GREEN MANURE TERMINATION METHOD IMPACT ON SOIL CARBON AND SOIL BIOLOGY DYNAMICS

by

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Abstract

No-till green manure (Gmr) termination creates a unique situation compared to conventional no-till agriculture due to the biomass of surface mulch generated. However, the impact this practice has on soil organic carbon (SOC) and soil organisms has been little researched and is the focus of the following thesis.

Tillage regimes of fall tilled, spring tilled, or no-till were applied to a spring planted Gmr within a four-year grain rotation in two trials. Changes in three SOC pools were characterized for three years after GMr termination. Soil carbon was predicted to increase under no-till. This was seen only in Trial 2 where total carbon was greater under no-till compared to fall and spring till (by 2.4 Mg C ha^{-1} and 2.3 Mg C ha^{-1}, respectively) and the effect remained significant all three years. The more labile permanganate oxidizable carbon was least responsive to termination.

Soil microbial biomass (SMB), earthworms, beetles, and spiders were also analyzed after Gmr termination. It was predicted that soil organism abundance would increase under no-till termination. SMB was highest in the spring after Gmr termination in rotations that included hairy vetch/oat Gmr, regardless of tillage. Earthworm densities were negatively affected by tillage (88% and 86% less in Trial 1 and 2, respectively) but densities recovered three years after tillage to that of no-till plots.

To further understand the impacts of tillage and presence of mulch on soil organisms a study with three levels of tillage (tilled, no-till, or fallow) and mulch present or absent was conducted. No effects of tillage were seen but earthworm density was greater under mulch in two out of three trials by 31% and 76% and spiders by 43% in one trial. Opiliones were analyzed in one trial only and were 29% less with mulch.

This research has shown that the same soil benefits seen in conventional no-till management cannot be assumed in no-till Gmr termination. SOC in rotations that have high C inputs, such as Gmr, could be buffered against the impacts of tillage. Impacts on soil organisms vary by taxa and are a result of the mulch created, not tillage itself.

List of Abbreviations Used

ANOVA	Analysis of variance
C	Carbon
DEN DF	Degrees of freedom in the denominator
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization
Gmr	Green manure
HVO	Hairy vetch/oat
LSD	Least significant difference
N	Nitrogen
NUM DF	Degrees of freedom in the numerator
POMc	Particulate organic matter carbon
POXc	Permanganate oxidizable carbon
RC	Red clover
RNA	Ribonucleic acid
SMC	Soil microbial biomass
SOC	Soil organic carbon
TOC	Total organic carbon

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Chapter 1. Introduction

1.1 General Introduction

On a global scale erosion, salinization, compaction, soil sealing, and acidification are some very real threats to soil that have resulted in significant crop yield declines (FAO and ITPS, 2015). In a time when the need to dramatically increase agricultural yields is a common refrain in scientific and popular literature alike, this raises serious concerns around our ability to meet production needs. Atlantic Canada is also experiencing its own soil woes. The soil organic carbon (SOC) in our soil is declining, partially due to cropping systems moving away from pasture and forage and toward annual crops (Agriculture and Agri-Food Canada, 2016; Nyiraneza et al., 2017).

The current and possible future impact of soil degradation on food security and agricultural sustainability has not gone unnoticed. Since the mid-90s the term soil health has been used increasingly in scientific literature. Although difficult to define, it is generally accepted that soil health combines measurements of physical, chemical, and biological soil properties and includes sustainability and multiple ecosystem functions of soil (Andrews et al., 2003; Doran and Parkin, 1994; Romig and Garlynd, 1996). Initiatives like 4 per 1000 launched by France in 2015 aim to tackle soil degradation and SOC loss on a large scale (Ministere de l'agriculture et de l'alimentation, 2018). The National Corn Growers Association in the United States has initiated the Soil Health Partnership with goals to sustain soil function and protect off-site resources such as water (Soil Health Partnership, 2016). The American non-profit organization The Soil Health Institute focuses on the sustained use of soils and protection of the environment (Soil Health Institute, 2016).

1.2 Tillage and Soil

No-till management is one strategy to improve soil. Tillage can be detrimental for soil by degrading soil structure, reducing organic matter, and increasing erosion (Conant et al., 2007; FAO, 2015). A review by Kay and VandenBygaart (2002) found increased macropores under no-till management. No-till management increased aggregate stability in the top 20 cm of soil in a long-term (21 year) Brazilian crop rotation study, although no increase was seen from 20-40 cm (Filho et al., 2002). Another study out of Brazil found tillage in a wheat (*Triticum aestivum* L.)/soybean (*Glycine max* L.) rotation decreased SOC by 16% compared to no-till management in the top 25 cm of soil (Bayer et al., 2002). A study of corn (*Zea mays* L.)-soybean systems under 31 years of varying tillage intensities showed that the highest level of tillage decreased water-stable aggregates, SOC, and potentially mineralizable N (Karlen et al., 2013).

There have been arguments against the widely claimed benefits of no-till on SOC, stating it actually reduces SOC in deeper soil layers (Baker et al., 2007; Du et al., 2017) and surface SOC gains are easily lost by even a single instance of tillage (Conant et al., 2007). Yang et al. (2008) found no-till significantly increased SOC from 0-5 cm but below 5 cm depth SOC was actually decreased at two of three sites under no-till, resulting in similar total amounts of SOC from 0-20 cm. Luo et al. (2010) found that any cultivation reduced SOC compared to uncultivated soil and while no-till increased carbon above 30 cm it decreased it below 40 cm.

Soil organisms are also impacted by tillage. A Brazilian study found that no tillage cropping systems had greater crop yields and a soil microbial biomass (SMB) that was up to 100% greater than cropping systems where the soil was disturbed (Silva et al., 2010). A long-term field experiment in Switzerland found tilled plots had less arbuscular mycorrhizae fungal richness compared to no-till or reduced till plots (Jansa et al., 2003). Thirty-one years of full inversion tillage decreased SMB by 64% compared to no-till (Karlen et al., 2013). A meta-analysis of soil management strategies found increased SMB and enzyme activity under no-till compared to full-inversion tillage (Zuber and Villamil, 2016). Reduced tillage increased the number of collembola in wheat fields in Ireland (Sousa et al., 2006). In a comparison of earthworm abundance under standard tillage or no tillage over a three-year period earthworm numbers were greater in no-till, and the effect strengthened by the third year of continued tillage or no-till (Johnson-Maynard et al., 2007). There is a trend of negative impacts of tillage on soil biology, with decreasing diversity and abundance.

Enhanced soil biological diversity and activity is another claimed benefit of conventional no-till agriculture (FAO, 2015), which can enhance several ecosystem functions. A review by Brussaard et al. (2007) found increased soil diversity corresponded to plant disease suppression. A microcosm study by Wagg et al. (2014) showed a decline of multiple ecosystem functions with declining diversity and changing composition of the soil organisms, including decreased carbon sequestration, decreased decomposition, and increased P leaching.

1.3 Organic Production and Soil

The negative consequences of tillage and the adoption of no-till agriculture in many conventional production systems has led to some criticism of organic production, which relies heavily on tillage for weed control (Trewavas, 2004). However, many studies comparing organic and conventional production systems find increased soil health under organic management (Lynch, 2014; Lynch et al., 2011). In a meta-analysis of pairwise comparisons of organic versus conventionally managed fields, organic management had 3.5 Mg C ha^{-1} more SOC than conventional management (Gattinger et al., 2012). A Danish study found that although organic rotations had greater inputs of carbon, SOC was similar to that of a conventional rotation. However, soil respiration and SMB were greater in the organic rotations, indicating more rapid cycling of labile SOC compared to conventional management (Chirinda et al., 2010). Aggregate stability in an organic rotation with tillage was similar to a conventional no-till rotation at 0-6 cm and was increased compared to conventional no-till at 6-20 cm after 4 years (Loaiza Puerta et al., 2018). A long-term European study has shown organic farming increases soil health, not only by increasing SOC and SMB, but also fostering beneficial soil biological interactions that lead to increased biological control and nutrient cycling (Birkhofer et al., 2008).

1.4 Green Manures and Soil

Green manures (Gmr) are another management practice that can be used to improve soil health (Biederbeck et al., 2005; Woodley et al., 2014). Gmr are grown to be left in the field rather than have the plant biomass removed. They are often a nitrogen fixing crop grown in rotation to return nitrogen and organic matter to soil and are often employed under organic management. Use of Gmr has been shown to decrease weed biomass (Harker and Blackshaw, 2009; Mirsky et al., 2011; Sainju and Singh, 2001), decrease N leaching (Thorup-Kristensen et al., 2012), increase crop N use efficiency

(Aulakh et al., 2000), increase microbial activity (Biederbeck et al., 2005; Elfstrand et al., 2007), and decrease occurrence of plant disease and pests by interrupting their life cycle (Abawi and Widmer, 2000; Bulluck III and Ristaino, 2002; Viaene and Abawi, 1998). A recent meta-analyses of the fertility impact of Gmr in cropping systems in Eastern Canada and Northeastern United States found that use of Gmr can increase cash crop yields by up to 27% (Charles et al., 2017). A long-term study in Belgium found that after 51 years of returning crop resides SOC, labile C, and SMB were all increased (Buysse et al., 2013).

Performance of Gmr crops vary with species of Gmr. When comparing faba bean *(Vicia faba* L.) and field pea (*Pisum sativum* L.) Gmr, Shirtliffe and Johnson (2012) found that faba bean produced lower Gmr biomass and had greater weed biomass. Lower C:N ratios and amount of N fixed by faba bean resulted in greater SMB and β-glucosidase compared to chickling vetch (*Lathyrus sativus* L.) (Lupwayi and Soon, 2016). Use of hairy vetch (*Vicia villosa* Roth) Gmr resulted in greater levels of root rot in bean compared to rye (*Secale cereal* L.) (Abawi and Widmer, 2000). Sweetclover (*Melilotus* spp.) has been shown to inhibit weed growth through the release of allelochemicals (Blackshaw et al., 2001; Harker and Blackshaw, 2009).

Very few studies have examined the residual benefits of Gmr to soil and crop beyond the first year after their incorporation (Lynch, 2015). Use of Gmr continued to affect SMC and β-glucosidase activity up to three years after the Gmr (Lupwayi and Soon, 2016). A Manitoban study found no benefit of N added from a Gmr two years after incorporation (Cicek et al., 2015). Another Manitoban study comparing a pea/oat (*Avena sativa* L.) Gmr to an oat Gmr over two rotation phases (wheat in the first year followed by fall rye the next year) found that fall rye had greater above-ground biomass and N uptake after pea/oat, showing there can be multi-year Gmr effects (Cicek et al., 2014).

1.5 No-Till Green Manure Management

Traditionally, termination of Gmr required tillage, which has led to increased interest in reducing or eliminating tillage in Gmr systems for the soil health benefits (Halde et al., 2017; Silva and Delate, 2017). Leaving the Gmr on the soil surface reduces tillage and also creates a mulch that can contribute to non-mechanical weed control (Shirtliffe and Johnson, 2012). Crop rollers operate by rolling over crops and crushing stems while leaving Gmr biomass rooted to the soil. Using no-till management to terminate a Gmr creates a unique situation compared to conventional no-till agriculture. Mulch biomass left behind by a Gmr can reach up to 7.6 Mg ha^{-1} (Halde et al., 2014). The mulch itself is unique to other mulches as it remains rooted to the soil. There is also a lot of variety in species of Gmr grown and when it is grown, as it can be a full-season crop planted in spring or an overwinter crop planted in fall after harvest (Halde et al., 2014; Rivers et al., 2018; Vaisman et al., 2014).

The bulk of research on no-till Gmr termination has focused on the yield of the following cash crop and weed control. Use of cereal rye as a no-till Gmr in the upper Midwest of the United States has had some success in soybean agriculture, although sufficient rye biomass (at least 8 Mg ha^{-1}) is required and there is some yield depression compared to tilled soybeans. In the same region hairy vetch has been no-till managed with corn, but problems with insect infestation followed, possibly due to use of the Gmr as an oviposition site (Silva and Delate, 2017). In the mid-Atlantic United States corn and soybean yields in organically managed no-till Gmr systems showed no difference or an increase with tilled organic production, with greater success in more southern locations (Wallace et al., 2017). Vegetable and cotton (*Gossypium barbadense* L.) yields in a Californian experiment with rolled Gmr resulted in an 80% decline in yields due to late crop planting and low emergence due to the presence of the Gmr mulch (Luna et al., 2012). Weed control in corn planted into crimped hairy vetch impacted yields in a study out of Maryland (Teasdale et al., 2012). The above studies involved overwinter Gmr crops. Full season Gmr crops in Manitoba, Canada have been shown to require 6 Mg kg^{-1} mulch biomass to obtain adequate weed control, which was best achieved with hairy vetch (Halde et al., 2014). Adoption of no-till Gmr management in Europe has been slower than in North America, largely due to inconsistent weed control and lack of knowledge (Vincent-Caboud et al., 2017).

An area of no-till Gmr termination that has been less researched is how soil and SOC is impacted by this management strategy. Terminating a Gmr with a crop roller has been shown to decrease soil N over conventional tillage (Sainju and Singh, 2001) and also increase asynchrony between N supply and crop N demand (Vaisman et al., 2011). A Missouri study found no pattern in SOC changes in the top 15 cm of soil between tilled and no-tilled Gmr, although they did see signs of N immobilization in no-till (Clark et al., 2017). Shallow inversion tillage and no-tillage of winter oilseed radish *(Raphanus sativus* (L) Domin) did not impact soil N or N lost through leaching in a UK study (Cooper et al., 2017). Outside of the Gmr work undertaken in Manitoba (Halde and Entz, 2014; Vaisman et al., 2011), these Gmr studies have been on fall seeded overwintering Gmr, not full season.

There are very few studies of the response of soil organisms in the no-till Gmr research. A study out of Pennsylvania that compared cover crop type and tillage and included roller crimped overwinter Gmr, found no impact of tillage on arthropod activity-densities. Although by the third year of the study arthropod community composition was affected, with more evenness in tilled compared to no-till (Jabbour et al., 2015). Another study out of Pennsylvania had greater captures of predatory arthropods in no-till hairy vetch and triticale (× *Triticosecale*) Gmr compared to no-till cereal rye, but a tilled treatment was not included so the impact on rolling the Gmr itself was not assessed (Rivers et al., 2018).

For a management practice that is adopted in large part for the benefit to soil and soil health, there is little evidence on how no-till Gmr termination impacts the soil. Producers are looking to make informed choices about farm management. The impacts of combining no soil disturbance, changing the distribution of Gmr biomass in the soil, and covering the soil with a tightly woven mulch rooted to the soil are unlikely to mimic other management systems where these factors occur in singularity. A conceptual visualization of the interactions occurring in a Gmr system, whether managed by tillage or by no-till methods, is presented in Figure 1.1. Several factors will influence the outcomes of Gmr termination method and will vary by climate, soil type, Gmr species, and crop rotation.

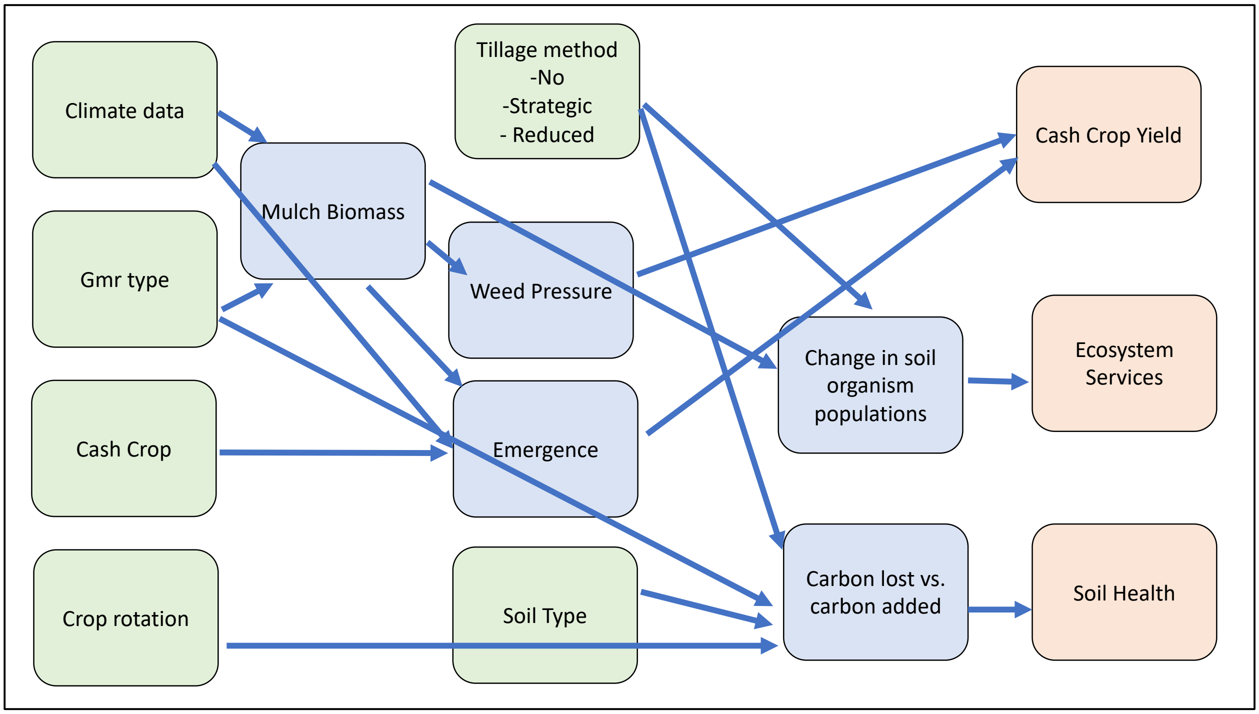


Figure 1.1 A simplified conceptual model of the outcomes of no-till Gmr termination. Green boxes are information to be entered into the model, blue boxes are intermediate calculations done by the model, and the orange boxes are the desired output. Arrows indicate what information is needed to calculate the outcomes.

