y = C_1 + C_0(1-e^{-kt})\) (Jones, 1984) (Pseudo R\(^2\) ranging from 0.81 to 0.96), where polysaccharide peak area decreased from 2 to 72 d (Fig. 2.1; Fig. 2.3). A second derivative and curve fitting technique was used to resolve overlapped spectra (Painter et al., 1985). After normalization of spectral curves, the percentage change of the polysaccharide peak area was 60.7% from day 2 to 15, and 13.1% from day 15 to 72 respectively (Fig. 2.1; Fig. 2.3). The reduction in polysaccharide peak area over time was attributed to microbial decomposition of carbohydrates, where heterotrophic microorganisms cleaved C-O linkage in polysaccharides. The rapid decay of plant-derived polysaccharides support previous research which suggested that non-humic organic molecules, such as polysaccharides released directly from cells of fresh plant residues constitute an easily decomposable C fraction (Kuo et al. 1997). Although spectroscopic studies are limited on crop residues and polysaccharide decay, these decreasing trends were consistent with Tseng et al. (1996), who measured a 50% decrease in polysaccharide peak height in a 325 h bench-scale solid substrate composting study where crop residues were used as a feed material. Calderón et al. (2011) performed mid-infrared spectral interpretation of fractionated fresh and incubated soil to determine changes in SOM over time, and found that the light fraction showed higher C losses compared to other soil fractions, where light fraction C decreased 65% on average during an 800 d incubation.

The labile polysaccharide pool (C\(_1\)) was small (ranging from 22.02 to 43.60±2.202%) relative to the potentially decomposable polysaccharide pool (C\(_0\)) in all samples (Table 2.3). The potentially decomposable C pool (C\(_0\)) did not show a significant interaction between cover crop
and corn stover (Table 2.3). The cover crop × corn stover interaction was significant (P=0.0007) for the labile polysaccharide pool, with NC+S, NC-S and OSR&rye having the highest polysaccharide peak area, and OSR+S, OSR-S and oat-S having the lowest polysaccharide peak area. These results suggest that the labile polysaccharide pool was highest in cover crop-corn stover treatment combinations with small C inputs, indicating that nitrogen may have been a limiting factor for carbon mineralization, and may be due to the fact that crop residues were added in small amounts and contained relatively similar C:N ratio. Previous studies have shown that nitrogen availability strongly controlled microbial extracellular polysaccharides in soils amended to cover crop residues; Roberson et al. (1995) found that wooly pod vetch (*Vicia dasycarpa* L.) performed better than a moderate N fertilization in stimulating microbial extracellular polysaccharides production and improving soil structure. N concentrations in these non-legume cover crops were likely to have resulted from residual fertilizer N after summer crop harvest. Although plant biomass amounts added to the incubations were calculated from aboveground plant biomass, studies have shown that roots can contribute as much as 40% of the above ground cover crop biomass (Kuo et al. 1997). The use of non-leguminous cover crops over the short-term is not likely to affect SMN concentrations.

### 2.3.2. Carbon Mineralization

First order decay models $y = A(e^{-kt})$ were fitted (Pseudo $R^2$ ranging from 0.68 to 0.86) to cumulative evolved C (Table 2.4). Peak C mineralization rate was observed at day 2 for all cover crop-corn stover treatment combinations. Rapid C mineralization was attributed to adequate soil conditions (moisture and temperature), thus conducive to high levels of microbial activity early in the incubation. The potentially mineralizable C pool (A) did not show a significant interaction between corn stover and cover crop amendments (P=0.0807), suggesting that there was no
difference in the impact of stover removal between the cover crop treatments. The main effects of cover crop and corn stover were, however, significant (P=<0.0001 for both). No cover treatments were significantly different than cover crop amendments, with a potentially mineralizable C pool of 1.2 mg C g⁻¹, whereas cover crop treatments showed no differences amongst each other (17.1 mg C g⁻¹ higher than NC). Treatments receiving a corn stover amendment showed a 54.1% higher potentially mineralizable C pool than those without corn stover (20.3 and 9.3 mg C g⁻¹, respectively). With the exception of the NC treatments, field rates were similar for cover crop-corn stover treatment combinations, with C loading rates of 0.83 to 2.04 g C kg⁻¹ (Table 2.2).

2.3.3. Polysaccharide Decomposition and C Mineralization Rate

The decay rate of crop residues is mainly affected by the availability of C in the substrate, the chemical composition of the carbon substrate, as well as soil moisture and temperature (McGill et al. 1981). The decomposition of SOM is dependent on the size of the available C and N pools as they, in turn, affect the size of the microbial biomass pool (Reinertsen et al., 1984). Lignin content of cover crop residues is another important consideration as it is considered to be involved in protecting plant carbohydrates from microbial degradation (Herman et al., 1977). Crop residues in this experiment were unlikely to contain high lignin content because they were harvested during the vegetative stage. Crop residues were also ground to the same size, thus reducing the impact of particle size on decomposition rate. Soil texture also plays an important role in SOM decomposition, as clay is an important soil constituent for carbohydrate retention; low clay content may increase the rate of decomposition compared to soil with high clay content (Gupta, 1982).
A positive correlation (r=0.53, P=0.0006) was observed between the labile polysaccharide pool and the polysaccharide decomposition rate, further emphasizing that microbial activity was dependent on the size of the readily decomposable C pool. Polysaccharide decay rates were highest in the no cover and lowest in OSR treatments (Table 2.3). These results were also consistent with CO₂ evolution profile (Table 2.4), which also showed highest decay rate with NC and lowest with OSR treatments. The interaction between cover crop and corn stover was significant for polysaccharide decomposition (P=0.0016), signifying that the cover crop treatments produced different polysaccharide decomposition rate with and without the amendment of corn stover, likely a reflection of the chemical composition of the plant material as well as the availability of C in the substrate. A significant interaction was also observed for carbon evolved (P=0.0012). The rate constant (k) was significant for the cover crop and corn stover interaction (P=0.0012). With the exception of NC+S, NC-S and oat+S, cover crop-corn stover treatment combinations were not significantly different between each other (Table 2.4). Indeed, NC+S, NC-S and oat+S showed the higher mineralization rate compared to the other treatments. These treatments received the lowest amount of C inputs, and as such, heterotrophic microorganisms were likely less limiting on soil nitrogen sources. Chemical half-life of evolved C were lowest in NC+S, NC-S and oat+S and highest for OSR&rye+S, OSR&rye-S, oat-S and rye+S (Table 2.3). These results suggest that oat and mixture of oilseed radish + cereal rye contributed the least to C mineralization when considering similar C availability and C:N ratio between different crop residues. These cover crop-corn stover treatment combinations reduced C mineralization compared to NC, thereby promoting soil carbon sequestration. This is likely a short term effect, as easily mineralizable C will mineralize if N is not limiting. Although treatment differences were similar, the correlation between C mineralization rate and
polysaccharide decomposition rate was not significant (Table 2.5). In addition, the correlation between chemical half-life for evolved C and polysaccharide-C band half-life was not significant (Table 2.5). Similar mineralization rate among cover crop-corn stover treatment combinations was attributed to the relatively similar C:N ratio (ranging from 16.16 to 35.19) of the cover crop amendments as well as the similar biomass amounts (ranging from 0.74 to 2.04 g C kg$^{-1}$) added for each. Carbon mineralization results for the different crop residues and C input were consistent with polysaccharide decomposition curves mentioned previously as treatment differences from first-order model parameters were similar.