1.6 Overview of Research Presented in the Dissertation

This dissertation aims to fill a void in our understanding of no-till Gmr systems. The main research question is how does this unique combination of changes at the soil surface influence the soil ecosystem. This will not only provide information for producers on the value of this management practice but will also provide a better understanding of the soil ecosystem under agricultural management. The experiments are designed to learn more about the impacts of a no-till Gmr system in areas where there is currently little information available: SOC and soil organisms.

The first research chapter, Chapter 2, describes the SOC dynamics after Gmr termination in an organic grain rotation. Soil carbon is a major influencer of soil physical, chemical, and biological properties and many soil ecosystem functions. Three termination treatments were included; fall-tilled, spring tilled, and no-till of a spring-seeded hairy vetch/oat Gmr using a crop roller. Total SOC, particulate organic carbon, and permanganate oxidizable carbon were measured at two soil depths (0-5 cm and 5-15 cm) for three years to understand the sensitivities of the different SOC pools to Gmr termination method.

Chapter 3 discusses the responses of soil organisms to the three Gmr termination treatments from Chapter 2 and includes data from a second field site in Carman, MB. Soil organisms impact many soil ecosystem functions and are closely linked to SOC dynamics. SMB was analyzed for one growing season after Gmr termination to look at the effect of termination method and the ability of soil microbes to recover over a short time period. Soil macrofauna (earthworms, beetles, and spiders) dynamics were also analyzed over multiple growing seasons throughout the crop rotation to assess the impacts of Gmr termination method and the resilience of these organisms to disturbance.

The final research chapter, Chapter 4, investigates the impacts of tillage and Gmr mulch in a factorial experiment to better understand the relative influence of these factors on soil organisms. Three levels of tillage (tilled, no-till, and fallow) of a hairy vetch/oat Gmr and two levels of mulch (mulch presence or absent) were tested at two sites, one in Nova Scotia and one in Manitoba. Earthworms, beetles, spiders, and (at one site) opiliones were analyzed for one growing season. This experiment was designed to expand on the findings of the previous chapter and shed light on how the different components of Gmr termination method influence soil organisms.

The focus of my research is on the impacts of Gmr termination method to the soil ecosystem. Impacts on yield and weed biomass have been better represented in the body of research on no-till Gmr and therefore are not presented in this dissertation. Data on wheat yield (the cash crop following the Gmr) and weed biomass in the wheat rotation phases were collected for the Bible Hill site and can be found in Appendix A (Figures A1-4).

Chapter 2. No-till Green Manure Termination Influences Soil Organic Carbon Distribution and Dynamics

2.1 Introduction

Soil organic carbon stocks in Eastern Canada are declining. At the same time, soil carbon in Western Canada has been increasing and this increase is attributed to the adoption of reduced tillage and no-till agriculture (Agriculture and Agri-Food Canada, 2016). No-till production has been adopted by many conventional farmers on a global scale, and has been successful in stopping degradation or even improving soil properties and soil carbon stocks (Agriculture and Agri-Food Canada, 2016; Lehman et al., 2017; So et al., 2009). However, there has been criticism of the claim that no-till is a long-term solution for carbon storage. Conant et al. (2007) and VandenBygaart (2016) found that even infrequent tillage events in a mostly no-till system (not an uncommon occurrence) can cause declines in accumulated soil organic carbon (SOC). There is also evidence that while conversion to no-till from full inversion tillage increases SOC in the surface layers, it either has no influence or reduces SOC in lower soil layers (Kay and VandenBygaart, 2002; Krauss et al., 2017; Luo et al., 2010; Peigné et al., 2007).

Within organic agriculture there is continued interest in adopting no-till practices, due in part to criticism of an over-reliance on tillage (Trewavas, 2004), the conversion to organic by no-till conventional producers (Halde et al., 2017), fuel cost savings (Zikeli and Gruber, 2017), and a general interest in improving soil health (Lynch, 2014). Adoption by farmers has been slow due to inconsistent weed control and access to equipment, such as no-till seeding drills and crop rollers (Halde et al., 2017; Vincent-Caboud et al., 2017), and in particular for Eastern Canada the availability of manure for fertility (Halde et al., 2017). In organic agriculture, tillage is used for weed control, to terminate green manures (Gmr), and incorporate manure and other organic amendments. Crop rollers (Figure 2.1) are an alternative method to terminate a Gmr crop over tillage. Crop rollers, also called blade rollers or roller crimpers, roll over crops and use dull blades to crush or break stems while leaving plant material attached to the soil surface. The Gmr plant biomass then functions as a mulch and can suppress weed growth (Blackshaw et al., 2001; Shirtliffe and Johnson, 2012).

Figure 2.1. Crop roller used to no-till terminate hairy vetch/oat Gmr (I and J Manufacturing, Gordonville, PA). The barrel of the roller is filled with water to create more downward force.

The majority of studies on no-till Gmr systems have focused on the performance of the cash crop and weed control (Halde et al., 2015; Shirtliffe and Johnson, 2012; Silva and Delate, 2017; Vaisman et al., 2011; Wallace et al., 2017). Adequate mulch biomass is required for weed control in a no-tillage Gmr system. Halde et al. (2014) suggest 6.0 Mg ha^{-1} dry biomass in the spring as a minimum for weed control. The amount of Gmr biomass also directly relates to the amount of C added to the system. Gmr biomass is influenced by planting date (Teasdale et al., 2004), plant species (Halde et al., 2014;

Vaisman et al., 2014), decomposition rates of initial biomass (Halde and Entz, 2016), and termination method (Astier et al., 2006; Mooleki et al., 2016). Many Gmr studies are conducted in mesic environments and use fall planted Gmr (Peigné et al., 2015; Silva, 2014; Teasdale et al., 2004), which is not feasible in more frigid environments.

Of the organic cropping system studies that have examined the influence of tillage on SOC, many have not specifically looked at no-till Gmr termination within the organic no-tillage system, which is only one way to reduce tillage in organic production (Podolsky et al., 2016). However, leaving a large amount of biomass on the soil surface creates a unique situation compared to many no-till or reduced till systems. A meta-analysis of organic tillage studies, ranging in duration from one to twenty years, in Europe and North America found an increase in SOC stocks of 143 g m^{-2} with any reduction in tillage compared to deep-inversion tillage (Cooper et al., 2016). A Missouri study found no pattern in changes to SOC in a three-year study comparing a tilled and no-till overwinter Gmr between crops in a wheat (*Triticum aestivum* L.)—corn (*Zea mays* L.) soybean (*Glycine max* (L.) Merr.) rotation (Clark et al., 2017). In Switzerland, a study with a grass/clover mix Gmr and winter wheat rotation observed an increase (17% and 11% at two sites) in SOC after 13 years under reduced tillage (skim/chisel plow) compared to full-inversion tillage but SOC was also stratified, with gains only being observed in the surface 10 cm and a slight loss in the 20-50 cm depth increment (Krauss et al., 2017).

The objective of this study was to examine SOC distribution and dynamics as influenced by different methods of Gmr termination – fall tilled, spring tilled, and no-tilled - of a spring planted Gmr within a four-year organic grain rotation. Three SOC pools (total organic carbon (TOC), particulate organic matter (POMc), and permanganate oxidizable (POXc)) were measured at two soil depths (0-5 cm and 5-15 cm) for three years after Gmr termination. I hypothesized that no-till Gmr termination would increase SOC, especially in the top 5 cm of soil, and the more liable pools (POMc and POXc) would be more responsive to termination method than TOC.

2.2 Methods

2.2.1 Site Description and Treatments

The experiment was established at the Brookside experimental site for organic agriculture at the Dalhousie Agricultural Campus in Truro, Nova Scotia (45°23’24.72” N; 63°15’16.15” W). Soil at the site was a sandy loam (Orthic Humo-Ferric Podzol). The research site was drained by tiles installed at 0.8 m. Mean annual temperature was 6.1°C and annual precipitation was 1168 mm, which includes a mean snowfall of 157 cm (10-year average) and potential evapotranspiration of 512 mm annually. Annual total growing degree-days (number of degrees the mean temperature is above 5°C) average 1746 and the frost-free period was 109 days (Environment Canada, 2017a).

The experimental design was a randomized split-plot blocked design with three blocks. Each block contained four plots (14 by 75 m) which contained four subplots (14 by 16 m). Since 2003, the site was organically managed and conventionally tilled (full inversion tillage used annually) and had a four-year rotation consisting of red clover (*Trifolium pratense* L.)-red clover-spring wheat-soybean where the red clover served as a Gmr. Commencing in 2013, red clover was replaced by hairy vetch/oat (*Vicia villosa* Roth and *Avena sativa* L.) seeded in a 30:70 ratio in three of the rotation sequences (the fourth treatment remained in the prior red clover Gmr rotation and was not included in the present study). The new fully-phased four-year rotation consisted of hairy vetch/oat- spring wheat-fallow/fall rye (*Secale cereal* L.)-soybean where wheat and soybean were grain crops and fall rye was a cover crop used for weed control. The hairy vetch/oat Gmr was a full season cover crop and the following year spring wheat was planted and harvested for grain, and wheat stubble was left in the third year to avoid soil disturbance and no-till seeded to a fall rye cereal cover crop in late summer, which was rolled and soybean was planted into the rolled rye biomass in the fourth and final year of the rotation. Seeding rates and varieties are given in Appendix B (Table B1). The partial fallow year was included to help detect changes in SOC in the treatments beyond the first year. The main plot treatment was Gmr termination method and sub-plots contained each phase of the rotation. The present study reports on the subplots seeded to hairy vetch/oat Gmr in 2013 (hereafter called Trial 1-2013-2016) and in 2014 (Trial 2, 2014-2017). For the three years following Gmr termination, SOC dynamics were tracked. These years will be referred to as Yr1 (wheat phase), Yr2 (partial fallow/rye phase), and Yr3 (soybean) for each trial.

The standing hairy vetch/oat Gmr biomass on all plots was rolled in late August. Rolling terminated the oat but hairy vetch continued to grow until cold temperatures caused winter-kill. The three termination methods were: (i) no-till (rolled only in late summer), (ii) spring till (one tillage pass with moldboard plow followed by one post-emergent tine weeding pass after seeding, rolled only in fall) and (iii) fall till (tilled with a moldboard plow in October and post-emergent tine weeding after seeding). Thus both no-till and spring till plots were covered all winter with Gmr biomass while the fall till terminated plots were bare (Table 2.1). Post-emergent tine weeding was done in the wheat plots for the two tilled treatments only. In each year, soil was sampled in May. See Table 2.1 for the full sequence and dates of all field operations for both trials and Figure B1 in Appendix B for a diagram of the field layout.

Table 2.1. Schedule of field operations for Trial 1 and Trial 2. Tillage refers to full soil inversion with a moldboard plow to approximately 15 cm depth. No tillage operations occurred after the initial green manure termination and the system remained no-till.

Field Operation	Date Trial 1 (D/M/Y)	Date Trial 2 (D/M/Y)	Treatments Applicable
Gmr planting	29/5/13	20/5/14	All
Gmr biomass sampling	16/08/13	11/08/14	All
Gmr rolling	20/8/13	13/8/14	All
Fall tillage	30/10/13	30/10/14	Fall till only
Mulch biomass sampling	25/04/14	4/5/15	No-till and Spring till only
Spring tillage	1/5/14	15/5/15	Spring till only
Soil sampling –wheat phase Yr1	14/5/14	21/5/15	All
Wheat planting	16/5/14	5/6/15	All
Post-emergent tine weeding	31/5/14	26/6/15	Fall and Spring till only
Soil sampling-partial fallow[†] phase Yr2	21/5/15	18/5/16	All
Fall rye planting	4/9/15	26/8/16	All
Soil sampling-rye/soybean phase Yr3	18/5/16	25/5/17	All
Fall rye rolling	23/6/16	12/6/16	All
Soybean planting	23/6/16	15/6/16	All

[†] Refers to wheat stubble followed by fall rye phase after wheat.

2.2.2 Green Manure and Soil Sampling

Gmr was sampled each year for above ground biomass and carbon and nitrogen content. Prior to rolling in late summer, three 0.25 m^2 quadrats of the above-ground biomass were removed from the vetch/oat plots. The biomass from the quadrats was pooled and manually separated into vetch, oat, or weeds for analysis. Spring mulch in the no-till and spring tilled plots (prior to spring tillage) was also sampled by the above method. All plant samples were dried at 55°C for 3-5 days until constant mass, and ground and analyzed by combustion for C and N (varioMax CN by Elementar, Germany).

In both trials, soil was sampled in May using a 5 cm diameter slide hammer to a depth of 15 cm. This depth was chosen because it matched the plow depth and was where differences were expected to be detectable between termination treatments. Six cores were taken per plot. All cores were divided into 0-5 cm and 5-15 cm, pooled by depth, air dried, and sieved to 2 mm. A reference soil sample was also taken for each block as a benchmark of an undisturbed soil. This consisted of a grassy area approximately 2 m by 56 m meters, one for each block, to the south of the trials that bordered a fence shared with a pasture where soil had been undisturbed for many years. The reference areas were soil sampled using the method described above. Bulk density was determined by using the mass of the collected soil samples and the volume of the slide hammer. Initial soil parameters are given in Table 2.

Table 2.2. Soil properties for 0-15 cm at experiment initialization in 2013 sampled by block (Trial 1) and 2014 (Trial 2). Values are ± one standard error. pH was determined by mixing air dried soil and water in a 1:1 soil slurry. Soil texture was determined by the hydrometer method. Nutrient levels were measured by the Nova Scotia Department of Agriculture†.

Soil Property	Trial 1	Trial 2
TOC (g kg^{-1})	2.0 ± 0.1	2.4 ± 0.1
pH	5.71 ± 0.05	5.60 ± 0.04
Silt%	32.7 ± 2.6	30.3 ± 2.3
Sand%	63.0 ± 3.7	67.3 ± 2.8
Clay%	4.3 ± 1.2	2.7 ± 0.6
Bulk Density (g mL^{-1})	1.28 ± 0.02	1.22 ± 0.03
P (kg ha^{-1})	182 ± 42	154 ± 48
K (kg ha^{-1})	234 ± 27	186 ± 53
Calcium (kg ha^{-1})	2345 ± 136	1974 ± 606
Magnesium (kg ha^{-1})	510 ± 109	429 ± 138
Sodium (kg ha^{-1})	28 ± 2	19 ± 6
Sulfur (kg ha^{-1})	38 ± 1	26 ± 8
Aluminum (ppm)	1433 ± 95	1221 ± 371
Boron (ppm)	<0.50	<0.50
Copper (ppm)	0.74 ± 0.05	0.59 ± 0.18
Iron (ppm)	230 ± 24	187 ± 56
Manganese (ppm)	33 ± 4	35 ± 9
Zinc (ppm)	1.32 ± 0.25	0.9 ± 0.28

†Determination of Mehlich III Extractable Major and Trace Metal Ions in Soil by ICP-OES (Mehlich, 1984)

2.2.3 SOC Analysis

TOC was measured by dry combustion. Soil was ground by placing in 75 mL squared-sided glass bottles with metal rods and rolling on a roller mill for 72 h. Approximately one gram of dry soil was then ignited at 900°C and evolved C and N were measured (varioMax CN by Elementar, Germany). Carbonates are not present in this soil at the depth sampled so total C is equivalent to TOC (Carter et al., 2003). POMc was determined through fractionation as described by Gregorich and Beare (2007). Briefly, 25 g of soil was dispersed in a solution of sodium hexametaphosphate and passed through a sand-sized sieve (53 μm). All material collected on the sieve was considered the POM fraction. The collected POM fraction was ground and analyzed for C and N by combustion as for TOC. POXc was determined by potassium permanganate oxidation by the method described in Weil et al. (2003). Briefly, 2.5 g of dry ground soil and 18 mL distilled water were mixed with 2 mL of a 0.2 M $KMnO_4$ solution. The labile carbon reacts with the permanganate to bleach the solution. The concentration of labile C, or POXc, was then determined through light spectroscopy at 550 nm using a predetermined standard curve (Jenway 6505 US/Vis Spectrophotometer, Essex, GB). With respect to the present trials, SOC will be used when referring to soil organic carbon in general or to all three carbon pools collectively. TOC will only be used to refer to the whole soil carbon obtained by combustion analysis. These carbon pools were chosen as TOC can be slow to respond to management changes while the more labile pools can be more sensitive to short-term changes (Cates et al., 2016) and are important to soil functions like nutrient cycling and sustaining soil life (Lynch, 2014).

2.2.4 Statistical Analysis

All SOC data was analyzed by a repeated measures ANOVA using Proc Mixed in SAS 9.4 with year as the repeated measure and included termination method, depth, their interactions with each other and year, and block. Data were checked for normality and constant variance and the covariance structure was determined using the lowest AIC value. The covariance structures used in this analysis were compound symmetry and heterogeneous compound symmetry. All factors were considered fixed effects except for block which was a random effect. Level of significance was set at $\alpha=0.05$. LSMEANS and PDIFF statements were used to separate means and create letter groupings for all significant terms in Proc Mixed. When there were no significant differences between sampling depth or year, data were combined. Although the design was a split-plot, each Trial only sampled one of the four split plots (Figure B1). Therefore, the split was not included in the statistical model.

2.3 Results

2.3.1 Green Manure

Hairy vetch/oat Gmr aboveground biomass averaged 5.4 ± 0.9 Mg ha^{-1} and 6.7 ± 0.4 Mg ha^{-1} dry weight in Trial 1 and Trial 2, respectively, before rolling in late summer. Aboveground biomass of mulch remaining before spring tillage in the spring tilled and no-till plots was 4.9 ± 0.3 Mg ha^{-1} and 4.2 ± 0.6 Mg ha^{-1}, for Trial 1 and 2 respectively. This amounts to an overwinter loss of 0.5 Mg ha^{-1} (18% of pre-rolling biomass) in Trial 1 and 1.5 Mg ha^{-1} (37% of pre-rolling biomass) in Trial 2, not accounting for some continued vetch growth that occurred after late summer biomass sampling. Based on the carbon percentage and biomass of mulch before rolling, Trial 1 added approximately 2.6 ± 0.4 Mg ha^{-1} of C and Trial 2 added 2.9 ± 0.1 Mg C ha^{-1} to the system.

2.3.2 SOC Pools

Bulk density was found to be unaffected by termination method or by depth so it was determined that using an equivalent soil mass correction such as proposed by Wendt and Hauser (2013) was unnecessary (Appendix A, Figure A1).

In Trial 1, there were no significant differences in TOC (0-15 cm) among the three termination methods within each of the three years, only between the reference samples and all Gmr plots (Table 2.3, Figure 2.2). The Gmr plots had greater TOC than the reference samples in Yr1. This same pattern of greater TOC in the Gmr plots over the reference samples after Gmr termination was repeated in Trial 2 for Yr1 and Yr2. In Trial 2, while no treatment interactions with year or depth for TOC were significant, termination method alone was significant (P<0.001). No-till plots had greater TOC than the spring tilled and reference samples, regardless of year (Figure 2.2b). In Trial 1, termination x depth was significant (P=0.0082, Table 2.3) but this was only because the reference samples had less TOC at the 5-15 cm depth and there was no differences between the three termination methods.

The TOC C:N was greater in the no-till plots compared to the spring tilled plots in Yr2 only of Trial 1 (P=0.0382, Table 2.3, Figure 2.2c). In Yr3 no-till was greater than fall tilled plots in Trial 2 (P=0.0049, Figure 2.2d). A similar pattern occurred for C:N of the POM but the effect was not significant (data not shown).

The POMc pool was significantly affected by termination dependent on depth in Trial 1 (Table 2.3, Figure 2.3a, P=0.0049). No-till plots had significantly more POMc in the top 5 cm than in 5-15 cm, the same pattern as the reference samples. No-till plots also had greater POMc than fall or spring till plots at 0-5 cm, but it was not significant. Only termination method alone was significant in Trial 2, with no-till plots having greater POMc than all other termination treatments (P=0.0027, Table 2.3, Figure 2.3b). The same pattern can be seen in Trial 2 as in Trial 1 in the 0-5 cm soil depth, with more POMc in no-till compared to fall and spring till plots. There is a slight stratification of POMc in no-till plots, but it was not significant in Trial 2.

In both trials, POXc was significantly affected by termination method by depth (Table 2.3, Figure 2.3). POXc was highly stratified in the reference samples only, unlike POMc which saw stratification under both no-till plots and in reference samples.

Table 2.3. ANOVA table for all carbon parameters; TOC (Mg C ha^{-1}), C:N of SOC, POMc (Mg C ha^{-1}), and POXc (Mg C ha^{-1}) for Trial 1 and Trial 2.

TOC	Trial 1				Trial 2			
Effect	Num DF	Den DF	F Value	P Value	Num DF	Den DF	F Value	P Value
Termination	3	45	4.70	0.0061	3	43	12.02	<.0001
Year	2	45	7.56	0.0015	2	43	42.48	<.0001
Depth	1	45	95.40	<.0001	1	43	103.02	<.0001
Termination*Year	6	45	1.42	0.2276	6	43	2.26	0.0556
Termination*Depth	3	45	6.21	0.0013	3	43	0.86	0.4696
Termination*Year* Depth	6	45	2.08	0.0742	6	43	1.43	0.2264
Block	2	45	8.74	0.0006	2	43	2.16	0.1279
SOC C:N								
Termination	3	45	6.93	0.0006	3	42	1.00	0.4017
Year	2	45	123.8	<.0001	2	42	9.46	0.0004
Depth	1	45	0.43	0.5157	1	42	2.20	0.4838
Termination*Year	6	45	2.46	0.0382	6	42	3.69	0.0049
Termination*Depth	3	45	0.43	0.1598	3	42	0.37	0.7752
Termination*Year* Depth	6	45	0.61	0.7216	6	42	0.87	0.5260
Block	2	45	10.12	0.0002	2	42	4.81	0.0131
POMc								
Termination	3	45	0.19	0.9023	3	42	5.52	0.0027
Year	2	45	4.72	0.0138	2	42	23.83	<.0001
Depth	1	45	8.89	0.0046	1	42	3.44	0.0705
Termination*Year	6	45	1.66	0.1525	6	42	1.25	0.3023
Termination*Depth	3	45	4.90	0.0049	3	42	1.59	0.2053
Termination*Year* Depth	6	45	1.05	0.4003	6	42	0.24	0.9615
Block	2	45	0.87	0.4242	2	42	4.08	0.0240
POXc								
Termination	3	43	1.61	0.2011	3	44	11.71	<.0001
Year	2	43	5.06	0.0106	2	44	14.18	<.0001
Depth	1	43	22.90	<.0001	1	44	3.42	0.0712
Termination*Year	6	43	0.48	0.8191	6	44	1.47	0.2094
Termination*Depth	3	43	6.92	0.0007	3	44	5.69	0.0022
Termination*Year* Depth	6	43	0.60	0.7278	6	44	0.69	0.6579
Block	2	43	1.67	0.2004	2	44	0.42	0.6624

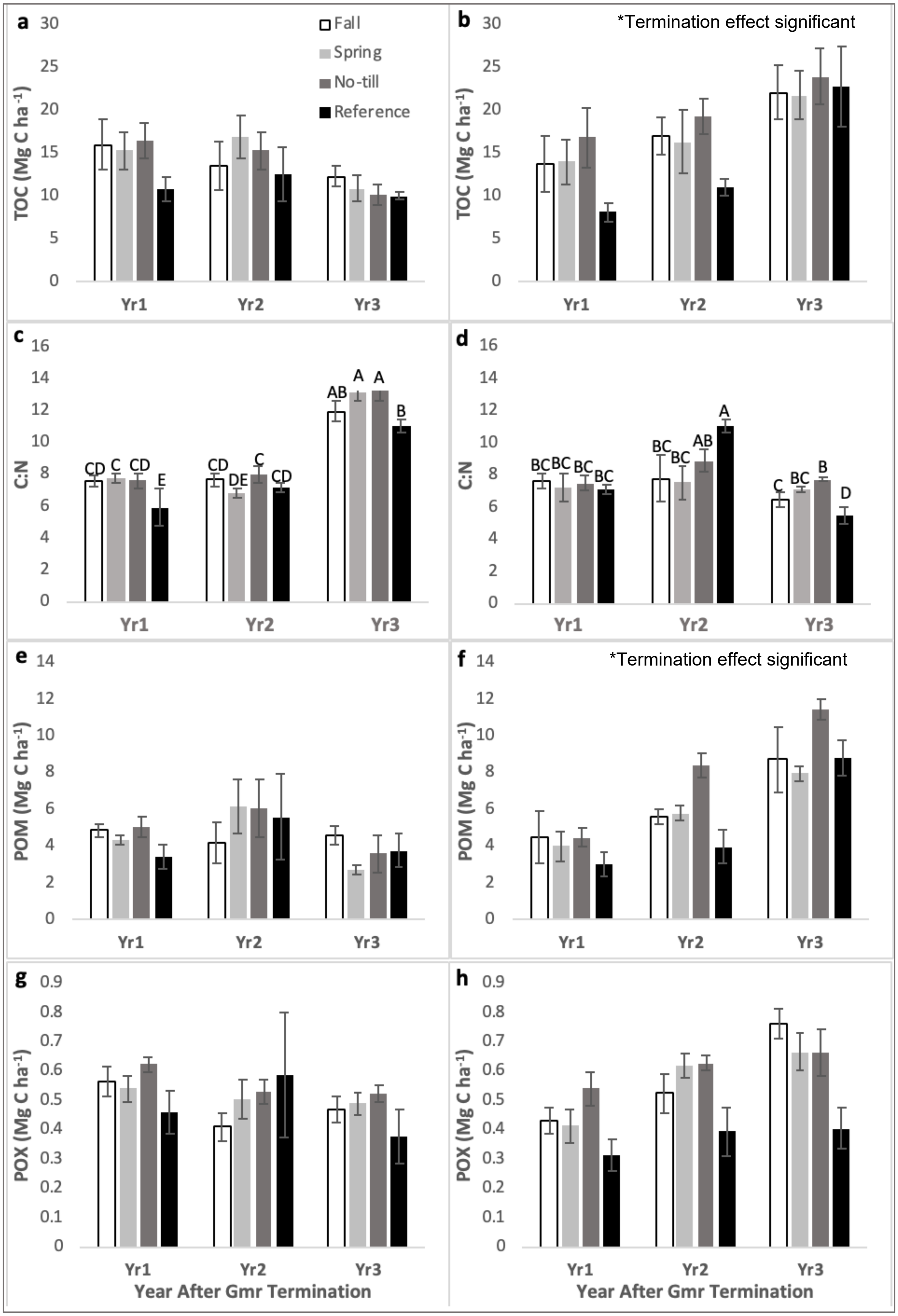

a
b
*Termination effect significant
Fall
Spring
No-till
Reference
TOC (Mg C ha^{-1})
0
5
10
15
20
25
30
Yr1
Yr2
Yr3
c
d
C:N
0
2
4
6
8
10
12
14
16
CD
C
CD
E
CD
DE
C
CD
AB
A
A
B
BC
BC
BC
BC
BC
BC
AB
A
C
BC
B
D
e
f
*Termination effect significant
POM (Mg C ha^{-1})
0
2
4
6
8
10
12
14
g
h
POX (Mg C ha^{-1})
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Year After Gmr Termination

Figure 2.2. SOC pools by year for (a) TOC for Trial 1 (Mg of C ha^{-1}), (b) TOC for Trial 2, (c) C:N of TOC for Trial 1, (d) C:N of TOC for Trial 2, (e) POMc for Trial 1 (Mg of C ha^{-1}), (f) POMc for Trial 2, (g) POXc for Trial 1 (Mg of C ha^{-1}) and, (h) POXc for Trial 2. Treatments are (i) no-till (rolled only), (ii) spring (tilled only in spring), (iii) fall (tilled in fall), and (iv) reference (undisturbed field margin).Yr1 is the wheat phase, the year following Gmr termination, yr2 is the partial fallow phase, and Yr3 is the soybean phase. Data is combined across all depths (0-15 cm) Different letters indicate significant differences by LSD at $\alpha=0.05$ and are only included when the effect of termination x year was significant. Bar represent one standard error.

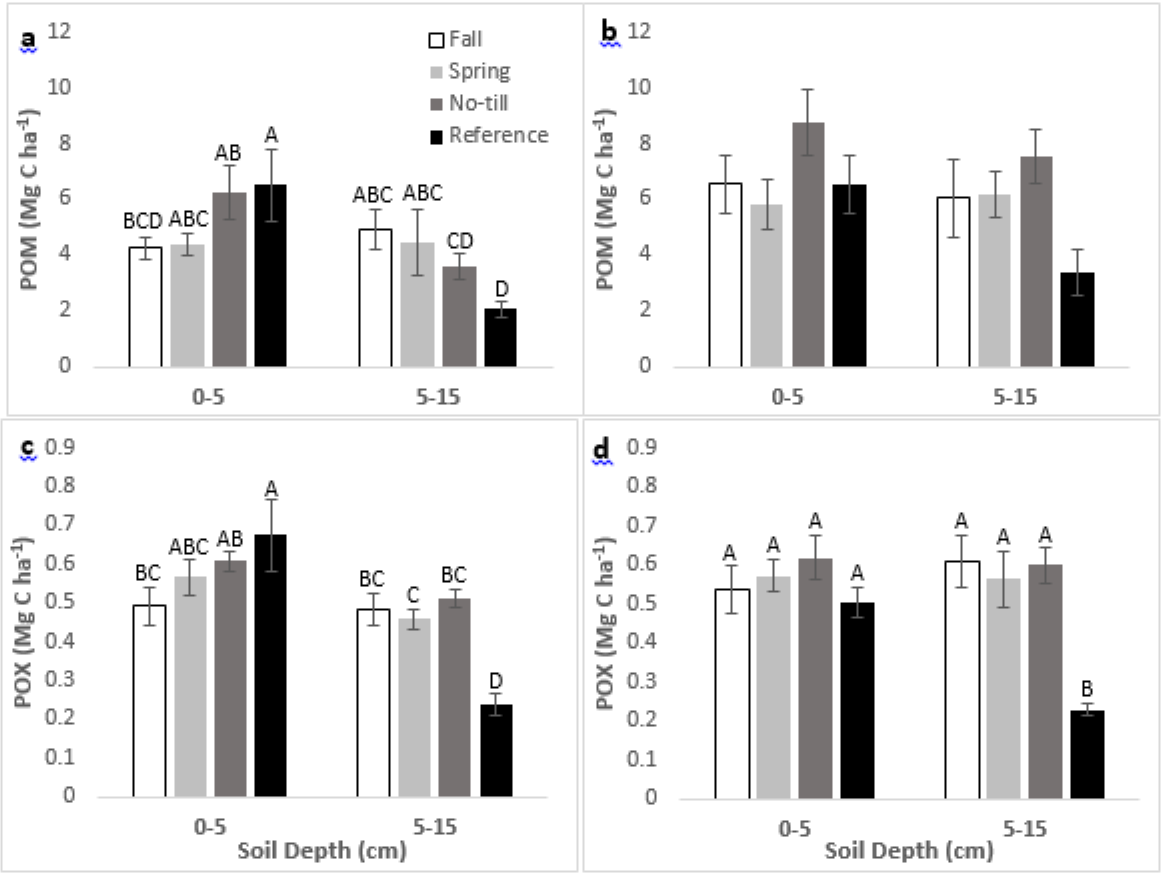


Figure 2.3. Carbon pools by depth for 0-5 cm and 5-15 cm. Values are combined from Yr1, Yr2, and Yr3 as POMc and POXc were found to be not significantly different across the three years. (a) POMc (Mg C ha^{-1} by depth in Trial 1, (b) POM in Trial 2, (c) POXc for Trial 1 (Mg of C ha^{-1}), and (d) POXc for Trial 2. Treatments are (i) no-till (rolled only), (ii) spring (tilled only in spring), (iii) fall (tilled in fall), and (iv) reference (undisturbed field margin). Different letters indicate significant differences by LSD at α=0.05. Bars represent one standard error.

2.4 Discussion

The amount of spring mulch biomass from the Gmr (4.9 ± 0.3 Mg ha^{-1} in Trial 1 and 4.2 ± 0.6 Mg ha^{-1} in Trial 2) was similar to that obtained in a study in Manitoba also using a spring planted hairy-vetch/oat as Gmr by Halde et al. (2015) which was 4.5 Mg ha^{-1}. The greater decomposition rate of Gmr mulch overwinter in Trial 2 (37% compared to only 18% in Trial 1) could be attributed to moisture level of the mulch. Trial 2 had greater initial biomass when measured in the previous year, which may have caused it to take longer to dry out. A similar occurrence happened in Halde and Entz (2016) where greater mulch biomass in litter bags decomposed faster than lower biomass levels. The differences in estimated overwinter decomposition could also be due to different growth rates after the initial sampling of fall biomass in September, which was not measured.