Although the literature is limited with regard to SOM decomposition studied through DRIFT, these results follow previous studies using wet chemistry techniques that have showed higher decay rate of plant material with high availability of C in the substrate, and the chemical composition (low C:N ratio) of the plant material (Honeycutt et al., 1993; Kuo et al., 1997). Kuo et al (1997) reported low rate constants (average of 0.0169), and by consequence a high half-life (average 45.25 d) for carbohydrates in soil amended with rye, ryegrass, vetch and canola aboveground biomass.

2.3.4. Aliphatic and Aromatic

The effects of cover crop, corn stover and cover crop × corn stover, incubation time × cover crop, incubation time × corn stover and incubation time × cover crop × corn stover were not significant (P≥0.0608) for relative peak areas of 2930, 1620, 1530 and 1159 cm$^{-1}$ (Table 2.6). Incubation time (d) was significant for all bands studied; aromatic-C peak 1620 cm$^{-1}$ contributed the most to relative peak area, followed by aliphatic-C peak 2930 cm$^{-1}$ and aromatic-C peak 1530 cm$^{-1}$. The peak at 1159 cm$^{-1}$, which was assigned to C-O bonds of both polyalcoholic and ether functional groups (Spaccini et al. 2001) was not discernable as a distinct peak in all crop residue
treatments, and therefore not used to calculate total relative peak area. Demyan et al. (2012) found the relative contribution of the 1159 cm$^{-1}$ peak to be higher for the control than for the treatment amended with farmyard manure and mineral fertilizer; thereby indicating that the band may not be indicative of organic compounds of greater lability.

The interaction of incubation time × cover crop was significant (P=0.0153) for the relative peak area at 2930 cm$^{-1}$; differences were only found between no cover and OSR at day 0.5, where no cover had 2.29±0.00190% higher aliphatic-C peak area compared to OSR (Table 2.6). This was largely attributed to a greater input mass compared to the other cover crop treatments. Relative peak area was highest at day 2 (26.1±0.001%), levelled at day 7 (3.1±0.001%), and increased slightly at day 43 (4.0±0.001%). These results are consistent with previous studies that have attributed spectral peak area at 2930 cm$^{-1}$ to organic components of greater lability. Demyan et al. (2012) reported a positive correlation between aliphatic stretching peak at 2930 cm$^{-1}$ and hot-water extractable C. Spaccini et al. (2001) found that aliphatic bands corresponding to the region of 2920 and 2860 cm$^{-1}$ can be used to study mineralization rates of corn residues in different soil particle fractions. Given that the 2930 cm$^{-1}$ band is located near the large O-H bands, spectral inferences due to water adsorption were reduced by freeze drying the samples and conducting a baseline correction on the resulting spectra.

The peak at 1530 cm$^{-1}$ showed a higher relative peak area at day 2 (2.16±0.035%), levelled at day 7 (0.79±0.035%), and increased slightly at day 43 (0.99±0.035%) (Table 2.6). In contrast, the peak at 1620 cm$^{-1}$ showed a lower relative peak area at day 2 (71.66±0.005%) and day 4 (75.50±0.005%), levelled at day 7 (95.99±0.005%), and decreased slightly at day 43 (94.89±0.005%). Peak area for 1530 cm$^{-1}$ was the smallest out of the three peaks studied, thus contributing less to total relative peak area. Similar to the peak at 2930 cm$^{-1}$, significant
differences were measured at day 2, 4 and 43. However, given that peak area was small compared to the other peaks studied, the evidence for attributing SOM quality characteristics to the 1530 cm\(^{-1}\) peak was less clear. Previous research has shown a negative correlation between peak area at 1530 cm\(^{-1}\) and SOM fractions, thus attributing it to more stable components in soils (Demyan et al., 2012). Relative peak area for 1620 cm\(^{-1}\) was the highest out of the three peaks studied, thus contributing the most to total relative peak area. Fluctuations in relative peak area over time were attributed to the aliphatic peak at 2930 cm\(^{-1}\) and less to 1620 cm\(^{-1}\). As such, the peak at 1620 cm\(^{-1}\) was more indicative of a recalcitrant C pool. These results follow previous research that has shown a negative correlation between peak area at 1620 cm\(^{-1}\) and SOM fractions, thus attributing it to more stable components in soils (Demyan et al., 2012).

### 2.3.5. N Mineralization

Soil mineral N levels were significant for the cover crop x corn stover interaction (P=0.0010) (Table 2.7). The addition of corn stover increased nitrogen immobilization within the cover crop treatments. The addition of corn stover, C:N ratios increased to a range of 23 to 35 (Table 2.2). Results indicated net N immobilization in corn stover amended plots. However, immobilisation levels were low < 2.5 mg N kg\(^{-1}\), and crop requirements are relatively high, hence it is likely that N recommendations will not be affected by corn stover amended in these cropping systems. These results follow expected trends where cover crop-corn stover treatment combinations having the lowest C:N ratios showed higher nitrogen mineralization, with the exception of cereal rye. OSR-S having the highest N mineralization (4.75±0.029 mg N kg\(^{-1}\)) and no cover+S (2.11±0.0292 mg N kg\(^{-1}\)) having the lowest N mineralization. The corn stover × incubation days interaction was also significant (P=0.0002) (Fig. 2.4.). Soil organic N concentration can affect the soil N mineralization potential (Stanford and Smith, 1972); however,
in this study, residual nitrogen concentrations were unlikely to be different between subplots following squash harvest as they received the same amount of inorganic N at the start of growing season.

2.3.6. Microbial Biomass C

The interaction of sampling time × cover crop × corn stover was not significant (P=0.0692), indicating that different crop residues did not display difference in SMC over time (Fig. 2.5.). Given that the size of the available C and N pools govern decomposition rates (Reinertsen et al., 1984), these results contrasted with polysaccharide decomposition and carbon mineralization rates, which showed treatment differences between the various crop residues amended. Peak microbial activity was also not observed in the first week of incubation (Fig. 2.5.), which contrast with previous study by Wyland et al. (1996) which observed a rise and fall in MBN during the first week following cover crop incorporation. Soil MBC was significantly different among sampling dates (P=0.0063) and was 30.88% higher at day 36 than at initial peak of day 2 (Fig. 2.5.). The MBC in whole-soil was positively correlated with total cover crop C content (r = 0.55, P <0.05, n = 9). The positive correlation between cover crop C content and MBC suggested that increase C from plant residue increased readily decomposable C pool. Although small changes in soil C inputs have been shown to enhance active SOM pools such as MBC, The addition of organic C inputs from crop residues did not increase MBC among cover crop-corn stover combinations. Soil moisture and temperature were maintained constant throughout the incubation period; microbial activity is more likely to have been influenced by the amount, the composition and the soil microbial community composition (Meentemeyer, 1978; Melillo et al., 1982; Parton et al., 2007).
Management practices that are influential on the size of the microbial biomass C pool include: the use of organic amendments, cropping system, fertilization and crop residue-management practices (Bolton et al., 1985). Crop residues, in particular, provide organic C input and help replenish the readily decomposable the C pool, which serves as a food source for the soil microbial community (Bosatta and Agren, 1999). Microbial biomass may also be more influenced by organic inputs than by changes in SOM, as microbial biomass is positively affected by the size of the soluble C pool (Ocio and Brookes, 1990; Reinertsen et al. 1984).

In this study, cover crop-corn stover biomass inputs were relatively low (ranging from 3.10 to 7.15 Mg ha\(^{-1}\)), thus organic carbon inputs were low (ranging from 0.74 to 2.04 g C kg\(^{-1}\)) (Table 2.2). Previous research have reported that cover crop C inputs increased MBC under conventional tillage (Sainju et al. 2002; Acosta-Martinez et al. 2011). However, cover crop C content was much higher, suggesting that higher availability soil organic C is needed to affect MBC. Salinas-Garcia et al., (2001) observed MBC concentrations higher near the soil surface of plots mulched with 33, 66, and 100% of stover compared with unmulched control. Roldán et al., (2003) reported that plots with ≤33% of stover cover had lower (322 mg C kg\(^{-1}\)) MBC than those with 66% (426 mg C kg\(^{-1}\)) and 100% (654 mg C kg\(^{-1}\)) of cover.