TOC was greater in the no-till plots in Trial 2 regardless of year. This was calculated to be 2.4 ± 1.2 Mg C ha^{-1} more than the fall tilled plots and 2.3 ± 1.3 Mg C ha^{-1} soil more than the spring tilled plots. This is a reasonable result considering the amount of C added to the system by the Gmr (more than 4 Mg ha^{-1}). The differences could be accounted for by tillage causing the decomposition of added and existing SOC (Conant et al., 2007) and the movement of organic matter below the plow layer (Angers and Eriksen-Hamel, 2008). Inversion depth of the moldboard plow in the present study was approximately 15 cm, which could have put organic matter, including some of the Gmr biomass, below the sampling depth when the Gmr was incorporated.

The higher soil C:N ratio in the no-till treatment by Yr2 (Trial 1) and Yr3 (Trial 2) could reflect different rates of decomposition or N loss over time. Leaving biomass on the soil surface slows down decomposition but could also speed up N loss through exposure to surface water runoff and ammonia volatilization. Halde and Entz (2016) found Gmr litter bags placed on the soil surface lost 46.4% of their N content in the first 30 days. The difference by one year in C:N response (Yr2 in Trial 1 and Yr3 in Trial 2) and that the effect on TOC was seen in Trial 2 only could be due to climatic differences. In Yr1 of trial 2 the spring snow melt occurred much later than usual which could have delayed initial decomposition of mulch biomass in Trial 2 (field site is typically snow-free by the end of April (Environment Canada, 2017a) but in 2015 snow was present in May).

As expected, POMc was concentrated at the soil surface under no-till Gmr termination in Trial 1. When considering both depths together in Trial 1, fall, spring, and no-till plots had very similar levels of POMc (24.4 ± 1.6%, 22.1 ± 2.2%, and 24.6 ± 2.5% of TOC, respectively). POXc was stratified in the references plots and there was a slight stratification in the no-till treatment visible in Figure 2.3c for Trial 1, but it was not significant. No stratification occurred for TOC except in the reference samples. A review by Peigné et al. (2007) compared conservation tillage to conventional tillage and found that carbon and organic matter tended to be higher in the surface soil but similar levels in untilled layers. Similar trends were found by Kay and VanderBygaart (2002) and Carr et al. (2013).

Both POMc and POXc have been proposed as more sensitive than TOC to management changes. Cates et al. (2016) found POMc more responsive to management than TOC and Plaza-Bonilla et al. (2014) found POMc more sensitive than both TOC and POXc. Culman et al. (2012) found POXc to be as effective as TOC and POMc at detecting site changes. In a comparison of multiple soil C pools, Morrow et al. (2016) found POXc to be the most effective parameter at detecting differences between different cropping systems. Simonsson et al. (2014) and Ladoni et al. (2015) found POM fractions to be more variable than TOC and less able to detect cropping system differences. Our results found treatment differences in TOC and POMc in Trial 2, but POXc was relatively consistent between different termination methods and appears to be less responsive to the termination treatments.

A stronger response was expected in the more labile pools (POMc and POXc) compared to TOC, but that was not the case for POXc. A study that added mulch over three years (roughly 2.7 Mg C ha^{-1} per year) was able to detect changes in TOC within the three year period (Mulvaney et al., 2017). In organic Gmr rotations the high levels of added biomass could be buffering the more labile carbon pools against short term changes caused by tillage. The relatively high POMc fraction (~25% of TOC) across all treatments indicates this is a 'well fed' soil, in terms of organic matter. This could also be contributing to the stratification seen under no-till termination in the labile pools. If POXc is relatively buffered against tillage changes, differences might only be detectable at the very surface of the soil where the large amount of plant biomass was located after no-till termination.

Taking such large amounts of organic material and either incorporating them into the soil with tillage or leaving them on the soil surface was expected to influence the carbon pools in the soil through different distribution and decomposition rates. In general, when significant differences were found, no-till Gmr termination had greater C than the two tilled treatments. Sampling to a deeper depth could help track the fate of the carbon in the soil and would be better able to determine if SOC is being lost from the system or distributed to lower depths with tillage.

Chapter 3. Soil Macrofauna Resilience Under Different Green Manure Termination Methods

3.1 Introduction

No-till green manure (Gmr) systems hold interest as a method of soil improvement for organic production. However, little is known about the impacts of no-till termination of Gmr on life in the soil. Soil microbes and soil fauna influence many important soil functions and are often included in soil health evaluations (Ferris and Tuomisto, 2015). Soil organisms control nutrient cycling and decomposition, help create soil structure, and can both cause and prevent plant diseases (Setälä et al., 2005). Understanding how no-till Gmr termination influences soil organisms will allow for a more complete evaluation of the impacts of this management practice.

Soil microbial biomass is frequently measured along with many other measures of soil microbial abundance and community composition such as microbial quotient, phospholipid fatty acid analysis, DNA or RNA analysis, or enzyme assays (Bini et al., 2013; Pérez-Brandán et al., 2014; Pieper et al., 2015). However, soil microbes can respond inconsistently to disturbances (Wardle, 1995), have relatively short-term responses (Kaurin et al., 2018), or can be resistant to disturbances and management practices altogether (Marshall et al., 2011). Detecting influences of management practices on the microbial community composition and diversity can be difficult due to their high temporal variability (Lauber et al., 2013) and the strong influence of inherent soil properties such as pH (Bainard et al., 2016). A synthesis of studies examining impact of tillage on soil organisms found a mild negative response of microbial biomass, and inconsistent responses by nematodes and microarthropods, which were inhibited or stimulated by tillage (Wardle, 1995). A meta-analysis of 62 global studies on tillage and microbial properties found relatively greater soil microbial biomass (SMB) and enzyme activity under no-till compared to other forms of tillage excepting chisel till, which was similar to no-till (Zuber and Villamil, 2016).

Study of organisms with larger body size could give a clearer picture of changes in soil health and the soil community as larger-bodied organisms tend to fluctuate less temporally and are less resilient compared to soil microbial populations due to their longer life cylces (Lynch, 2014; Postma-Blaauw et al., 2010; Wardle, 1995). In the above-mentioned synthesis of tillage studies by Wardle (1995), earthworms and Coleoptera (beetles), and to a lesser extent Araneae (spiders) were most often negatively affected by tillage, compared to smaller-bodied organisms. In a study of organic potato (*Solanum tuberosum* L.)/grain/forage rotations, Nelson et al. (2009) found that the earthworm population took the entire five year rotation to recover to levels of adjacent undisturbed pasture after a sharp decrease following the tillage-intensive potato phase of the rotation. Forest fires continued to affect the distribution of macrofauna five years after burning in a study in Western Russia (Korobushkin et al., 2017).

Earthworms are frequently used as an indicator species for soil ecosystem health and have been shown to be highly influenced by tillage. Peigné et al. (2009) showed that earthworm abundance increased when annual cropping systems moved from conventional tillage to reduced or no-tillage but over the short duration of the study (three years) there was no detectible improvement in soil structure associated with greater numbers of earthworms. In a comparison of conventional tillage and no-tillage over a three-year period earthworm numbers were greater under no-till, and this effect strengthened after the third year (Johnson-Maynard et al., 2007).

Beetles (Coleoptera) and spiders (Araneae) are additional larger-bodied organisms that can serve as indicators of soil health. Coleoptera population dynamics are closely linked to soil; both adult and immature stages hibernate in soil (Larochelle and Larivière, 2003). This makes them highly susceptible to soil disturbance, such as tillage, although studies on soil cultivation and beetles have reported inconsistent results (Holland and Luff, 2000). Beetle and arachnid populations can be reduced by direct mortality with tillage or by emigration after habitat alteration by tillage, with arachnids showing more sensitivity to this disturbance than beetles (Thorbek and Bilde, 2004). In Pennsylvania, throughout a three-year crop rotation of soybean (*Glycine max* (L.) Merr.)-wheat (*Triticum aestivum* L.)-corn (*Zea mays* L.), the majority of beetles captured in pitfall traps were in a rolled hairy vetch (*Vicia villosa* Roth)/triticale (×T*riticosecale* Wittmack) cover crop compared to a wheat or cereal rye (*Secale cereal* L.) stand (Rivers et al., 2017). Carabidae are regarded as beneficial carnivores and granivorous insects in agricultural systems. Exclusion barriers against beetles resulted in lower predation rates of onion fly (*Delia antiqua* Meigen) pupae (Menalled et al., 1999) and greater populations of cereal aphids (*Sitobion avenae* F.) in field trials (Collins et al., 2002). Arachnids also play an important role as pest predators (Baba et al., 2018; Royauté and Pruitt, 2015) and are sensitive to disturbances (Pearce and Venier, 2006).

No-till Gmr management is unique to other no-till or reduced tillage systems. The relatively large amount of surface mulch biomass left rooted to the soil, plus the micro-environmental changes created by the mulch, could influence soil biota in unexpected ways. The objective of this study was to look at how no-till termination of Gmr in an organic grain rotation affects soil microorganisms and selected soil invertebrates. Microbial biomass, earthworms, ground beetles, and spiders were sampled for three years after termination of Gmr by three methods – no-till (crop rolled only), spring tilled (soil covered overwinter), and fall tilled (soil bare overwinter). We hypothesized that there would be relatively greater abundance of macrofauna in the no-till treatment and population recovery of beetles and spiders would be more rapid from tillage in subsequent rotation phases due to their greater mobility compared to earthworms. Experiments were conducted between 2013-2017 in Bible Hill, Nova Scotia and Carman, Manitoba.

3.2 Methods

3.2.1 Site Descriptions and Experimental Design

3.2.1.1 Bible Hill, NS

This research was conducted on the same research plots described in Chapter 2 at the Brookside experimental site for organic research in Bible Hill, NS, with the exception that the red clover (*Trifolium pratense* L.) rotation that was excluded in the previous chapter is included in this study for beetle and spider analysis. See Chapter 2 for a full site description. Briefly, there were four main plots (14 by 75 m) within three blocks and within each were four subplots (14 by 16 m with a 1 m grassy, mowed buffer between each subplot). Main plot treatments were Gmr (red clover or hairy vetch) termination method (n=4), and sub-plots contained each phase of the four-year rotation so that all phases were present each year (Figure B1). One main plot was in a conventionally tilled four-year organic grain rotation of red clover-red clover-wheat-soybean. The other three main plots had a four-year rotation of hairy vetch/oat (*Avena sativa* L.)-wheat-fallow/fall rye-soybean. The rotation that included hairy vetch/oats will be referred to HVO and the rotation with red clover as the Gmr will be referred to as RC. Varieties and seeding rates can be found in Table B1. The subplots seeded to HVO or those in the second year of RC in 2013 make up Trial 1 and those in 2014 make up Trial 2. Rotation phases will be referred to as Yr1 (wheat phase), Yr2 (partial fallow/rye phase for the HVO rotation or soybean phase for the RC rotation), and Yr3 (soybean for the HVO rotation or first year RC for the RC rotation) for each trial.

The three termination treatments for HVO were the same as described in the previous chapter with the inclusion of the red clover rotation as a fourth treatment. They were: (i) no-till (crop rolled only), (ii) spring till (one tillage pass with moldboard plow followed by post emergent tine weeding after spring seeding of wheat, crop rolled only in fall), (iii) fall till (tilled with a moldboard plow in the fall and post-emergent tine weeding after spring seeding of wheat), and (iv) red clover (tilled with a moldboard plow in the fall of the second year of red clover and post-emergent tine weeding after spring seeding of wheat). Reference samples, as described in Chapter 2 were also sampled for soil biota and were included as a treatment in the analysis. Dates of field operations can be found in Table 2.1 in Chapter 2, except for seeding information of clover. Clover was seeded on 8 May 2012 (Trial 1) and 6 May 2013 (Trial 2). The RC termination was done by moldboard plow at the same time as the fall tilled HVO treatments. Two years of RC followed by a fall incorporation by a moldboard plow is a typical Gmr practice for this region. Hairy vetch is less common but has shown a lot of promise as a Gmr for biomass and weed control (Halde et al., 2014; Vaisman et al., 2014)

3.2.1.2 Carman, MB

A second experimental site was established at the University of Manitoba Ian N. Morrison Research Farm located 70 km southwest of Winnipeg in Carman, MB (49° 29'53.200"N, 98°01'47.100"W). Mean annual temperature was 3.5°C and annual precipitation was 445 mm, which includes a mean snowfall of 100 cm (Environment Canada, 2017b). Soils in Carman are classified as Black Chernozems and are loamy fine sand.

The experimental design was a four replicate RBD in a split-split plot arrangement with two main plots (26 by 10 m). Each main plot had three 8 by 10 m sub plots with a 1m buffer between each plot. The main plots were Gmr type (n=2) while the subplots were Gmr termination method at three levels: (i) no-till (crop rolled only), (ii) spring till (one tillage pass with moldboard plow followed by post emergent tine weeding after spring seeding of wheat, crop rolled only in fall), (iii) fall till (tilled with a moldboard plow in the fall and post-emergent tine weeding after spring seeding of wheat). The two Gmr crops were a typical N supplier for the region (forage pea (*Pisum sativum* L.)/oat mixture and a high N supplier (hairy vetch/barley (*Hordeum vulgare* L.)) each followed by a wheat rotation phase. However, due to time restrictions only the plots that had previously been hairy vetch/barley were sampled for earthworms and that is what is presented in this chapter. Varieties and seeding rates are given in Table B1. The rotation did not continue past the wheat phase as occurred at the Bible Hill site. The two-year sequence of Gmr-wheat was conducted twice, once in 2013/2014 (Trial 1) and again in 2014/2015 (Trial 2), at two locations within a few meters from each other. Dates of field operations can be found in Table 3.1 and initial soil properties can be found in Table 3.2 and a diagram of one block of the experiment can be found in Appendix B (Figure B2).

Table 3.1. Schedule of field operations for Trial 1 and Trial 2 for Carman, MB site. Tillage refers to full soil inversion with a moldboard plow to approximately 15cm depth.

Field Operation	Date Trial 1 (D/M/Y)	Date Trial 2 (D/M/Y)	Treatments Applicable
Green Manure Planting	29/5/13	23/5/14	All
Green Manure Rolling	26/7/13	23/7/14	All
Fall Tillage	3/10/13	2/10/14	Fall till only
Spring Tillage	8/5/14	8/5/15	Spring till only
Wheat Planting	8/5/14	9/5/15	All
Earthworm Sampling	17/6/14 & 18/6/14	16/6/15	All

Table 3.2. Soil properties for both trials at Carman, MB at initiation of trial. Values are ± one standard error. No bulk samples were collected at the start of Trial 2 so a bulk density value for Trial 2 is not available.

Soil Property	Trial 1	Trial 2
TOC %	2.01 ± 0.13	1.96 ± 0.07
pH	4.61 ± 0.05	5.34 ± 0.04
Silt%	19.45 ± 2.64	19.41 ± 0.53
Sand%	75.75 ± 2.67	72.19 ± 0.27
Clay%	4.80 ± 0.17	8.40 ± 0.66
Bulk Density (g mL^{-1})	1.75 ± 0.03	na

3.2.2 Soil Microbial Biomass

SMB in the wheat phase was determined from the pre-plant soil sample described in Chapter 2 in addition to other in-season soil samples. The pre-plant soil sample was taken with a 5 cm slide hammer on 14 May 2014 for Trial 1. Additional soil samples were taken with a 2.5 cm soil corer on 2 July 2014 and 21August 2014 representing two weeks after spring tillage, two months after spring tillage, and four months after spring tillage. Ten soil cores were taken per plot, divided into two depths, 0-5 cm and 5-15 cm, and pooled. Soils were kept at 4°C until they were analyzed. SMB data is only included here from Yr1 of Trial 1 due to equipment limitations that occurred during analysis.

The chloroform-fumigation method was used for SMB-C (Vance et al., 1987). Two 25 g subsamples were taken from each soil sample and one subsample was fumigated with chloroform for 24 h in a vacuum desiccator. Extraction was done by shaking both soil subsamples with 75 mL of 0.5M K_2SO_4 for one hour and passing the supernatant through filter paper. The extractants were frozen until analysis for dissolved organic carbon by thermal oxidation (Thermalux 3.1.1 Scientific Analytical, Tewkesbury, UK). Before oxidation samples were sparged with HCl to remove any inorganic carbon, which has been shown to be created in the chloroform-fumigation process (Rotbart et al., 2017). The difference in carbon between the fumigated and non-fumigated extracts is assumed to represent the SMB.

3.2.3 Earthworm Sampling

Data on earthworm population dynamics were collected in Yr1 through Yr3 (wheat phase, partial fallows/fall rye phase, and soybean phase) for the Bible Hill site but only in Yr1 (wheat phase) for the Carman site due to the termination of this rotation after the wheat phase. Earthworms were sampled by manual extraction in the spring of each year (Table 3.3). Sampling was only done on days with no precipitation. Two 0.5 m^2 quadrats were randomly placed in the plot away from the edges and excavated with a shovel to a depth of 15 cm. Soil was placed on a 1 cm sieve and manually checked for all earthworms. After soil was thoroughly checked it was returned to the hole in the plot. Earthworms were kept cool and were counted and weighed within 48 h of collection.

Additional tillage took place at Bible Hill in the RC rotation in Yr3 before earthworm sampling could take place due to the asynchrony in the Yr3 phases between the HVO rotation and the RC rotation (in Yr3 RC rotation was returning to first year red clover and was tilled before seeding). Therefore, the RC rotation is not included in the earthworm analysis for Bible Hill.

Table 3.3. Earthworm sampling dates for Bible Hill, NS site along with air temperature and relative humidity at the start of sampling each day.

Rotation Phase	Trial 1	Temp (°C)	Rel. Hum. (%)	Trial 2	Temp (°C)	Rel. Hum. (%)
Wheat (Yr1)	9/6/2014 &	19.6	46	9/6/2015 &	15.9	84
	10/6/2014	20.8	49	12/6/2015	16.7	76
Fallow/Fall Rye (HVO[†]) or	9/6/2015 &	15.9	84	22/6/2016 &	17.9	81
Wheat (RC[ǂ]) (Yr2)	12/6/2015	16.7	76	23/6/2016	17.5	73
Fall Rye/Soybean (HVO) or First year Red Clover (RC) (Yr3)	21/6/2016	22.2	56	5/6/2017	10.1	76

[†] HVO refers to the rotation with hairy vetch/oat as the green manure

[ǂ] RC refers to the rotation with red clover as the green manure

3.2.4 Beetle and Spider Sampling

Beetles and spiders were collected in Yr1 and Yr2 at the Bible Hill site and Y1 at the Carman site using pitfall traps. Due to significant loss of samples during courier shipping from Manitoba, only beetle data for Bible Hill are presented. Two pitfall traps were established 1 m apart in the middle of each plot so that the top of the trap was flush with the soil surface. A partition between the two traps was created with black plastic lawn edging (11.5 cm high) dug into the soil several centimeters to increase capture rates by guiding beetles into traps. Each trap consisted of two nested plastic cups (300 mL, 8 cm wide at top and 5 cm wide at bottom) and a rain cover made from a square of plywood placed over the trap opening held up from the soil surface by four nails. Traps were filled with a brine solution to euthanize beetles and prevent escape by more mobile species. Traps were opened for 48 h every two weeks, weather permitting, from June to August and captured beetles and spiders were frozen until analysis. When there was heavy rainfall traps would overflow and those samples were not kept but the plots were resampled the following week. There were also problems with crows dislodging the pitfall traps. When this happened, those samples were discarded. Traps also collected opiliones, but they were infrequent so are not included in this study. Data from pitfall traps represents activity-density because capture depends on not only the abundance of the organism but also the level of activity (Winder et al., 2001).

The most abundant beetles present were and *Harpalus* ssp. (specifically *H. pensylvanicus* Degeer and *H. rufipes* DeGeer) and *Pterostichus melanarius* Illiger. Their numbers were also tallied separately for activity-density.

3.2.5 Microclimate Monitoring

Data on soil temperature and moisture levels were taken at 10 cm depth every two weeks using handheld probes at three locations in each plot (Delta-T SM150T Soil Moisture Sensor, Cambridge, UK) in all plots once the soil was no longer frozen until the end of June for the Bible Hill site only.

3.2.6 Statistical Analysis

Earthworm population density and biomass for the Bible Hill site were analyzed by repeated measures using Proc Mixed in SAS 9.4 with sample date as the repeated measure and included termination method and block in the model. Termination method was a fixed effect and block was a random effect. Data were checked for normality and constant variance and the covariance structure was determined using the lowest AIC value. Earthworm density residuals did not meet the assumptions of normality and constant variance and was log transformed for Trial 1 and a square root transformation in Trial 2 before analysis. SMB data was also analyzed using repeated measures in the same manner except sampling date was the repeated measure.

Beetle and spider captures (Bible Hill site only) were analyzed as a cumulative activity-density (June-August) for all Yr1 captures and for all Yr2 captures. An ANOVA was conducted using the general linear model function in Minitab® 17.3.1.

For all analyses, level of significance was set at α=0.05. LSMEANS and PDIFF statements were used to separate means and create letter groupings for all significant terms in Proc Mixed. When there were no significant differences between sampling depth (0-5 cm and 5-15 cm) or year (Yr1, Yr2, and Yr3) data were combined (SMB data only). Although the site design was a split-plot, each trial only sampled one of the four split plots that corresponded with the correct phase of the rotation within each main plot (Figure B1). Therefore, the split was not included in the statistical model.

3.3 Results

3.3.1 Soil Microclimate

Soil microclimate (10 cm depth) for the Bible Hill site varied more among termination methods in Trial 1 than Trial 2 during the sampling period (Figure 3.1). In Trial 1 soil temperature and moisture were significantly affected by Gmr termination method (P=0.0080 and P=0.0005, respectively). Soil temperature in no till plots was significantly lower than fall tilled or clover plots (by 0.9°C in both cases) and wetter than all other termination methods (by 1.9% compared to spring tilled, 4.5% compared to fall filled, and 7.5% compared to clover). In Trial 2 only moisture was significantly affected (P<0.0001) with no-till and spring tilled plots being wetter than fall tilled and clover, and fall tilled plots being significantly wetter than clover plots.

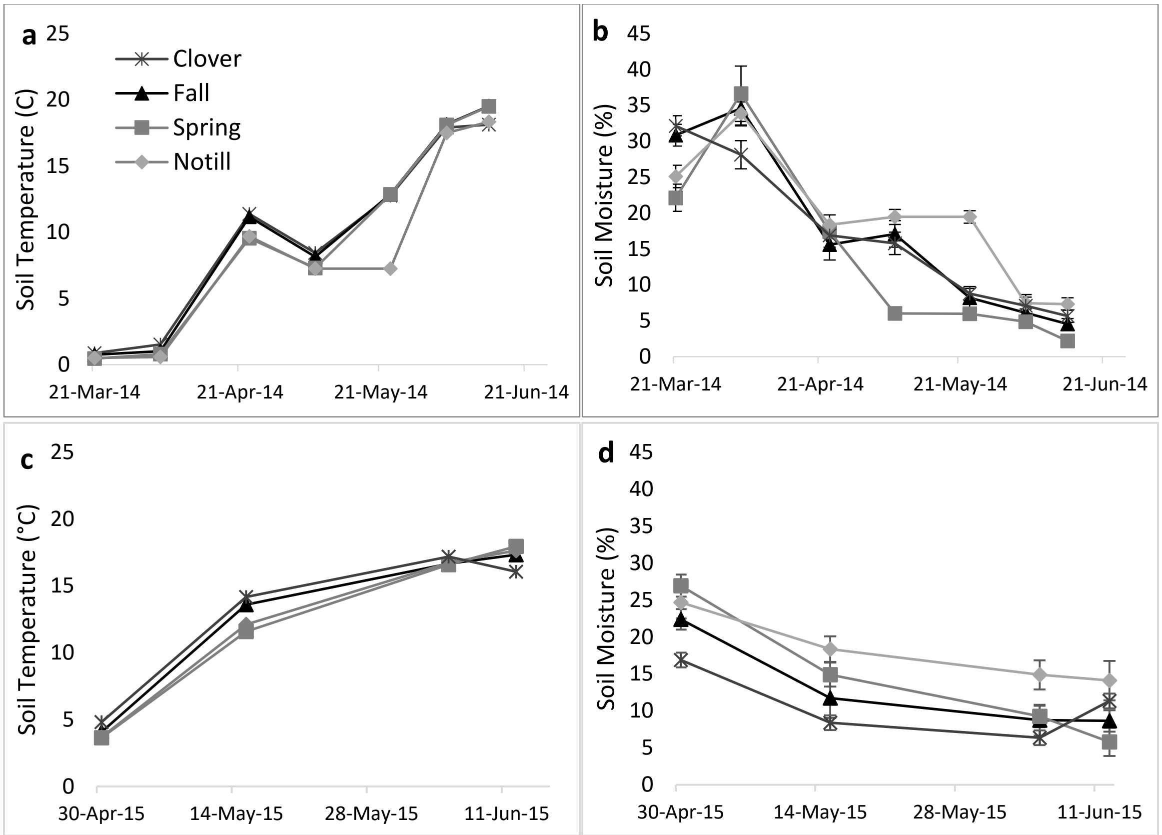


Figure 3.1. Microclimate conditions in Bible Hill, NS showing (a) soil temperature (°C) Trial 1, (b) soil temperature (°C) Trial 2, (c) soil moisture (%) Trial 1, and (d) soil moisture (%) Trial 2. Values are the average of nine values, three taken from each treatment in three blocks. Error bars represent one standard error. Error bars are not presented on soil temperature because they were too small to be clearly visible.

3.3.2 Microbial Biomass

SMB-C was significantly affected by termination method and sampling date in Trial 1 (Table 3.4). Fall-till and no-till plots had significantly greater SMB-C than all other termination treatments two weeks after spring tillage. Spring tilled plots also had high SMB-C values at the two-week sampling time. Clover and reference samples at this sampling date and all other sampling dates produced much lower SMB-C values.

Table 3.4. ANOVA table for microbial biomass carbon at Bible Hill for Trial 1. Termination refers to termination method, which were (i) clover (tilled in fall), (ii) fall

(tilled in fall), (iii) spring (tilled only in spring), (iv) no-till (rolled only), and (v) reference (undisturbed field margin). Depth refers to soil depth, which were 0-5 cm and 5-15 cm. Time is the sample date, which were two weeks after spring tillage was completed, two months after spring tillage, and four months after spring tillage.

Effect	Num DF	Den DF	F- Value	P-Value
Time	2	56	15.52	<.0001
Termination	4	56	3.67	0.0101
Termination*Time	8	56	3.73	0.0015
Depth	1	56	0.21	0.6499
Depth*Time	2	56	0.30	0.7435
Termination*Depth	4	56	0.41	0.8013
Termination*Depth*Time	8	56	0.39	0.9212
Block	2	56	1.46	0.2417

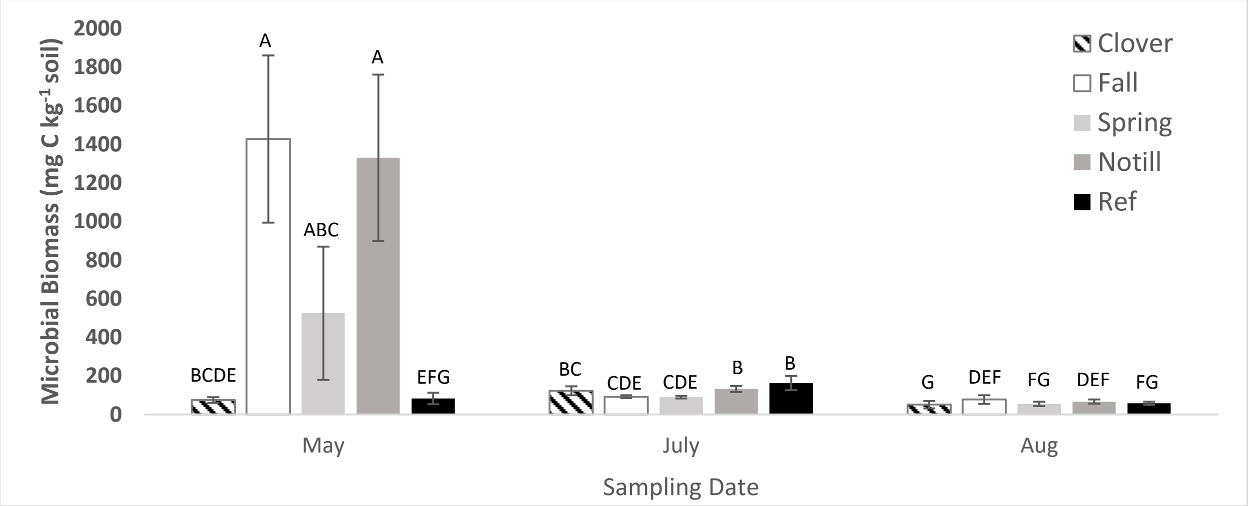


Figure 3.2. Soil microbial biomass carbon in Bible Hill, NS by termination method and sampling date for Trial 1. Termination methods are (i) clover (tilled in fall), (ii) fall (tilled in fall), (iii) spring (tilled only in spring), (iv) no-till (rolled only), and (v) reference (undisturbed field margin). May is two weeks after spring tillage, July is two months after spring tillage, and August is four months after spring tillage. Different letters indicate significant differences by LSD at α=0.05. Bars represent one standard error.

3.3.3 Earthworms

3.3.3.1 Bible Hill, NS

Earthworm abundance (earthworms/m^2) and biomass (g) had similar responses to treatments, so only abundance is presented. In Trial 1, both spring and fall tilled plots had lower earthworm abundance compared to no-till in Yr1 and Yr2 (Table 3.5, Figure 3.3). By Yr3 of the rotation, there were no differences between the three termination methods. The same pattern occurred in Trial 2, although only the overall termination effect was significant (Table 3.5), not the interaction between termination and year. In all years the reference samples had greater earthworm abundance than all cultivated plots except in 2014 (Yr1 Trial 1).

Table 3.5. ANOVA table for earthworm abundance at Bible Hill. Termination refers to termination method, which were i) fall (tilled in fall), (ii) spring (tilled only in spring), (iii) no-till (rolled only), and (iv) reference (undisturbed field margin).

	Trial 1				Trial 2			
Effect	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
Year	2	22	18.74	<.0001	2	22	5.5	0.0115
Termination	3	22	18.69	<.0001	3	22	24.6	<.0001
Termination x Year	6	22	6.43	0.0005	6	22	2.3	0.071
Block	2	22	0.13	0.8799	2	22	1.9	0.1735

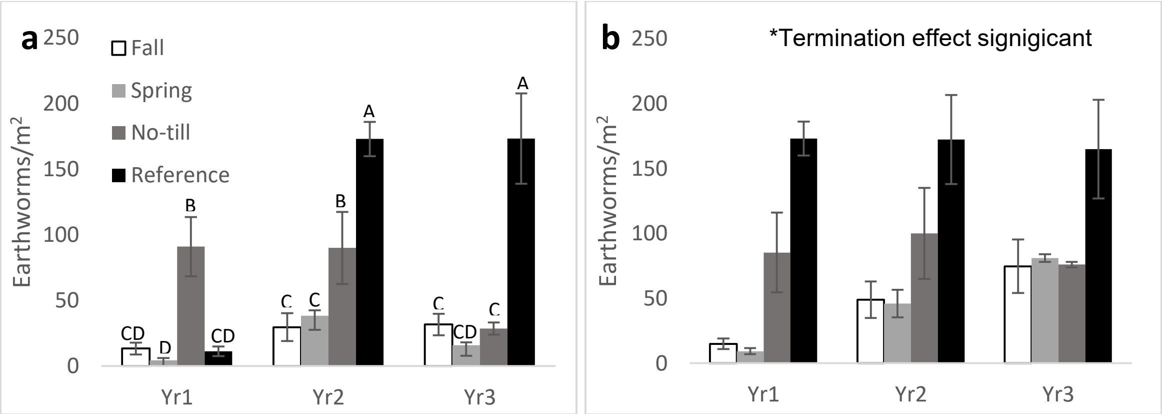


Figure 3.3. Earthworm abundance (per m^2) in Bible Hill, NS by year after Gmr termination showing (a) Trial 1 and (b) Trial 2. Treatments are (i) fall (tilled in fall), (ii) spring (tilled only in spring), (iii) no-till (rolled only), and (iv) reference (undisturbed field margin). Yr1 is the wheat phase, the year following Gmr termination, yr2 is the partial fallow phase, and Yr3 is the soybean phase. Different letters indicate significant differences by LSD at α=0.05 and are only included when the effect of termination x year was significant. Bars represent one standard error.

3.3.3.2 Carman, MB

Earthworms were not significantly affected by treatments in either trial (P=0.634 in Trial 1 and P=0.620 in Trial 2, Figure 3.4). Because only one reference sample was taken per block in Carman they were not included in statistical analysis but are included in the figure for comparison. Overall earthworm abundance in Carman was much lower than in Bible Hill, with about seven times higher abundance of earthworms in Bible Hill (average of 9.4 earthworms/m^2 in Carman compared to 67.8 earthworms m^2 in Bible Hill).