2.4. SUMMARY AND CONCLUSIONS

DRIFT spectroscopy used successfully to semi-quantitatively assess SOM decomposition from cover crops and corn stover amendments, in a whole-soil, by measuring polysaccharide-C bands regularly over the length of an incubation study. The addition of crop residues in low amounts (but at quantities similar to typical field biomass production) and with similar C:N ratios resulted in lower C mineralization and polysaccharide decomposition in cover crop-corn stover treatments compared to the no cover. Results indicate that cover crop-corn stover
treatment combinations, such as OSR and OSR&rye, having a higher C content and lower C:N ratio than the other cover crop treatments showed the highest C mineralization despite low levels of residual N in the soil. Therefore, organic inputs originating from cover crop and corn stover residues were likely to help maintain soil organic carbon pools in the short-term. The retention of corn stover with these cover crops will likely immobilize soil N, thus reduce plant available N. However, immobilisation levels were low, and crop N requirements would be relatively high, therefore the retention of corn stover was unlikely to affect N recommendations in vegetable and grain production. Accordingly, research is required to assess, through field studies, the impact the long-term cover cropping in corn stover removal systems and its influence on labile and recalcitrant C pools as well as N dynamics.
Table 2.1. Chemical composition of aboveground plant biomass amended to soil in a 72 d incubation study.

<table>
<thead>
<tr>
<th>Cover crop†</th>
<th>Corn stover</th>
<th>Biomass composition</th>
<th>Amendment rate†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Organic C % Total N C:N</td>
<td>Total biomass Mg ha⁻¹</td>
</tr>
<tr>
<td>No cover</td>
<td>Amended</td>
<td>41.4 0.73 57.1</td>
<td>3.55</td>
</tr>
<tr>
<td>Oat</td>
<td>None</td>
<td>41.1 1.63 25.3</td>
<td>3.10</td>
</tr>
<tr>
<td>Oat</td>
<td>Amended</td>
<td>41.5 1.18 35.2</td>
<td>6.65</td>
</tr>
<tr>
<td>Oilseed radish &amp; cereal rye</td>
<td>None</td>
<td>60.6 2.34 24.9</td>
<td>3.50</td>
</tr>
<tr>
<td>Oilseed radish &amp; cereal rye</td>
<td>Amended</td>
<td>41.1 1.25 32.9</td>
<td>7.05</td>
</tr>
<tr>
<td>Oilseed radish</td>
<td>None</td>
<td>34.9 1.67 21.0</td>
<td>3.60</td>
</tr>
<tr>
<td>Oilseed radish</td>
<td>Amended</td>
<td>40.2 1.79 22.5</td>
<td>7.15</td>
</tr>
<tr>
<td>Cereal rye</td>
<td>None</td>
<td>41.9 2.59 16.2</td>
<td>3.35</td>
</tr>
<tr>
<td>Cereal rye</td>
<td>Amended</td>
<td>41.2 1.83 22.5</td>
<td>6.90</td>
</tr>
</tbody>
</table>

†Amendment rates were based on mean cover crop above-ground biomass harvested late October to early November 2009-2011 and mean corn stover removal in 2011 at the field trial site at Ridgetown, Ontario.
Table 2.2. Diffuse Reflectance Infrared Fourier Transform (DRIFT) peak positions, widths and proposed assignments.

<table>
<thead>
<tr>
<th>Peak position (cm⁻¹)</th>
<th>Peak width (cm⁻¹)</th>
<th>Proposed molecular assignment</th>
<th>Relative hypothesized stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>2930</td>
<td>210</td>
<td>C–H-stretch of aliphatic†</td>
<td>Labile</td>
</tr>
<tr>
<td>1620</td>
<td>80</td>
<td>C=C and/or COO⁻ stretch of aromatic†</td>
<td>Recalcitrant</td>
</tr>
<tr>
<td>1530</td>
<td>26</td>
<td>C=C-stretch of aromatic†</td>
<td>Intermediate</td>
</tr>
<tr>
<td>1159</td>
<td>22</td>
<td>C–O bonds of poly-alcoholic and ether‡</td>
<td>Unknown, likely non-labile</td>
</tr>
<tr>
<td>1106</td>
<td>67</td>
<td>C–O bonds of polysaccharides§</td>
<td>Labile</td>
</tr>
<tr>
<td>1056</td>
<td>24</td>
<td>C–O bonds of polysaccharides§</td>
<td>Labile</td>
</tr>
<tr>
<td>1036</td>
<td>35</td>
<td>C–O bonds of polysaccharides§</td>
<td>Labile</td>
</tr>
</tbody>
</table>

†Baes and Bloom (1989)
‡Senesi et al. (2003)
§Tseng et al. (1996)
Table 2.3. First order decay model \(y = C_1 + C_0(e^{-kt})\)‡ for polysaccharide-C spectral peak area region (950 to 1170 cm\(^{-1}\)) in soil amended with and without cover crops and corn stover biomass.

<table>
<thead>
<tr>
<th>Cover crop</th>
<th>Corn stover</th>
<th>(C_1)</th>
<th>(C_0)</th>
<th>(k)</th>
<th>(t_{1/2})</th>
<th>Pseudo (R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>---- KM† ----</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No cover</td>
<td>None</td>
<td>758 abc 2760</td>
<td>0.3 a</td>
<td>2.5 cde</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>No cover</td>
<td>Amended</td>
<td>866 ab 2730</td>
<td>0.3 a</td>
<td>2.0 e</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>Oat</td>
<td>None</td>
<td>519 d 1840</td>
<td>0.2 bc</td>
<td>3.3 bcd</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Oat</td>
<td>Amended</td>
<td>714 abcd 2000</td>
<td>0.2 bc</td>
<td>3.5 bcd</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Oilseed radish</td>
<td>None</td>
<td>961 a 2760</td>
<td>0.2 cd</td>
<td>3.5 bc</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>Oilseed radish</td>
<td>Amended</td>
<td>644 bcd 2860</td>
<td>0.2 cd</td>
<td>4.7 ab</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>Oilseed radish &amp; cereal rye</td>
<td>None</td>
<td>572 cd 1410</td>
<td>0.1 d</td>
<td>9.9 a</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>Oilseed radish &amp; cereal rye</td>
<td>Amended</td>
<td>567 cd 2210</td>
<td>0.1 d</td>
<td>11.3 a</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Cereal rye</td>
<td>None</td>
<td>707 bcd 3250</td>
<td>0.3 ab</td>
<td>2.5 de</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>Cereal rye</td>
<td>Amended</td>
<td>650 bcd 2450</td>
<td>0.2 cd</td>
<td>4.5 ab</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>se</td>
<td>0.03 470.9 0.02</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Contrast estimate

<table>
<thead>
<tr>
<th></th>
<th>Contrast estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover crop – no cover</td>
<td>0.09** -396 -0.2** -4.74**</td>
</tr>
</tbody>
</table>

Effect

<table>
<thead>
<tr>
<th></th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover crop</td>
<td>&lt;0.0001 0.0911 &lt;0.0001 &lt;0.0001</td>
</tr>
<tr>
<td>Corn stover</td>
<td>0.8374 0.8762 0.0123 0.0222</td>
</tr>
<tr>
<td>Cover crop*corn stover</td>
<td>0.0001 0.6088 0.0016 0.0006</td>
</tr>
</tbody>
</table>

* Means within a column with a different letter were significantly different at \(P<0.05\) according to the Tukey’s means separation test.