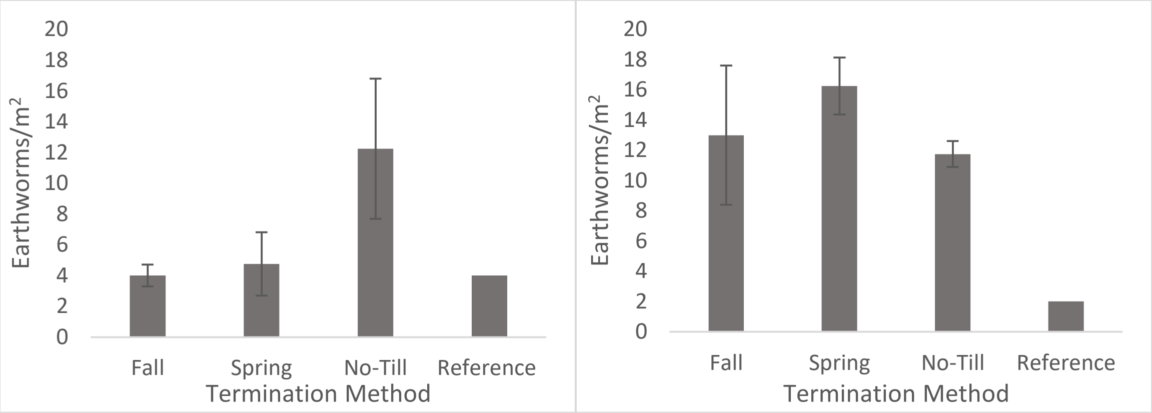


Figure 3.4. Earthworm abundance (per m^2) at Carman, MB in the year following Gmr showing (a) Trial 1 and (b) Trial 2. Treatments are (i) (i) fall (tilled in fall), (ii) spring (tilled only in spring), (iii) no-till (rolled only), and (iv) reference (undisturbed field margin). At Carman the reference only has one sample per trial and is included for visual comparison only.

3.3.4 Beetles

A total of 5111 beetles were caught over both trials in Yr1 and Yr2 for the period of June-August at Bible Hill; 3165 in Trial 1 and 1946 in Trial 2 (Table 3.6). More beetles were captured in Trial 1, partly due to Trial 1 having 12 sample collections and Trial 2 having 11 (due to weather restricting collection in Trial 2) and partly due to the increase in crow predation each year of beetle collection (pers. obs.). Termination method had no effect on beetle captures in either trial (Table 3.7), although there was a non-significant trend for lower activity-density in beetles in no-till plots (Figure 3.5). *P. melanarius* captures were unaffected by termination method. *Harpalus* ssp. captures were significantly affected by termination method in Trial 2 only and only in Yr1 (P=0.009). Significantly fewer *Harpalus* ssp. were captured in no-till and reference samples than both clover or fall tilled plots and spring tilled plots has fewer captures than clover plots.

Table 3.6. Cumulative captures of all beetles, *P. melanarius*, *Harpalus* sp., and spiders in Yr1 and Yr2 from June to August for both trials for Bible Hill.

Taxa	Trial 1	Trial 2	Total
Total Beetles	3165	1946	5111
P. melanarius	1013	549	1358
Harpalus ssp.	1146	666	1762
Spiders	790	369	1159

Table 3.7. ANOVA output for effect of termination method on cumulative beetle and spider captures. Yr1 is the cumulative captures for the year after the green manure termination (wheat phase) and Yr2 is the cumulative captures for the year after the wheat phase (partial fallow/fall rye). Total is the cumulative captures over both Yr1 and Yr2.

	Trial 1			Trial 2		
	DF	F-Value	P-Value	DF	F-Value	P-Value
Total Beetles						
Yr1	4	2.70	0.108	4	1.05	0.439
Yr2	4	1.09	0.421	4	0.66	0.639
Total	4	1.83	0.216	4	1.3	0.347
Pterostichus melanarius						
Yr1	4	3.68	0.055	4	0.72	0.599
Yr2	4	0.79	0.562	4	0.76	0.575
Total	4	2.27	0.150	4	0.65	0.640
***Harpalus* ssp.**						
Yr1	4	2.37	0.139	4	7.22	0.009
Yr2	4	1.24	0.367	4	0.23	0.912
Total	4	1.89	0.206	4	2.37	0.140
Spiders						

Yr1	4	2.47	0.128	4	6.17	0.014
Yr2	4	4.75	0.029	4	1.02	0.453
Total	4	6.75	0.011	4	4.23	0.040

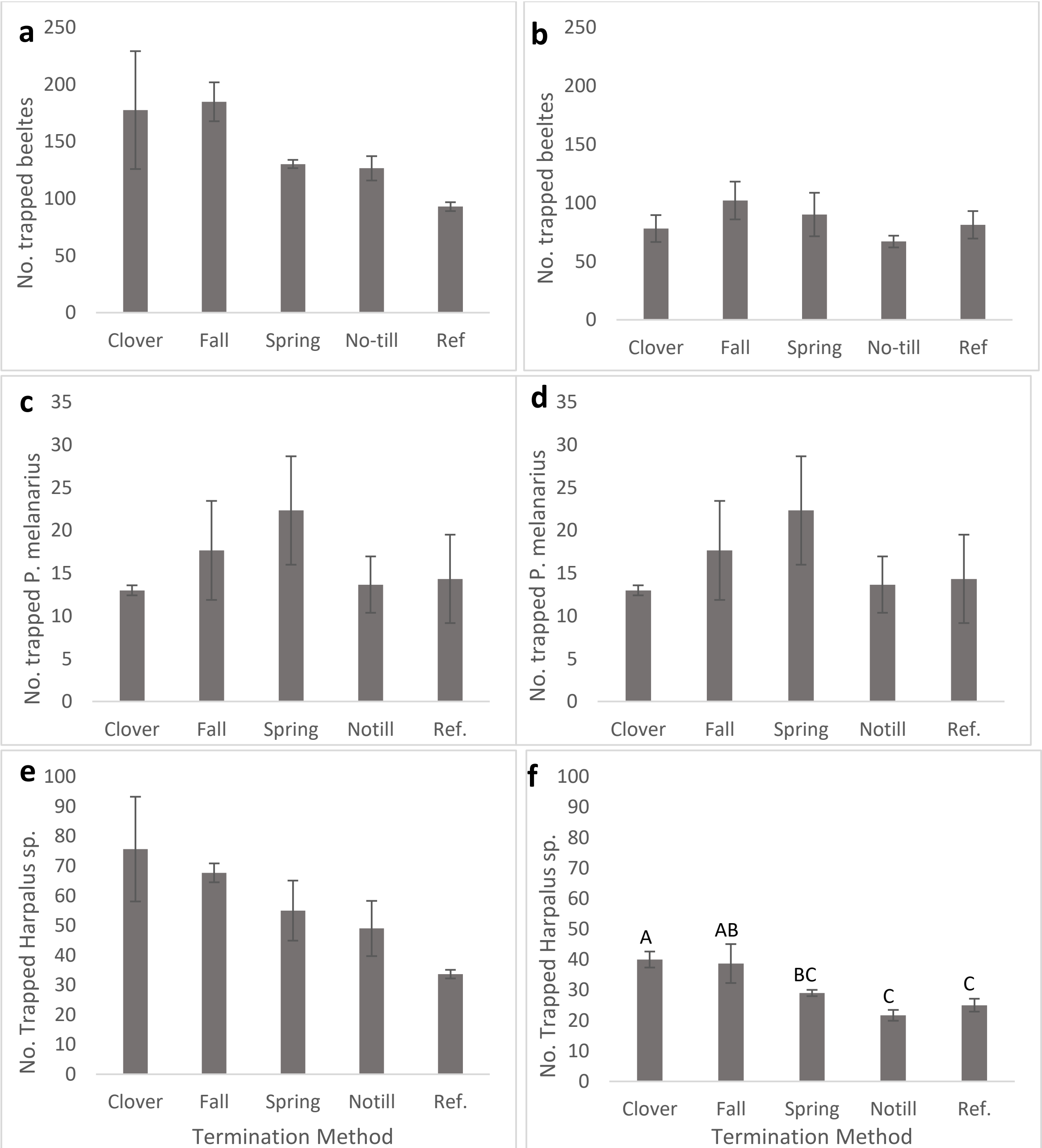


Figure 3.5. Cumulative beetle captures from Yr1 of both trials showing (a) beetles Trial 1, (b) beetles Trial 2, (c) *P. melanarius* Trial 1, (d) *P. melanarius* Trial 2, (e) *Harpalus* ssp. Trial 1, and (f) *Harpalus* ssp. Trial 2. Treatments are (i) clover (2 years of red clover fall tilled), (ii) fall (HVO tilled in fall), (iii) spring (HVO tilled only in spring), (iv) no-till

(HVO rolled only), and (v) reference (undisturbed field margin). Different letters indicate significant differences by LSD at α=0.05 and are only included when the effect of termination x year was significant. Bars represent one standard error.

3.3.5 Spiders

A total of 1159 spiders were caught over both Trials in Bible Hill. Spiders were significantly affected by termination method in Trial 1 (Table 3.7). Reference samples had a greater number of spiders caught than any tilled plots (fall tilled, spring tilled, or clover plots) and no-till plots had great capture rates than spring tilled or clover plots (Figure 3.6). Termination method was significant in the model for Trial 2 (Table 3.7) but this was due to significantly greater capture rates in reference samples and not due to the three termination methods. There were no significant differences between the four termination methods.

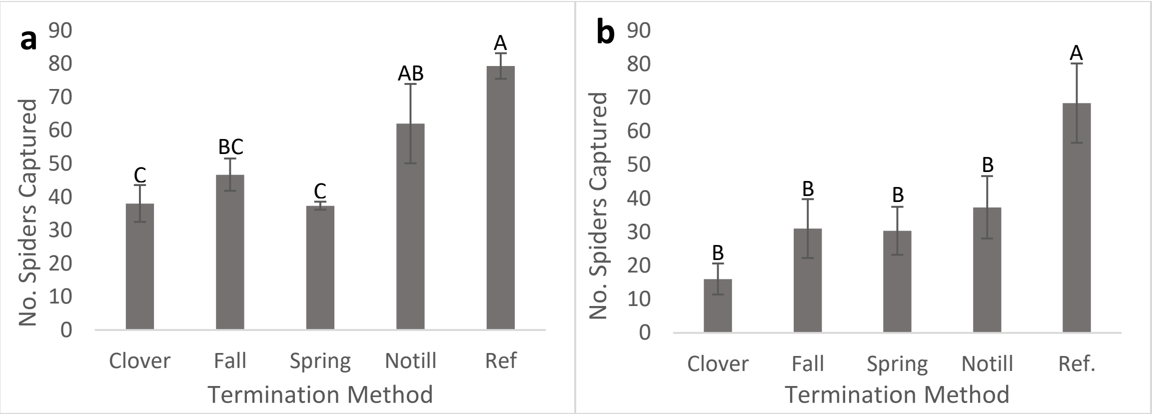


Figure 3.6. Cumulative spider captures showing (a) spiders trapped in Trial 1, Yr1 and Yr2 combined and (b) spiders trapped in Trial 2, Yr1 and Yr2 combined. Treatments are (i) clover (2 years of red clover fall tilled), (ii) fall (HVO tilled in fall), (iii) spring (HVO tilled only in spring), (iv) no-till (HVO rolled only), and (v) reference (undisturbed field margin).Yr1 is the wheat phase, the year following Gmr termination, yr2 is the partial fallow phase, and Yr3 is the soybean phase. Different letters indicate significant differences by LSD at α=0.05. Bars represent one standard error.

3.4 Discussion

I found SMB in the range of 50-200 mg C kg^{-1} soil except at the two-week after termination sampling time in plots that had previously been under hairy vetch/oat, where values were much greater. The lower range is similar to a Gmr study that also took place on a sandy loam soil (Liang et al., 2014). However, the values in fall tilled, spring tilled, and no-till plots two-weeks at the first sampling data are much greater than those reported in Liang et al. (2014). This could be due to the quick decomposition of organic material as the Gmr breaks down in the spring. Liang et al. (2014) used a fall planted Gmr that was a mixture of legumes and was still living in the spring whereas the present study used a spring planted Gmr that had winter-killed, likely leading to faster decomposition once spring temperatures increased. The higher values in the fall tilled and no-till plots relative to the spring tilled plots could be due to the increased time since disturbance, as the spring tilled plots were the most recently tilled. The lack of SMB flush in clover and reference samples in May indicates the HVO Gmr is contributing to the SMB flush. Crop type and quality of crop residues have been shown to influence SMB (Liang et al., 2014). The flush of SMB in the present study was short lived and two months later SMB values were lower in all plots. While there are significant differences after at the two- and four-month sampling times, the effects are largely idiosyncratic. In a meta-analysis of crop effects on SMB, McDaniel et al. (2013) found a stronger effect of crop type than tillage, similar to the present study.

Tillage significantly decreased earthworm abundance but the population recovered by the third year after treatments in Bible Hill, as predicted. This is similar to a five-year organic potato/grain/forage rotation where earthworm numbers were lower in the potato phase but recovered to reference field (permanent pasture) levels by the fourth year of the rotation (Nelson et al., 2009). However, in the present study earthworm abundance in the Gmr plots did not reach the levels in the reference area, as in Nelson et al. (2009). In Trial 1, earthworm abundance in fall and spring tilled plots did not increase to the level in no-till, as in Trial 2, but rather abundance in no-till decreased to the level of fall and spring tilled. This could be due to the higher temperature on the day of sampling for Yr3 of Trial 1 (22.2°C, Table 3.3). Optimal temperature for earthworm function is 10-20°C and they burrow deeper to escape higher temperatures (Curry, 2004).

Earthworms responded the same way to tillage in Carman in Trial 1, although high variation in earthworm abundance in no-till terminated plots resulted in this being non-significant. The overall lower earthworm populations at Carman are likely partially due to the coarser soil texture (72-76% sand in Carman compared to 63-67% sand in Bible Hill), which has been found in other studies (Eriksen-Hamel et al., 2009). The lower numbers of earthworms likely contributed to the inability to detect treatment effects.

Earthworms are often food limited (Curry, 2004) and the placement of organic matter at the soil surface could draw earthworms toward the soil surface. In a study of high and low corn residue under conventional no-till management, Abail and Whalen (2018) found greater earthworm abundance under high residue. Renkema et al. (2012) found greater earthworm abundance when compost was used as mulch on the soil surface compared to pine needle mulch or no mulch. It is also possible the lower temperature and greater soil moisture under no-till mulch observed in Trial 1 in Bible Hill and contributed to greater earthworm abundance in no-till plots, as those conditions are favourable to earthworms (Curry, 2004).

The only detectable effect of termination treatment on beetle activity-density was seen for *Harpalus* ssp. in Trial 2 in Bible Hill, with greater captures in tilled plots compared to no-till and reference. Jabbour et al. (2015) found that tillage intensity (full inversion vs. chisel plow) did not affect capture rates but did affect community composition of soil arthropods. They only saw an effect of tillage on soil arthropods when tillage treatments were maintained beyond one year, seeing only cover crop type effects in Yr1. The present study did not see a difference in activity-density due to cover crop type between fall-tilled plots with clover or with hairy vetch. Although it was only significant in Trial 2 for *Harpalus* ssp., the overall trend of beetle captures was higher under tilled termination of Gmr (clover, fall-tilled, and spring-tilled) compared to no-till and reference samples. In Pennsylvania, Rivers et al. (2017) have shown presence of rolled hairy vetch mulch late in the season (Aug-Oct) may benefit *Harpalus* ssp. as they are generally considered fall breeders and sensitive to fall tillage, but that was not seen in the present study. Blubaugh and Kaplan (2015) found greater number of in *Harpalus* ssp. larvae for plots with lower disturbance but did not see the same preference in adults, possibly due to the more limited mobility of the larvae. A groundcover trial in an apple orchard had activity-density of beetles in tilled and herbicide-treated plots compared to straw or pine bark mulch (Miñarro and Dapena, 2003). Mulch may create a physical barrier to movement, causing lower activity-density regardless of the number of beetles present, especially the rolled HVO that remained rooted to the soil.

If mulch is creating a physical barrier, it would be expected that smaller macrofauna would move through the mulch more easily, resulting in higher captures of smaller-bodied organisms. Larger beetles were shown to prefer un-mulched plots in a highbush blueberry study (Renkema et al., 2015). However, *Harpalus* ssp. are smaller than *P. melanarius* (Larochelle and Larivière, 2003) yet still had higher activity-density in tilled plots while *P. melanarius* showed no effect. More research is required to determine how rolled Gmr mulch affects macrofauna based on body size.

Spider captures showed the opposite trend to *Harplaus* sp., with greater capture rates in no-till and reference samples compared to tilled termination treatments. Arachnids are more sensitive to soil disturbance than carabids or staphylinids and seek refuge in plots with plant cover after soil disturbance (Thorbek and Bilde, 2004). Jiang et al. (2018) found no significant differences in Araneae comparing conventional tillage and no-till with or without corn stover mulch. Tamburini et al. (2016) found Araneae to be more sensitive to tillage than carabid beetles, as in the present study. Whalen et al. (2007) found greater Araneae abundance and lower Coleoptera activity-density in no-till corn-soybean compared to conventional till, supporting the findings of this study.

As hypothesized, SMB was affected by treatments but the effect was very transient. Earthworms appear to be the most sensitive of the macrofauna studied here to tillage. They showed the strongest negative response to the tilled termination treatments and took two full years after tillage to return to levels found in the no-till plots. In this type of organic grain rotation it appears occasional tillage could be performed without causing long-term damage to the earthworm population, similar to the findings in Nelson et al. (2009). No-till termination showed an advantage for spider abundance and a disadvantage for *Harpalus* ssp. giving no clear effect on possible natural predator benefits from no-till termination. Response of spiders and beetles to tillage in the no-till Gmr systems appears to be similar to conventional tillage systems. Analyzing larger-bodied organisms over entire rotations in agricultural systems may give a clearer picture of their population dynamics and the longer-term effects of disturbance on soil organisms.

Chapter 4. Impact of mulch and tillage on soil biology in a green manure system

4.1 Introduction

No-till green manure (Gmr) management is distinct from other no-till systems due to the large biomass and, in the case of a hairy vetch/oat (*Vicia villosa* Roth and *Avena sativa* L.) Gmr, the tightly woven mat of mulch left still rooted to the soil surface (Figure 4.1). This creates a unique physical environment at the soil surface. The biomass of the mulch created by a no-till full-season Gmr has been recorded as high as 7.6 Mg ha^{-1} with a hairy vetch/barley (*Hordeum vulgare* L.) mix in Carman, MB (Halde et al., 2014).

Incorporating no-till Gmr into crop rotations is expected to bring many of the benefits of conventional no-till agriculture (Trewavas, 2004). One of those benefits is improved soil biodiversity (FAO, 2018). However, the unique combination of lack of soil disturbance and presence of mulch could affect soil organisms in unexpected ways.

Figure 4.1. Hairy vetch/oat mulch before wheat planting in 2014 in Bible Hill, NS. Hairy vetch/oat had been planted in the spring of the previous year, was rolled in the late summer with a crop roller, and winter killed.

For many soil dwelling organisms, such as earthworms, tillage has consistently been associated with lower population abundance (Eriksen-Hamel et al., 2009; Johnson-Maynard et al., 2007; Wardle, 1995). The responses of beetles to tillage is less clear. A study by Holland and Luff (2000) found that 20 taxa preferred inversion tillage and 21 preferred minimum tillage. Spiders were captured more often in no-till compared to conventional till in a two year study of corn *(Zea mays* L.) in Spain (Rodríguez et al., 2006).

For organisms that move on the soil surface, such as beetles and spiders, habitat structure and microclimate changes created by the mulch could have a strong effect on movement and abundance. Mulch changes the soil environment directly below it, creating cooler, wetter conditions. Podolsky et al. (2016) found a decrease in 2.8°C under a barley–pea (*Pisum sativum* L.) rolled Gmr compared to full inversion tillage in the spring. Earthworms have been shown to be affected by microclimate changes, preferring cooler and wetter habitats (Curry, 2004). However, despite large differences in surface residue (7–9 Mg dry matter ha^{-1} year^{-1}), earthworm populations did not increase in no-till corn production (Eriksen-Hamel et al., 2009). Carabid beetles showed preference for blueberry plots mulched with compost over pine needle mulch or no mulch (Renkema et al., 2012).

Weed density studies have shown greater beetle populations with increased weed cover, often driven by the dominant species *Pterostichus melanarius* (Hummel et al., 2012; Kulkarni et al., 2017; Saska et al., 2014). However, as mentioned above, the mat of mulch left by a rolled Gmr presents a physical environment unlike that of dense weed cover. One of the few studies on beetles in rolled Gmr found high activity-densities in rolled hairy vetch/ triticale (x *Triticosecale* Wittmack) compared to other phases of the crop rotation (Rivers et al., 2017).

Chapter 3 showed that soil organisms can respond differently to no-till Gmr management. *Harpalus* ssp. had greater captures in tilled treatments but it is unclear if this is due to a deterrent effect of the Gmr mulch or by a preference for tilled soil. Spiders and earthworms showed a preference for undisturbed soil (no-till and reference samples). This could be due to the cover provided by the mulch and plant cover or the lack of soil disturbance. The objective of the current study was to investigate the effects on soil organisms of these two factors, soil disturbance and mulch. It was hypothesized that there would be an additive effect of mulch and tillage on soil organisms, but that mulch presence would be the more important factor driving soil organism responses due to its altering of the physical characteristics and microclimate of the soil surface.

4.2 Methods

4.2.1 Site Description and Treatments

Experimental plots were located at the same research sites as in the previous chapters (Chapter 2 and 3); at the Brookside Experimental Site for Organic Research in Bible Hill, NS and at the Ian N. Morrison Research Farm in Carman, MB. Plots were established adjacent to the plots used for previous chapters.

4.2.1.1 Bible Hill, NS

A split-plot design in four blocks was established in 2015 and again in 2016 at a second location a few meters from the first. Blocks were used to minimize variation in soil and drainage among replications and split-plots were used to accommodate the tillage equipment. Main plots were 10 m by 28 m with a 5 m buffer between plots. There were three treatments at the main plot level: i) tilled (full-inversion tillage with a moldboard plow to a depth of approximately 15 cm in late summer), ii) no-till (mowed only), and iii) fallow, which was left undisturbed after experiment initiation except for periodic mowing to control weeds. There were two treatments at the split-plot level: with (+) or without (-) mulch for a total of 24 plots. Full treatment descriptions and notations are explained in Table 4.1. Treatments were chosen to expand on the findings from Chapter 3. All plots were planted with a hairy vetch/oat (HVO)mixture as in the main experiment in the spring of the establishment year except fallow plots. In late summer all vetch plots were mowed with a hay mower. Aboveground biomass was then manually removed from the tilled plots and the no-till- plots. The plots under the tilled treatment were then tilled with a moldboard plow. The biomass that was removed from the tilled+ plots was then manually spread evenly over the plots to create a mulch layer. The fallow+ plots had the biomass from one of the –mulch plots spread over to create a mulch layer. After the initial tillage there was no more soil disturbance in any plots. The following year wheat (*Triticum aestivum* L.) was seeded on all plots except the fallow treatment. Dates of field operations can be found in Table 4.2. Mulch biomass was sampled the following spring on 22 April 2016 and 5 May 2017 from three 0.25 m^2 quadrats per plot and dried at 55°C until constant weight to determine mulch biomass.

Table 4.1. Treatment descriptions for Bible Hill and Carman. All six treatments were present at the Bible Hill site. The Carman site had only the first four treatments in the table; the fallow treatments were not included at that site.

Treatment	Soil Disturbance	Mulch Presence

Tilled-	Full inversion tillage to approximately 15 cm depth in late summer	Aboveground Gmr biomass removed before tilling
Tilled+	Full inversion tillage to approximately 15 cm depth in late summer	Aboveground Gmr biomass removed before tilling but placed back on the soil surface after tilling
No-till-	None	Aboveground biomass removed after mowing
No-till+	None	Aboveground biomass remained after mowing
Fallow-	None	None
Fallow+	None	Aboveground biomass from one (-) mulch plot added on the soil surface

4.2.1.2 Carman, MB

Experimental design at Carman was a RCBD in four blocks. Treatments were similar to Bible Hill with the exception of the fallow treatment, which was not included, giving 16 plots at this location. Only one trial was conducted which commenced with planting of hairy vetch/barley in spring 2015. Plot sizes at Carman were 10 m by 8 m with a 2 m buffer between plots. Wheat was planted the following year. No-till+ plots were undisturbed. Mulch from No-till- and Tilled- plots was cut with a hand sickle and removed. For Tilled+ the mulch was rolled up, tilled, and unrolled back over the plot, leaving the matted nature of the mulch intact. Varieties and seeding rates are given in Appendix B (Table B1)

Table 4.2. Schedule of field operations at Bible Hill, NS for 2016 trial and 2017 trial and for the trial at Carman, MB.

Field Operation	Date 2016 Trial (D/M/Y)	Date 2017 Trial (D/M/Y)	Date Carman Trial (D/M/Y)
Gmr Planting	9/6/15	27/5/16	5/6/15
Gmr Mowing and Tilling	26/8/15-28/8/15	7/9/16-9/9/16	13/10/15
Wheat Planting	18/5/16	24/5/17	29/4/16
Earthworm Sampling	23/6/16-25/6/26	6/6/17-8/6/17	15/6/16
Pitfall traps installed	31/5/16	7/6/17	14/6/16

4.2.2 Soil Organism Sampling

Earthworms were sampled with the same method as Chapter 3 in the spring by excavating two 0.25 m^2 quadrats per plot to depth of 15 cm and manually extracting earthworms which were counted and weighed within 48 h of collection. Beetles and spiders were sampled throughout June-August in the same manner as in the main experiment. Two pitfall traps were established 1 m apart in the centre of each plot with a partition in-between to guide beetles into the traps. Traps were opened for 48 h every two weeks, weather permitting. The contents of the pitfall traps were frozen until they could be analyzed. *Pterostichus melanarius* Illiger and *Harpalus* ssp. were counted, as in Chapter 3, but due to low captures *Harpalus* ssp. were not included for Carman. In addition to spiders there was a sufficient number of opiliones to include in the analysis of Carman as well. Opiliones are also predators and have been shown to attack agricultural pests (Newton and Yeargan, 2001; Rivers et al., 2018). Because macrofauna were sampled for one year only, the two Bible Hill trials will be referred to by year (2016 and 2017).

4.2.3 Statistical Analysis

Pitfall trap captures for beetles, spiders, and opiliones were analyzed as a cumulative value (June-August). Macrofauna were analyzed by an ANOVA using the general linear model function in Minitab® 17.3.1. Assumptions of the models were checked using a plot of residuals versus fits for constant variance and a combination of a normal probability plot and the Andersen-Darling test for normality. Data were transformed to meet the assumptions of normality and constant variance if required. Level of significance was set at $\alpha=0.05$. Fisher's LSD was used to separate means and create letter groupings for all significant terms.

4.3 Results

4.3.1 Bible Hill, NS

Biomass of the mulch remaining in the spring was 3.3 ± 0.5 Mg ha^{-1} for 2016 and 3.3 ± 0.2 Mg ha^{-1}. Earthworm abundance was significantly affected by treatments in 2017 only, with greater abundance in mulched plots (Table 4.3, Figure 4.2). 2016 also had greater earthworm abundance in mulched plots but the difference between treatments was not significant.

In 2016, 1077 beetles were captured, of which 20% were *Harpalus* ssp. and 11% were *P. melanarius*. In 2017, 1859 beetles were captured, of which 35% were *Harpalus* ssp. and 26% were *P. melanarius*. Beetle captures were not significantly affected by treatments. Four hundred and sixty-three spiders were captured in 2016 and 692 in 2017. Spider captures were significantly affected by treatments in 2016 only, with greater captures in plots with mulch (Table 4.3, Figure 4.2)

Table 4.3. ANOVA table for Bible Hill. Tillage had three levels, i) full-inversion tillage, ii) no-tillage, and iii) fallow. Mulch had two levels, mulch present (+) and no mulch present (-).

	2016			2017		
Source	DF	F-Value	P-Value	DF	F-Value	P-Value
Earthworms						
Tillage	2	1.47	0.261	2	2.64	0.104
Mulch	1	0.81	0.382	1	5.24	0.037
Tillage*Mulch	2	0.95	0.408	2	0.31	0.736
Block	3	2.1	0.143	3	4.5	0.019
Carabids						
Tillage	2	1.74	0.209	2	0.99	0.395
Mulch	1	1.24	0.284	1	0.08	0.776
Tillage*Mulch	2	0.59	0.565	2	0.14	0.873
Block	3	0.83	0.496	3	0.26	0.855
P. melanarius						
Tillage	2	2.81	0.092	2	0.21	0.813
Mulch	1	0.75	0.402	1	3.19	0.094
Tillage*Mulch	2	0.2	0.82	2	0.75	0.489
Block	3	1.16	0.356	3	8.5	0.002
Harpalus ssp.						
Tillage	2	0.46	0.638	2	1.29	0.304
Mulch	1	0.72	0.411	1	1.5	0.24
Tillage*Mulch	2	0.07	0.936	2	0.43	0.658
Block	3	3.08	0.06	3	1.41	0.279
Spiders						
Tillage	2	2.21	0.144	2	1.39	0.279
Mulch	1	8.73	0.010	1	0.34	0.568
Tillage*Mulch	2	0.45	0.646	2	1.01	0.387
Block	3	1.43	0.274	3	2.56	0.094

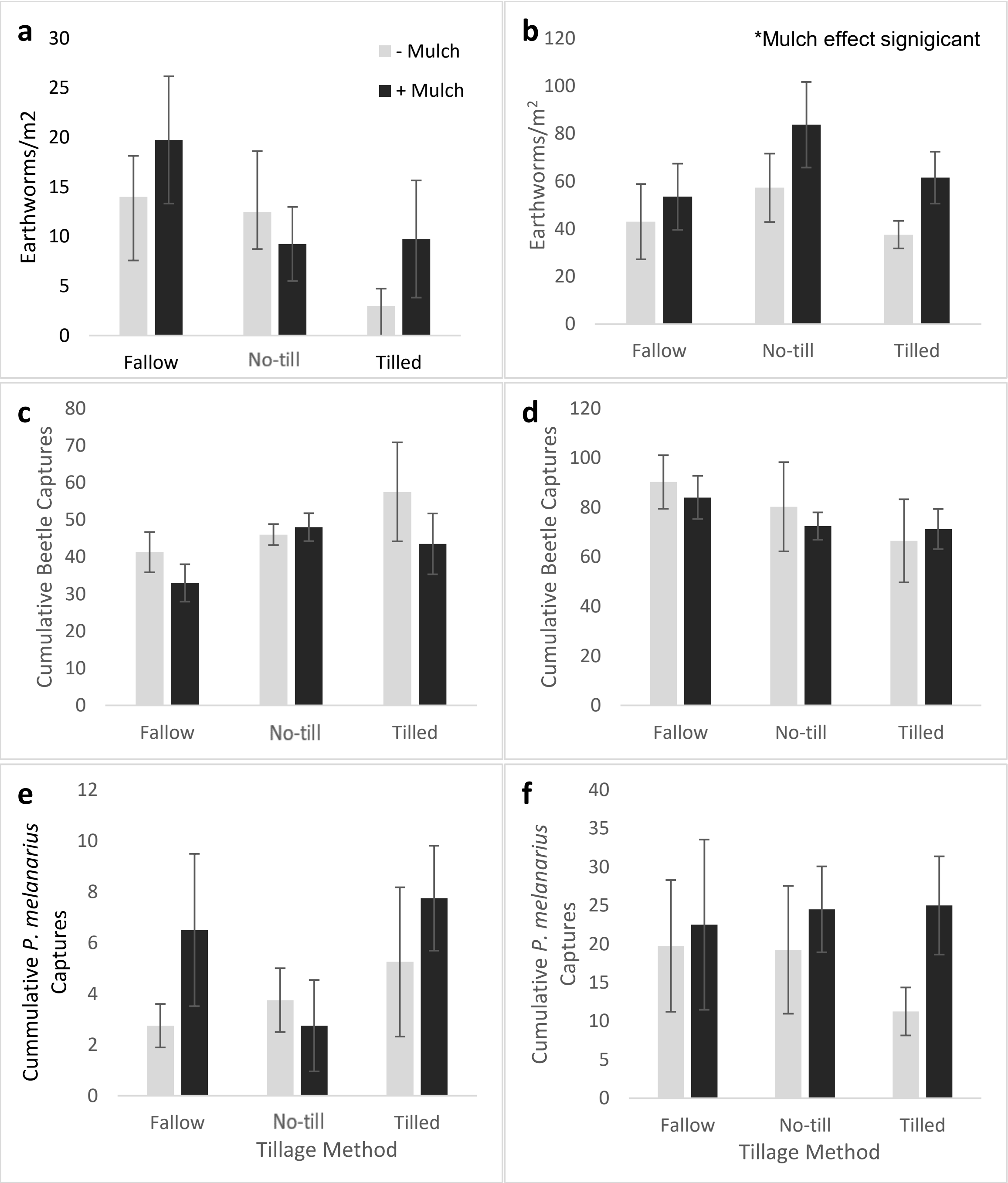
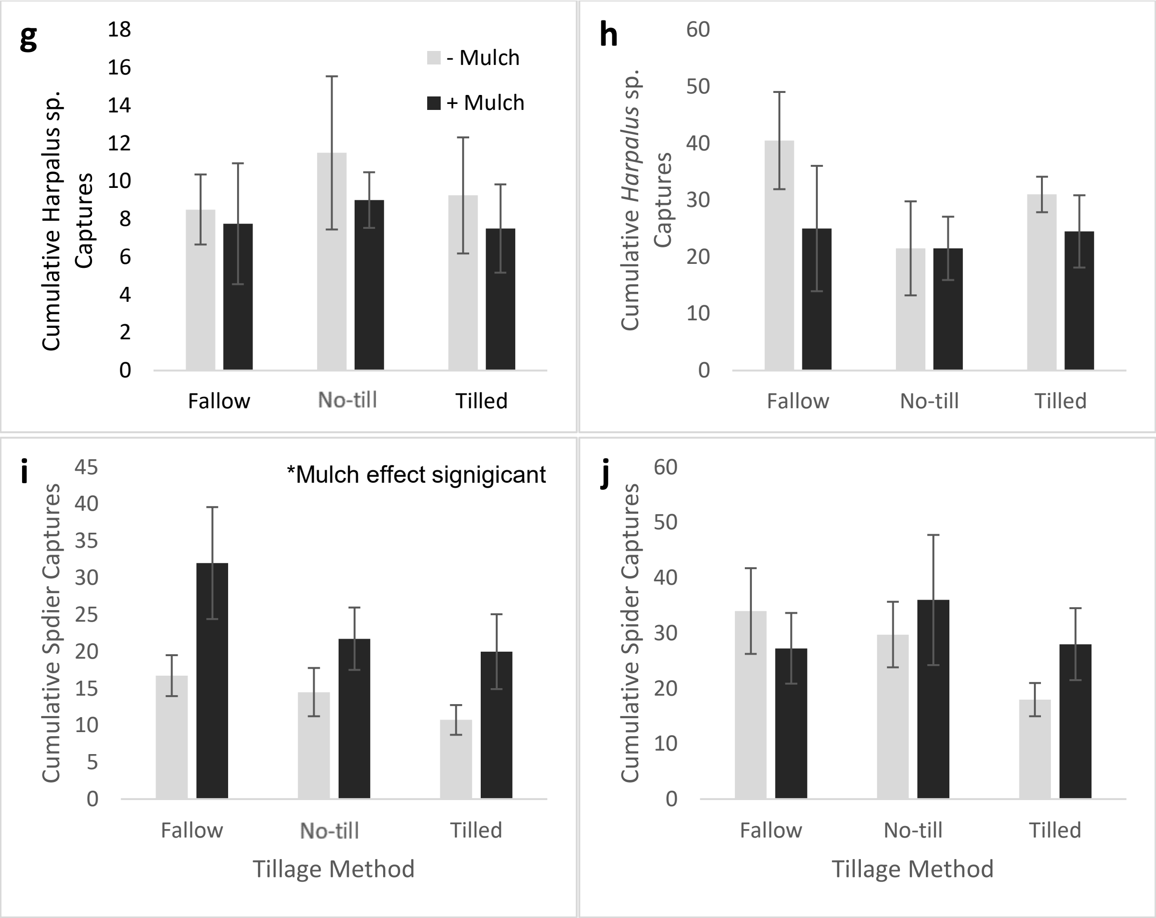