‡ \(y= C\) remaining, \(C_1= \) rapidly mineralizable C pool, \(C_0= \) the pool of potentially mineralizable C at 2 d, \(k=\) rate constant, \(t= \) incubation day.

† Kubelka munk (KM) units were the ratio of diffuse reflectance from the KBr-soil sample to KBr only sample.

*, ** indicates contrast differences at \(P < 0.05\) and \(P < 0.01\), respectively.
Table 2.4. First order decay model \( y = A(1-\exp^{-kt}) \) for evolved C in soil amended with fall cover crops with and without the addition of corn stover amendment.

<table>
<thead>
<tr>
<th>Carbon amendment</th>
<th>A (mg C g(^{-1}) soil)</th>
<th>k (mg C g(^{-1}) soil d(^{-1}))</th>
<th>( t_{1/2} ) (d)</th>
<th>Pseudo ( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No cover – corn stover</td>
<td>0.7</td>
<td>0.3 a</td>
<td>2.3 e</td>
<td>0.68</td>
</tr>
<tr>
<td>No cover + corn stover</td>
<td>1.6</td>
<td>0.3 a</td>
<td>2.2 e</td>
<td>0.72</td>
</tr>
<tr>
<td>Oat – corn stover</td>
<td>9.5</td>
<td>0.1 c</td>
<td>5.4 abc</td>
<td>0.70</td>
</tr>
<tr>
<td>Oat + corn stover</td>
<td>24.2</td>
<td>0.2 b</td>
<td>3.3 de</td>
<td>0.84</td>
</tr>
<tr>
<td>Oilseed radish + rye – corn stover</td>
<td>11.9</td>
<td>0.2 c</td>
<td>4.5 bcd</td>
<td>0.76</td>
</tr>
<tr>
<td>Oilseed radish + rye + corn stover</td>
<td>27.7</td>
<td>0.2 c</td>
<td>4.3 cd</td>
<td>0.86</td>
</tr>
<tr>
<td>Oilseed radish – corn stover</td>
<td>8.4</td>
<td>0.1 c</td>
<td>5.8 abc</td>
<td>0.81</td>
</tr>
<tr>
<td>Oilseed radish + corn stover</td>
<td>24.3</td>
<td>0.1 c</td>
<td>6.0 ab</td>
<td>0.73</td>
</tr>
<tr>
<td>Cereal rye – corn stover</td>
<td>16.1</td>
<td>0.1 c</td>
<td>6.3 a</td>
<td>0.69</td>
</tr>
<tr>
<td>Cereal rye + corn stover</td>
<td>23.7</td>
<td>0.1 c</td>
<td>5.5 abc</td>
<td>0.85</td>
</tr>
<tr>
<td>se</td>
<td>3.66</td>
<td>0.01</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

Contrast

<table>
<thead>
<tr>
<th>Estimate</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover crop – no cover-corn stover</td>
<td>17.1**</td>
</tr>
<tr>
<td>Effect</td>
<td></td>
</tr>
<tr>
<td>Cover crop</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Corn stover</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cover crop*corn stover</td>
<td>0.0807</td>
</tr>
</tbody>
</table>

\( a-e \) Means within a column followed by a different letter were significantly different at \( P<0.05 \) according to Tukey’s means separation test.

* \( P < 0.05; \) ** \( P < 0.01 \)
Table 2.5. Pearson correlation between first order model parameters for DRIFT and Evolved CO$_2$†.

<table>
<thead>
<tr>
<th>Model parameters</th>
<th>DRIFT vs Evolved CO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potentially Mineralizable Pool ($A,$ $C_0$)</td>
<td>-0.02133NS</td>
</tr>
<tr>
<td>Rate constant ($k$)</td>
<td>-0.04807NS</td>
</tr>
<tr>
<td>Half-life ($t_{1/2}$)</td>
<td>0.3514NS</td>
</tr>
</tbody>
</table>

† $r$ = Pearson correlation coefficient. NS = not significant
Table 2.6. Soil mineral nitrogen sampled periodically over in a 72 d incubation.

<table>
<thead>
<tr>
<th>C amendment</th>
<th>Mineral N mg N kg$^{-1}$ dry soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>No cover - corn stover</td>
<td>3.27 bc</td>
</tr>
<tr>
<td>No cover + corn stover</td>
<td>2.11 e</td>
</tr>
<tr>
<td>Oat - corn stover</td>
<td>3.78 ab</td>
</tr>
<tr>
<td>Oat + corn stover</td>
<td>2.31 e</td>
</tr>
<tr>
<td>Oilseed radish - corn stover</td>
<td>3.99 ab</td>
</tr>
<tr>
<td>Oilseed radish + corn stover</td>
<td>3.08 bcd</td>
</tr>
<tr>
<td>Oilseed radish and cereal rye - corn stover</td>
<td>4.75 a</td>
</tr>
<tr>
<td>Oilseed radish and cereal rye + corn stover</td>
<td>3.67 ab</td>
</tr>
<tr>
<td>Cereal rye - corn stover</td>
<td>2.49 cde</td>
</tr>
<tr>
<td>Cereal rye + corn stover</td>
<td>2.46 de</td>
</tr>
<tr>
<td>se</td>
<td>0.029</td>
</tr>
</tbody>
</table>

** Contrast **

<table>
<thead>
<tr>
<th><strong>Estimate</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover crop – stover vs control – stover</td>
</tr>
<tr>
<td>Cover crop + stover vs control + stover</td>
</tr>
<tr>
<td>Control + stover vs control - stover</td>
</tr>
<tr>
<td>Stover vs no stover</td>
</tr>
</tbody>
</table>

** Effect **

<table>
<thead>
<tr>
<th><strong>P value</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
</tr>
<tr>
<td>C amendment</td>
</tr>
<tr>
<td>Time*C amendment</td>
</tr>
</tbody>
</table>

abcd Means within a column followed by a different letter were significantly different at P<0.05 according to the Tukey’s means separation test.

** P < 0.01
Table 2.7. DRIFT relative peak area of labile and recalcitrant soil carbon fractions in near-surface soils (0-15 cm) amended with and without cover crops and corn stover.

<table>
<thead>
<tr>
<th>Time / day</th>
<th>Aromatic-C 1530 cm⁻¹</th>
<th>Aromatic-C 1620 cm⁻¹</th>
<th>Aliphatic-C 2930 cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.92 bc</td>
<td>94.70 c</td>
<td>4.18 c</td>
</tr>
<tr>
<td>2</td>
<td>2.16 a</td>
<td>71.66 a</td>
<td>26.09 a</td>
</tr>
<tr>
<td>4</td>
<td>1.98 a</td>
<td>75.50 b</td>
<td>22.40 b</td>
</tr>
<tr>
<td>7</td>
<td>0.79 bcde</td>
<td>95.99 cd</td>
<td>3.11 cde</td>
</tr>
<tr>
<td>10</td>
<td>0.85 bcd</td>
<td>95.10 cd</td>
<td>3.88 cd</td>
</tr>
<tr>
<td>15</td>
<td>0.65 cde</td>
<td>95.71 cd</td>
<td>3.52 cde</td>
</tr>
<tr>
<td>22</td>
<td>0.83 bcd</td>
<td>96.17 cd</td>
<td>2.89 de</td>
</tr>
<tr>
<td>29</td>
<td>0.94 bc</td>
<td>95.83 cd</td>
<td>3.16 cde</td>
</tr>
<tr>
<td>36</td>
<td>0.73 bcde</td>
<td>96.04 cd</td>
<td>3.17 cde</td>
</tr>
<tr>
<td>43</td>
<td>0.99 b</td>
<td>94.89 c</td>
<td>4.00 c</td>
</tr>
<tr>
<td>50</td>
<td>0.97 b</td>
<td>95.19 cd</td>
<td>3.68 cde</td>
</tr>
<tr>
<td>64</td>
<td>0.59 de</td>
<td>95.62 cd</td>
<td>3.67 cde</td>
</tr>
<tr>
<td>72</td>
<td>0.55 e</td>
<td>96.60 d</td>
<td>2.77 e</td>
</tr>
<tr>
<td>se</td>
<td>0.035</td>
<td>0.005</td>
<td>0.001</td>
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Effect

<table>
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<tr>
<th></th>
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<th></th>
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<td></td>
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<td></td>
<td></td>
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<td>Time</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<tr>
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<td>0.3402</td>
<td>0.4679</td>
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<tr>
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<td>0.1012</td>
<td>0.2495</td>
</tr>
<tr>
<td>Cover crop*corn stover</td>
<td>0.9735</td>
<td>0.0608</td>
<td>0.0664</td>
</tr>
<tr>
<td>Time*cover crop</td>
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<td>0.1816</td>
<td>0.0153</td>
</tr>
<tr>
<td>Time*corn stover</td>
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<td>0.6746</td>
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<tr>
<td>Time<em>cover crop</em>corn stover</td>
<td>0.7794</td>
<td>0.1843</td>
<td>0.4385</td>
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</tbody>
</table>

*a-d Means within a column followed by a different letter were significantly different at P<0.05 according to the Tukey’s means separation test.
Figure 2.1. Soil organic matter decomposition curve for each cover crop treatment with a) corn stover removal, and with b) corn stover retained.
Figure 2.2. Baseline corrected DRIFT spectra KBr diluted soil amended with cover crop residues at day 10 of incubation.
Figure 2.3. Baseline corrected DRIFT spectra of polysaccharide-C peak region (1050 to 1170 cm\(^{-1}\)) of KBr diluted soil amended with cover crop and corn stover residues at day 2, 4, 7, 15, 36 and 72 d of incubation.
Figure 2.4. Soil mineral N concentration during the 72-hr incubation with (retained) and without (removed) corn stover. Data pooled over cover crop amendments. \( P < 0.05 \), Tukey’s mean separation). *,** indicates P value between treatments at 0.05 and 0.01, respectively.
Figure 2.5. Soil microbial biomass carbon dynamics in a 72 day incubation study. Data pooled over corn stover and cover crop amendments.
3.1. INTRODUCTION

An overreliance on fossil fuels is a key issue in our society, and has prompted the use of cellulosic biomass for fuel production. Corn stover, which consists of above-ground biomass left after grain harvest, presents an opportunity as a biofuel feedstock when compared to other sources, such as woody biomass crops, forage crops or animal manure because of its high cellulosic content and high availability. However, high corn stover removal rates have shown detrimental effects on soil quality, with removal rates above 25% showing reductions in SOC as well as grain crop productivity (1.4 Mg ha\(^{-1}\) reduction in grain yield with 50% stover removal, and a 3.1 Mg ha\(^{-1}\) reduction with ≥75% stover removal) (Blanco-Canqui and Lal, 2009a). Kludze et al. (2013) assessed the sustainable removable crop residues for Southern Ontario at 1.1 Mg ha\(^{-1}\) yr\(^{-1}\) and 2.0 Mg ha\(^{-1}\) yr\(^{-1}\) for a corn-soybean and corn-soybean-wheat rotation, respectively.

Soil organic carbon is the main component of SOM, which moderates physical, chemical and biological soil processes (Blanco-Canqui and Lal, 2008). There are concerns that high removal rates may further impact soil quality (Blanco-Canqui and Lal, 2009b). The use of alternative cropping systems, such as cover crops, may present opportunities to increase SOC and to mitigate negative impacts of corn stover removal on soil physical properties. One of the primary physical characteristic influenced by SOM is soil structure through soil aggregation and aggregate stability (Tisdall and Oades, 1982). Soil aggregation dynamics are complex and result from the interaction of many factors including the environment, climate, soil management, plant influences, soil properties (mineral composition, texture, exchangeable ions, nutrient reserves, organic carbon, pedogenic processes, moisture availability), and microbial activity (Kay, 1998).
One of the most important biological processes for soil aggregation is the synthesis of organic compounds (e.g. polysaccharides) by soil microbes, roots and hyphae. These compounds act as “glues” and increase soil aggregation (Tisdall and Oades, 1982). Increased soil aggregation leads to improved water infiltration, decreases in soil surface erosion, enhanced field drainage and increase plant available water (Abiven et al., 2009; Blanco-Canqui, and Lal, 2007).

Fresh crop residues stimulate microbial activity and induce water-stable aggregation (WSA) (Angers and Chenu, 1998). Easily decomposable residues (e.g containing more polysaccharides) have been shown to increase WSA rapidly, whereas less decomposable residues have a gradual long-term effect on WSA (Abiven et al., 2009). Plant influences have also been attributed to root and hyphae enmeshment of soil particles and the release of organic compounds, including polysaccharides, which help bind particles together (Tisdall and Oades, 1982; Santos et al., 1997). Short-term studies have been used to assess rate of change in soil structural stability (Stone and buttery, 1989; Caron et al., 1992). The objective of this study was to assess the short-term effect of corn stover removal within a cover crop-corn stover rotation on soil structure and crop productivity after one year.

3.2. MATERIALS AND METHODS

3.2.1. Experimental Design

A field experiment was established in 2012-2013 to determine the effect of cover crops on corn stover removal systems by measuring soil structure and subsequent crop yield at the University of Guelph Ridgetown Campus in southwestern Ontario (42°46’N, 81°96’W, elevation 200m). A cover crop trial was conducted on a Brookston sandy loam (Orthic Humic Gleysol) soil with 3.5 to 3.8% OM and pH of 6.3 to 7.0 (Table 2.2). The experimental design consisted of
a randomized split-plot with four replications, where cover crops treatments planted in 2007-2010 served as the main plot factor (Fig. 3.1.). Treatments included a control with no cover, oat, cereal rye, oilseed radish, and a mixture of oilseed radish and cereal rye planted at 81, 67, 16, and 9+34 kg seed ha\(^{-1}\), respectively. Grain corn was grown in 2011 and 2012, the retention or removal of corn stover at grain harvest as the split-plot factor, where corn stalks were chopped and either retained on the soil surface or physically removed by hand. Corn residues were incorporated the following spring (2012 and 2013) once by disk tillage in late May. After tillage, the trial was planted with acorn squash (*Cucurbita pepo* var. *pepo* cv. Autumn Delight) at 10759 seeds ha\(^{-1}\).

Soil characteristics were determined from control plots (no cover-corn stover) (Carter and Gregorich 2008). Particle size distribution was determined by the hydrometer method (Day 1965). pH was determined using a 1:1 H\(_2\)O: soil ratio (v v\(^{-1}\)) (McLean 1982) and electrical conductivity (EC\(_{1:1}\)) was measured with a Hanna DiST\(^{®}\)3 portable EC Tester (Hanna Instruments, Smithfield, RI, USA). Soil C was determined by dry combustion using a LECO carbon analyzer. Soil inorganic C was measured by samples that had been placed in a muffle furnace at 420°C for a 24-hour period and the organic C was calculated as the difference between total C and inorganic C.

### 3.2.3. Soil Sampling

Sampling was conducted in late May (22\(^{nd}\) and 27\(^{th}\), for 2012 and 2013, respectively) and 30 d thereafter (21\(^{st}\) and 26\(^{th}\) of June, for 2012 and 2013, respectively). Composite soil samples were taken from each subplot and consisted of three 7.5-cm diameter cores at a 0-15 cm depth. Soil cores were homogenized in the field and sealed into plastic bags for transport. Samples were stored at 4°C until further processing. To ensure uniform water content, soil sampling was done
after a rain event to achieved soil moisture as close to field capacity as possible (Vilamil et al., 2006).

### 3.2.4. Water Stable Aggregates and Dispersibility of Clays

Aggregate stability was measured using the method of Pojasok and Kay (1990), in which a combination of wet sieving and turbidimetric measurements was used to assess the stability of macroaggregates and the dispersibility of clay. Samples were initially sieved at 4.75 mm to remove roots and stones. Two subsamples (5 g) were collected and wetted by capillarity on a wetting table for 90 min at a potential of -0.1 kPa. Wetted samples were washed with 40 mL of water into 50mL test tubes and rotated end-over-end (0.8 m diameter rotation, 25 rpm) for 10 min. The ensuing suspension was poured through a 0.25 mm sieve and the filtrate was collected in a 125-mL Erlenmeyer flask. The soil left on the sieve was oven-dried (105°C for 24hrs) and weighed. A correction was made for sand particles greater than 0.25 mm remaining on the sieve after dispersing the material on the sieve with 5% sodium hexametaphosphate. Percentage of aggregates stable to wet sieving was expressed as a percentage of the total oven-dried soil mass (Eq.3), where $A = \text{dry weight of stable soil larger than } 250 \mu m$, $B = \text{dry weight of sand fraction larger than } 250 \mu m$, and $C = \text{initial soil dry weight}$.

\[
WSA = \frac{A - B}{C - B} \times 100
\]  

Turbidity was measured using HACH 2100Q turbidimeter (Hach Company, Loveland, CO) to determine nephelometric turbidity unit (NTU) value from samples. The amount of suspended clay (mg mL$^{-1}$) was estimated from the NTU values using the calibration curve (Beer-Lambert-Bouger law). The turbidimeter was calibrated using standards containing 0, 200, 400 and 800 NTU; the instrument was zeroed using distilled water before analysis. A series of
dilutions (1:5; 1:7.5; 1:10; 1:12.5; 1:15; 1:17.5; 1:20) was made to derive the range of concentrations for the standard curve (NTU vs dispersed clays). The amount of dispersed clay (DC) was expressed as a percentage of the total clay present in the soil (Pojasok and Kay, 1990).

3.2.5. Vegetable Crop Productivity

Fruit yield was determined by harvesting mature fruit from a 3 m X 3 m section of the center row of each subplot. Plant and fruit biomass were harvested by hand, and weighed thereafter. Yield was calculated on a hectare basis. Estimates were generated for fruit yield as well as plant population.

3.2.6. Statistical Analysis

Water-stable aggregation, DC and squash fruit yield measurements among the ten cropping systems (cover crop-corn stover combination) were analyzed using an analysis of variance by the MIXED procedure of SAS (SAS Institute, version 9.3, Cary, NC, USA), with fixed effects as year, spring sampling time, cover crop, corn stover and the interaction of year x spring sampling time, year x cover crop, year x corn stover, spring sampling time x cover crop, spring sampling time x corn stover, year x spring sampling time x cover crop, year x spring sampling time x corn stover, spring sampling time x cover crop x corn stover, year x spring sampling time x cover crop x corn stover. Random effects were, block and the interactions of block x spring sampling time, block x corn stover, block x spring sampling time x corn stover. The assumptions of a variance analysis were met and included additive model effects, homogeneity of the error variance, errors were random, independent and normally distributed about a zero mean. Normality was assessed using Shapiro-Wilk test (Bowley 2008). The remaining assumptions were tested by plotting residuals of predicted by fixed effects (Bowley
An outlier test was performed using Lund’s table (Bowley 2008) of critical values for studentized residuals. Significance was determined with a type 1 error rate of 0.05.

3.3. RESULTS AND DISCUSSION

3.3.2. Weather Information

Weather data were compiled from 2012-2013 for Ridgetown, ON (Table 3.1). The first year of study, 2012, was characterised by a dryer than normal growing season (May to Sept.), with temperatures similar to the 30-yr mean. Monthly rainfall was unusually lower in April, May and June, compared to the 30-yr mean. The second year of study, 2013, was characterized by a slightly wetter and cooler growing season than 2012, but dryer than the 30-yr average; cooler temperatures were observed in June, August and September. In both years of study, the month of August and September were dryer and cooler than the 30-yr mean.

3.3.1. Soil Structure

There was a significant negative correlation between WSA and dispersibility of clay (r=-0.341, P<0.0001, N=180), thus confirming the theoretical application of the wet sieving method, where a decrease in soil aggregate stability showed an increase in total dispersed clay resulting from the disruption of clay particles at the surface of macroaggregates (Perfect et al., 1990; Caron et al., 1992).

Field sites underwent 4 years of cover crops, followed by one year of corn, where stover was either retained or removed in autumn. Incorporation of crop residues occurred the following spring. Thus, corn stover biomass was the predominant OM input in the cover crop trials in both years of study. Although corn stover biomass yields were 20±0.16% significantly higher (P=0.0072) in 2012 compared to 2011 (Table 3.2), WSA and dispersibility of clays were not
influenced by yearly differences in corn stover biomass (P≥0.0891) (Table 3.3), indicating a weak relationship between OM inputs and soil aggregation the following spring. Likewise, corn stover removal did not have a significant effect on soil aggregate (P=0.1571) and clay dispersibility (P=0.5818) after four years of cover crops. Cover crop treatments in this study did not show significant differences in soil aggregate stability (P= 0.8992) and clay dispersibility (P=0.3431) for both years, indicating a limited role in soil aggregation after 4 years of cover crop, one year of corn stover removal, when sampled in the spring following residue incorporation. The interaction between cover crop and stover was not significant for soil aggregate stability (P=0.3031) as well as clay dispersibility (P=0.4623), further indicating that corn stover removal did not influence soil structure in cover cropping systems in the short term. These results contrasted with a short-term study by Blanco-Canqui and Lal (2009a) that reported a decrease in aggregate stability resulting from high corn removal rates (≥50%) under no-till management after four years. Carbon sequestration is likely higher in no-till systems, thus making it difficult to compare relative contributions to soil aggregation. Conversely, a long-term study by Karlen et al., (1994) reported that complete stover removal did not impact aggregate stability after 10 yrs of stover management in silt loams. Similarly, Roldán et al., (2003) did not observe reduction in aggregate stability for complete stover removal in a no-till sandy loam after 5 consecutive years. The impact of crop residue removal on soil structural properties is most probably governed by differences in soil type, climate, and drainage conditions, and perhaps less on short-term cropping systems.

In this study, the addition crop residues did not influence on the soil aggregation stability, however, sampling time was significantly different (P≤0.0325) for both 2012 and 2013 (Table 3.3). These differences were mainly attributed to in-season soil disturbances resulting from
conventional disk tillage after the first sampling time (May), as June sampling showed a decrease in aggregate stability.

Stover-derived organic materials have been shown to increase specific surface area of soil particles and to promote soil aggregation (Kladivko, 1994; Blanco-Canqui and Lal 2007), and short-term effects may depend more on the amount of crop residue as well as the timing of incorporation. Additional OM inputs were met though incorporation of corn stover in the spring, thus cover crops would have contributed less to labile organic C pools. These results may indicate that recalcitrant C pools resulting from cover crop residue decomposition may have less of an impact on soil aggregation in the short-term. Angers et al., (1999) also reported a weak relationship between potato-cover crop sequences and aggregate stability in a sandy loam after 10 yrs. Soil texture is also likely to have played an important role in this experiment, as soil aggregation is more weakly related to microbial biomass and products in coarse textured soils compared to fine textured soils (Degens et al., 1996), thus may have influenced total aggregation. Ball-Coelho et al., (2000), reported a minimal effect of cover crop cereal rye on macroaggregate stability, and attributed this to inability of humic materials to bind sand grains. Although cropping systems and OM management likely have a larger impact on soil structure within coarser texture soils than finer textured soils (Carter et al., 1994), temporal variability due to climatic factors, which have an effect on soil temperature, moisture regimes, freeze-thaw and wet-dry cycles have been shown to be as influential on temporal variation in WSA as changes in management practices (Perfect et al., 1990).

3.3.2. Vegetable Crop Productivity

Corn stover removal systems did not show significant differences in squash fruit yield (P=0.6407) (Table 3.4). These results contrast with previous a previous study by Blanco-Canqui
(2009b), which showed a reduction in grain yield following corn stover removal. Squash root architecture have shown large differences compared to grain crops, such as maize, and may reflect different nutrient foraging strategies (Lynch, 1995). The interaction between corn stover and cover crop the treatment was also not a significant factor for fruit yield (P=0.3957). The nitrogen rate was significant (P<0.0001) for squash fruit yield (Table 3.4), with treatments receiving 140 kg N ha⁻¹ producing a 13% higher yield compared to treatments receiving no fertilizer. This is likely due to the amount of soil mineral nitrogen available to the squash crop. Nitrogen rate interaction with corn stover was, however, not significant for fruit yield (P=0.6407).

Cover crops did not significantly influence squash yield in both years of study and concur with previous study by Harrelson et al. (2007), which showed no detrimental effect of cover crop residues on pumpkin growth or yield. Cereal rye, however, used as a living mulch has been reported to reduce squash yield (Walters and Young, 2008). Squash yields may be more affected by weather conditions, field locations, soil type and fertility than corn stover removal systems, at least in the short run.

3.4. SUMMARY AND CONCLUSIONS

Corn stover removal in cover cropping systems did not influence soil aggregate stability and clays dispersibility when sampled the following spring. Subsequent squash crop yield was also not affected by corn stover removal in these cropping systems. The use of inorganic nitrogen fertilizer increased squash fruit yield, however interactions with corn stover and cover crops were not significant. One-time removal of corn stover did not affect crop yield and aggregate stability. The lack of effect of stover removal on crop yield and aggregate stability suggests that the one-time removal of corn stover in this soil may not impact soil quality in the short-term.
Further research should be directed at assessing the impact of long-term corn stover removal in cover cropping systems in degraded soils containing less SOM.
Table 3.1. Monthly mean temperature and total precipitation at the University of Guelph Ridgetown Campus, Ridgetown, ON, Canada, in 2012-2013 as compared to the 30-year mean.

<table>
<thead>
<tr>
<th></th>
<th>Rainfall</th>
<th></th>
<th>Temperature</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
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<tr>
<td></td>
<td>2012</td>
<td>2013</td>
<td>30 yr mean</td>
<td>2012</td>
<td>2013</td>
<td>30 yr mean</td>
</tr>
<tr>
<td>Jan.</td>
<td>55</td>
<td>71</td>
<td>66</td>
<td>-1.8</td>
<td>-2.6</td>
<td>-3.2</td>
</tr>
<tr>
<td>Feb.</td>
<td>32</td>
<td>69</td>
<td>62</td>
<td>-0.3</td>
<td>-4.0</td>
<td>-3.2</td>
</tr>
<tr>
<td>Mar.</td>
<td>52</td>
<td>26</td>
<td>43</td>
<td>7.7</td>
<td>0.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Apr.</td>
<td>32</td>
<td>102</td>
<td>88</td>
<td>7.0</td>
<td>6.2</td>
<td>6.7</td>
</tr>
<tr>
<td>May</td>
<td>34</td>
<td>64</td>
<td>75</td>
<td>15.5</td>
<td>15.1</td>
<td>14.8</td>
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<td>June</td>
<td>45</td>
<td>102</td>
<td>83</td>
<td>22.6</td>
<td>18.6</td>
<td>20.2</td>
</tr>
<tr>
<td>July</td>
<td>155</td>
<td>78</td>
<td>86</td>
<td>22.1</td>
<td>21.2</td>
<td>22.5</td>
</tr>
<tr>
<td>Aug.</td>
<td>73</td>
<td>53</td>
<td>86</td>
<td>19.8</td>
<td>19.3</td>
<td>21.4</td>
</tr>
<tr>
<td>Sep.</td>
<td>67</td>
<td>89</td>
<td>93</td>
<td>15.5</td>
<td>15.9</td>
<td>17.6</td>
</tr>
<tr>
<td>Oct.</td>
<td>103</td>
<td>95</td>
<td>72</td>
<td>10.2</td>
<td>11.4</td>
<td>11.0</td>
</tr>
<tr>
<td>Nov.</td>
<td>21</td>
<td>119</td>
<td>97</td>
<td>3.4</td>
<td>9.3</td>
<td>7.1</td>
</tr>
<tr>
<td>Dec.</td>
<td>55</td>
<td>62</td>
<td>65</td>
<td>1.3</td>
<td>-3.1</td>
<td>-2.2</td>
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Table 3.2. Corn stover dry biomass means for fall 2011 and 2012 in Ridgetown, ON, Canada.

<table>
<thead>
<tr>
<th>Cover crop</th>
<th>2011</th>
<th>2012</th>
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</thead>
<tbody>
<tr>
<td>No cover</td>
<td>2.93</td>
<td>4.17</td>
</tr>
<tr>
<td>Oat</td>
<td>2.87</td>
<td>2.88</td>
</tr>
<tr>
<td>Oilseed radish + cereal rye</td>
<td>2.68</td>
<td>3.44</td>
</tr>
<tr>
<td>Oilseed radish</td>
<td>2.31</td>
<td>3.10</td>
</tr>
<tr>
<td>Cereal rye</td>
<td>2.65</td>
<td>3.18</td>
</tr>
<tr>
<td>se</td>
<td>0.365</td>
<td>0.365</td>
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<thead>
<tr>
<th>Effect</th>
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<th>P value</th>
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</thead>
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<tr>
<td>Year</td>
<td>8.31</td>
<td>0.0072</td>
</tr>
<tr>
<td>Cover crop</td>
<td>1.55</td>
<td>0.2134</td>
</tr>
<tr>
<td>Year*cover crop</td>
<td>0.76</td>
<td>0.5567</td>
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</table>
Table 3.3. Impact of sampling time on soil structure in the spring of 2012 and 2013 at Ridgetown, Ontario, Canada†.

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Water Stable Aggregates</th>
<th>Dispersible clay</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>47.5 a</td>
<td>0.46 b</td>
</tr>
<tr>
<td>June</td>
<td>41.0 b</td>
<td>0.56 a</td>
</tr>
</tbody>
</table>

Se

<table>
<thead>
<tr>
<th>Effect</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>0.0891  0.4490</td>
</tr>
<tr>
<td>Time</td>
<td>0.0058  0.0325</td>
</tr>
<tr>
<td>Cover crop</td>
<td>0.8992  0.3431</td>
</tr>
<tr>
<td>Corn stover</td>
<td>0.1571  0.5818</td>
</tr>
<tr>
<td>Cover crop*corn stover</td>
<td>0.3031  0.4623</td>
</tr>
<tr>
<td>Year*time</td>
<td>0.3099  0.9957</td>
</tr>
<tr>
<td>Cover crop*time</td>
<td>0.3031  0.4623</td>
</tr>
<tr>
<td>Corn stover*time</td>
<td>0.3900  0.7879</td>
</tr>
<tr>
<td>Year<em>cover crop</em>corn stover*time</td>
<td>0.9350  1.0000</td>
</tr>
</tbody>
</table>

*Means within a column followed by a different letter were significant at P<0.05 according to Tukey’s multiple range test.

†Data were pooled means of two growing seasons with four replicates each.
Table 3.4. Impact of N fertilizer on squash fruit yield in 2012 and 2013 at Ridgetown, Ontario, Canada†.

<table>
<thead>
<tr>
<th>Nitrogen rate</th>
<th>Squash fruit yield</th>
<th>Shoot biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fresh</td>
<td>dry matter</td>
</tr>
<tr>
<td>kg ha⁻¹</td>
<td></td>
<td>Mg ha⁻¹</td>
</tr>
<tr>
<td>0</td>
<td>35.8 a</td>
<td>2.4 a</td>
</tr>
<tr>
<td>140</td>
<td>40.7 b</td>
<td>2.5 b</td>
</tr>
<tr>
<td>Se</td>
<td>0.89</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Effect: P value

Year: 0.6126 0.0400 <0.0001
Nitrogen rate: <0.0001 0.0388 0.0709
Cover crop: 0.1725 0.3405 0.8574
Corn stover: 0.6407 0.0796 0.5274
Cover crop*corn stover: 0.3957 0.4391 0.1603
Year*nitrogen rate: 0.4783 0.2751 0.0219
Cover crop*nitrogen rate: 0.1959 0.5715 0.2180
Corn stover*nitrogen rate: 0.2958 0.0760 0.4784
Year*cover crop*corn stover*nitrogen rate: 0.4727 0.2661 0.8987

*a-b Means within a column followed by a different letter were significant at P<0.05 according to Tukey’s multiple range test.

†Data were pooled means of two growing seasons with four replicates each.
Figure 3.1. Experimental design of field trial at Ridgetown in 2012. Similar design but different randomization was used in 2013.
CHAPTER 4: GENERAL SUMMARY AND CONCLUSIONS

DRIFT was a suitable technique to measure active organic components in soil over time, and should be used as such in the future. In this study, DRIFT spectroscopy was an effective method to semi-quantitatively assess SOM decomposition from cover crops and corn stover amendments, in a whole-soil, by measuring polysaccharide-C bands regularly over the length of an incubation study. Although treatment differences were similar between first-order model parameters ($C_1$, $k$, $t_{0.5}$) describing C mineralization and polysaccharide decomposition, the correlations between model parameters were not significant, further re-enforcing the fact that heterotrophic decomposition of labile organic pools may also be reflective of other labile carbon sources in addition to polysaccharides. The first hypothesis formulated: DRIFT spectroscopy can be used successfully to measure short-term SOM decomposition when compared to wet chemistry method (basal respiration) was partially accepted on the basis that treatment differences were similar, however first-order model parameters from polysaccharide decomposition curves did not correlate significantly with first-order model parameters from C mineralization curves.

Crop amendments which had a higher C content and lower C:N ratio, such as OSR and OSR&rye, showed the highest C mineralization despite low levels of mineral N in the soil. Results indicate that all cover crop-corn stover treatments combinations had a significantly lower C mineralization and polysaccharide decomposition rate compared to the no cover crop control in an incubation study, thus leading the conclusion that cover crops and corn stover residues reduced carbon losses compared to no cover over the short-term. Given the importance of SOC in mediating soil processes, cropping systems that contribute low quantities OM inputs (dry weight 3.10-7.15 Mg ha$^{-1}$) can still help maintain SOC and soil quality. These findings could
prove useful for growers in temperate climates with short growing seasons, such as Ontario. The second hypothesis: cover crops residues will increase soil labile organic carbon pools within a corn stover removal system, was rejected on the basis that corn stover removal within cover crop systems did not reduce SOC compared to the control (no cover-corn stover).

The lack of a significance effect of crop amendments on aromatic-C bands may suggest that these crop residues, at the quantities tested, did not influence the recalcitrant C pool over their short-term. This study was not long enough to fully express the cropping effects on recalcitrant C pools. Moreover, cover crop residues incorporated to the soil in the vegetative stages would be less likely to contain compounds resistant to degradation such as polymerized aromatic rings from lignin, or polymethylenic structures from lipids and waxes. Research is required to assess the impact the long-term (>20 yrs) cover cropping in corn stover removal systems and its influence on recalcitrant C pools.

The lack of significance in the interaction of cover crop x corn stover x N fertilizer rate on sqyash yield was indicative of relatively low immobilisation levels overall. However, the retention of corn stover with these cover crops will likely immobilize soil N, thus reduce plant available N. Crop N requirements, on the other hand, would be relatively high; the retention of corn stover is unlikely to affect N fertilizer recommendations in vegetable and grain production. The third hypothesis: cover crops will increase plant available N in corn stover removal systems was rejected because the contrast between cover crop and no cover in crop yield was not significant. Further research should assess plant available N originating from cover crop residues having a lower C:N ratio (legumes) over the long-term.
Corn stover removal in cover cropping systems did not influence soil aggregate stability and clays dispersibility in the following spring. Differences in soil aggregate stability were measured between May and June sampling times suggesting that the incorporation of residues had a higher influence on soil aggregation than corn stover management. The fourth hypothesis: cover crops will maintain soil structure in corn stover removal systems, was rejected on the basis that corn stover removal did not reduce soil structure compared to the control (no cover) in the following spring. Future research should be directed at assessing soil aggregation over the long-term (> 20 yr) in cover crop systems.

Squash yield was also not affected by corn stover removal or previous cover crop growth in these cropping systems. The use of inorganic nitrogen fertilizer increased squash fruit yield, however interactions with corn stover and cover crops were not significant indicating that crop residues did not influence nutrient uptake. The fifth hypothesis: cover crops will increase crop productivity in corn stover removal systems, was rejected because squash fruit yield did not increase as a result of the cover crop or corn stover. Further research should be directed at assessing the long-term impact of corn stover removal in cover cropping systems in degraded soils containing less SOM as well as in finer-textured soils.

The quantification of soil quality remains a highly contested concept within soil science. Many soil quality indicators may be used to characterize different soil processes. Crop residues may prove useful in maintaining or improving soil quality, however the selection of adequate testing method remains crucial in determining the actual soil quality benefits of using cover crops within crop rotations. Testing methods should imperatively include short and long-term soil quality indicators in order to determine the effect of crop residues on soil quality. In this study, the removal of corn stover did not reduce overall soil quality in the following spring or
after one year as measured by wet aggregate stability (Pojasok and Kay, 1990), soil mineral nitrogen (Maynard et al., 2008), microbial biomass C (Vance et al., 1987), carbon mineralization (Carter and Gregorich, 2008) and DRIFT (Wander and Traina, 1996). Fields with a relatively short-term history (3-5 years) of cover cropping may not have measurable soil quality benefits as those with a long-term history of cover cropping. Only a small portion (0.7% of annual terrestrial net primary production) of organic residues added to the soil will be converted into stable humic substances (Schlesinger 1990). Therefore, there is a need to conduct long-term studies to determine the impact of cover crops on overall soil quality. As such, the benefit of long-term research may help provide a more comprehensive understanding of the effects of crop residues on agricultural soils.
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