Figure 4.2. Soil organisms by tillage method and mulch presence at Bible Hill. Tillage had three levels, i) full-inversion tillage, ii) no-tillage, and iii) fallow. Mulch had two levels, mulch present (+) and no mulch present (-). (a) Earthworm abundance 2016, (b) earthworm abundance 2017, (c) cumulative captures for all beetles 2016, (d) cumulative captures for all beetles 2017, (e) cumulative captures for *P. melanarius* 2016, (f) cumulative captures for *P. melanarius* 2017. g. cumulative captures for *Harpalus* ssp. 2016, (h) cumulative captures for *Harpalus* ssp. 2017, (i) cumulative captures for spiders 2016 and, (j) cumulative spider captures for 2017.



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4.3.2 Carman, MB

Significantly more earthworms were collected in the mulched treatments regardless of tillage treatment (1.8 earthworms m^{-2} in un-mulched compared to 7.5 earthworms m^{-2} in mulched, Table 4.4, Figure 4.3). Three hundred and seventy-four beetles were captured, 32% of which were *P. melanarius,* neither of which were affected by treatments. Sixty-nine spiders and 479 opiliones were captured. Spider captures were not affected by tillage or mulch treatments (Table 4.4, Figure 4.3). Opiliones were significantly affected by mulch treatments, with greater captures in plots with no mulch (35.0 average captures per plot in un-mulched compared to 24.9 per plot in mulched).

Table 4.4. ANOVA table for Carman. Tillage had two levels, full-inversion tillage and no-tillage. Mulch had two levels, mulch present (+) and no mulch present (-).

Source	DF	F-Value	P-Value
Earthworms			
Tillage	1	1.30	0.283
Mulch	1	8.65	0.016
Tillage*Mulch	1	0.37	0.560
Block	3	3.04	0.086
Beetles			
Tillage	1	1.22	0.298
Mulch	1	0.03	0.872
Tillage*Mulch	1	2.74	0.132
Block	3	0.91	0.476
P. melanarius			
Tillage	1	0.01	0.912
Mulch	1	0.64	0.445
Tillage*Mulch	1	0.64	0.445
Block	3	2.26	0.150
Spiders			
Tillage	1	0.36	0.563
Mulch	1	0.07	0.803
Tillage*Mulch	1	2.12	0.179
Block	3	0.12	0.943
Opiliones			
Tillage	1	2.25	0.168
Mulch	1	5.66	0.041
Tillage*Mulch	1	0.46	0.516
Block	3	2.85	0.097

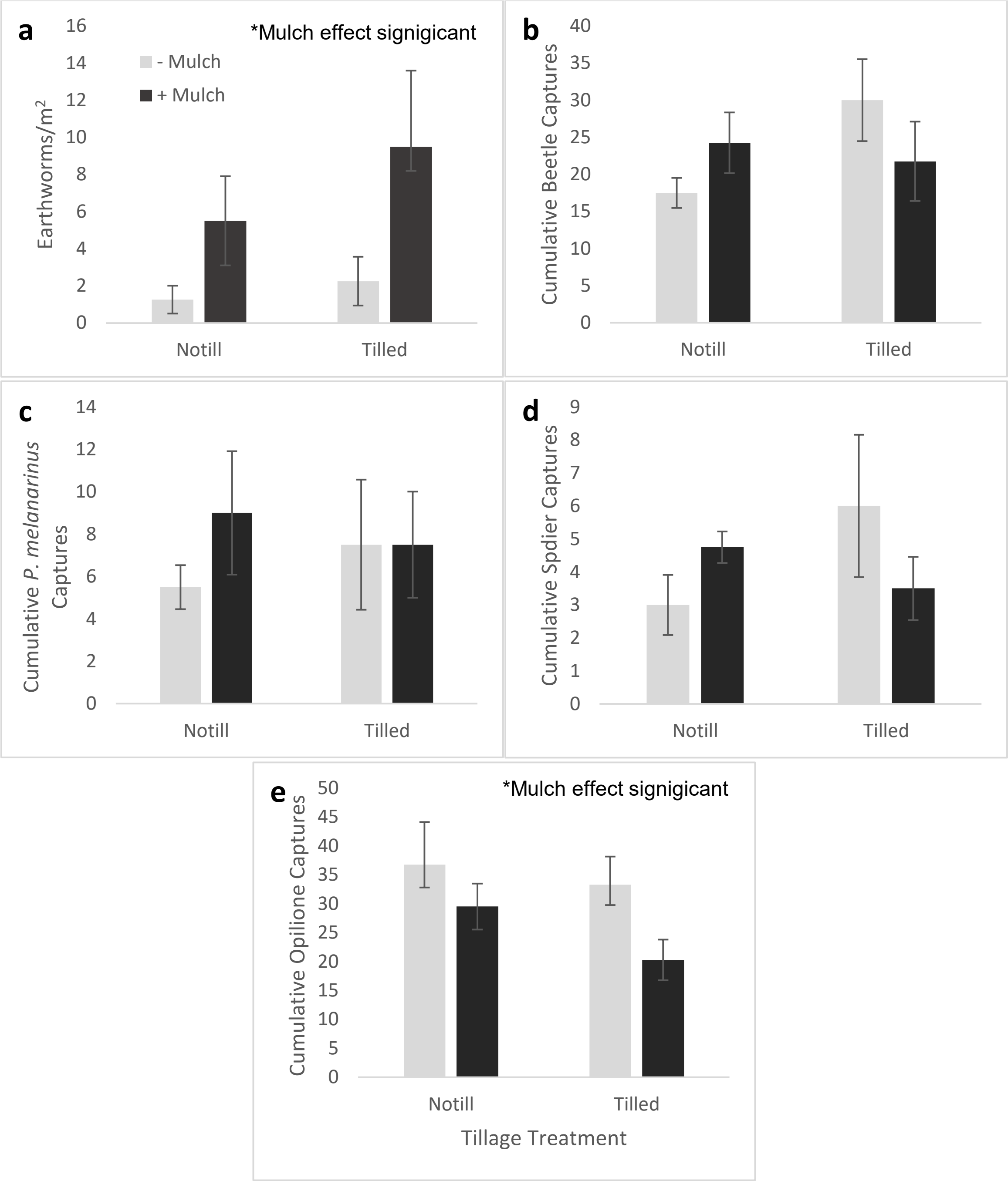

Figure 4.3. Soil organisms by tillage method and mulch presence at Carman. Tillage had two levels, full-inversion tillage and no-tillage. Mulch had two levels, mulch present (+) and no mulch present (-). (a) Earthworm abundance per m^{-2}, (b) cumulative captures for all beetles, (c) cumulative captures for *P. melanarius*, (d) cumulative captures for spiders and, (e) cumulative captures for opiliones.

4.4 Discussion

Mulch biomass at the Bible Hill site was lower than in the experiments from Chapter 2 (5.4 ± 0.9 Mg ha^{-1} from Trial 1 and 6.7 ± 0.4 Mg ha-^{1} from Trial 2). This is likely due to the different termination methods used. In these trials the Gmr biomass had to be mowed to implement the treatments. In the previous experiments the Gmr was only rolled, leaving it to continue to grow during the fall. Mowing has been shown to increase decomposition rate over rolling (Podolsky et al., 2016).

All macrofauna numbers were greater in 2017 than 2016 in Bible Hill. In total 273 earthworms were extracted in 2015 compared to 1346 in 2017, which is an 80% increase in 2017. Beetle captures were also 41% higher in 2017 and spiders were 37% higher. This is likely due to variation between fields sites, as opposed to climate differences between the years, as no year differences were evident from 2016 to 2017 in the trials in Chapter 3. The two sites in the present trial were adjacent to each other, but 2016 trial had been in a vegetable rotation five years previous while the site of the 2017 trial had been fallow for over ten years. Past fire disturbances has been shown to impact soil organisms for up to five years (Korobushkin et al., 2017) and past cultivation has been shown to impact the soil community composition for decades (de la Peña et al., 2016).

The two sites, Bible Hill and Carman, showed a similar response by earthworms to treatments, with earthworm collections being greater in mulched treatments regardless of tillage. Beetle captures at both sites were also similar in that there were no significant responses to treatments. Captures of spiders were greater in the mulch treatment in

Bible Hill in 2015, but there were no other effects of treatment on spiders in other experiments. Spider captures were much lower at Carman compared to Bible Hill (69 compared to 493 and 693).

As expected, presence of mulch had a stronger influence on earthworm abundance than tillage, even with mulch biomass that was 2-3 Mg ha^{-1} less than in the previous trials (see Chapter 2). It is surprising that tillage did not have any effect. In a Quebec study comparing no-till to full-inversion tillage with low or high corn residue, earthworms were affected by tillage but not by residue level, with greater earthworm abundance in no-till treatments (Eriksen-Hamel et al., 2009). A comparison of cropping systems in France did not find differences in earthworm abundance between conventional, organic, or 'living mulch' (reduced tillage and increased soil cover compared to conventional and organic) systems except in one year out of four where earthworm abundance was greater in the conventional system (Pelosi et al., 2009). The lack of tillage response by earthworms found here could be due to the timing of tillage. In Chapter 3 tillage occurred in either October or May whereas it the present study tillage happened in August. Earthworms could be lower in the soil profile in August due to the hotter temperatures (Curry, 2004), protecting them from tillage.

Beetle populations, in total or for *P. melanarius* or *Harpalus* ssp., were not significantly affected by tillage or mulch at either Bible Hill or Carman. Kulkarni et al. (2017) and Hummel et al. (2012) found a positive association with carabids (dominated by *P. melanarius*) and weed density, possibly due to the provided cover. *H. pensylvanicus* captures showed no response to tillage but had greater captures in Grm crops compared to soybean (Ward et al., 2011). Shearin et al. (2008) found increased *H.*

rufipes captures in Gmr plots compared to fallow plots, although the Gmr crop in that study was a living crop and not a rolled mulch. The nature of rolled HVO mulch (Figure 4.1) creates a very different physical environment compared to weed cover or living Gmr stands. The difference in findings between these studies and the present study could be due to the low-to-the-ground and matted nature of the Gmr mulch creating a barrier to movement. Pitfall traps only measure activity-density so it is possible that more beetles are present in mulched plots but they are not moving as much, and therefore less likely to be caught by a pitfall trap.

Spiders were positively affected by mulch presence in 2016 in Bible Hill only. In a Mediterranean olive orchard, incorporating ground cover increased captures of spiders over undisturbed plots (Cárdenas et al., 2012). Increasing crop diversity and reducing tillage did not affect wolf spiders in an Australian cotton (*Gossypium hirsutum* L.) cropping system (Rendon et al., 2015). Spider density was greater with more complex habitats in soybean fields in Miami (Schmidt and Rypstra, 2010), similar to the present study, as mulch presence arguably increases habitat complexity. The present study may differ from these studies not only in the treatments applied but also in the species of spiders present as they are from different regions of the world. The captures in Carman were lower than in Bible Hill (69 compared to 463 and 692), which would make detecting responses more difficult in Carman.

Opilions were negatively affected by mulch presence in the one trial where they were included. This is in contrast to Whalen et al. (2007) that found both spiders and opiliones were positively affected by the transition to no-till management in corn in Quebec. In soybean grown with a living alfalfa mulch in Iowa opilions increased more than three-fold compared to soybean with no mulch (Schmidt et al., 2007). As with the beetles, the negative effect of mulch could be due to limitation on movement by the physical barrier of the mulch. Body composition of spiders and opiliones differs, with opiliones having longer legs relative to body size. This could make movement though mulch more difficult for opiliones.

Further research in no-till Gmr systems on soil macrofauna abundance using methods other than pit fall traps would help determine if the mulch is hindering the capture of macrofauna in traps or if abundances are truly lower in the mulch. Responses of beetles, spiders, and opiliones could also be related to prey responses to treatments, as suggested in Rivers et al. (2018), although prey populations were not sampled in the present study. Further work could be done in this system on lower trophic level soil organisms to shed light on the mechanisms through which Gmr mulch influences predators.

Overall, mulch had a stronger influence on soil macrofauna than tillage. Tillage had no significant effects on the macrofauna in this study. It was expected that there would be an additive effect of mulch and tillage, but that was not the case. This leads to the conclusion that the same soil biological benefits seen in conventional no-till systems cannot be assumed for no-till systems that incorporate a no-till Gmr, as effects are largely driving by the mulch, which is unique to this system.

Chapter 5. Conclusions

Increased awareness of the extent and possible consequences of soil degradation combined with the promotion of conservation agriculture principles has led to increased desire by organic producers to reduce reliance on tillage. This has resulted in the development of no-till green manure (Gmr) management systems. The bulk of research on this management system has focused on cash crop performance and weed control (Silva and Delate, 2017; Vincent-Caboud et al., 2017; Wallace et al., 2017). Despite its roots in soil improvement and remediation, research on how no-till Gmr management influences soil is sparse. This prevents producers from making informed decisions and limits our understanding of how combining no-till practices with often large amounts of Gmr mulch biomass are affecting important soil properties and functions.

My thesis was designed to address the gap in knowledge created by the lack of soil-based studies in no-till Gmr systems. The main objective was to understand how the unique combination of conditions (lack of soil disturbance and mulch biomass) affect the soil ecosystem. I conducted Gmr termination studies at two Canadian sites, one in Bible Hill, Nova Scotia and one in Carman, Manitoba. The first study at the Bible Hill site was designed to follow changes in soil organic carbon (SOC) over three years after various Gmr termination strategies were implemented: fall-till, spring-tilled, or no-till termination. The second study used the same experimental setup and assessed the soil biology effects after Gmr termination. The same treatments were used at the Carman site to compliment the findings in Bible Hill and to allow comparison of responses in two different soil types and climates. Finally, another factorial study was designed for the same two locations to examine at the relative influence of the physical disturbance of tilling versus the presence of mulch created by no-till Gmr termination.

5.1 Soil Organic Carbon Dynamics

In Chapter 2 SOC dynamics were presented for the Bible Hill location only as this site had multiple years of data to follow the changes over time of various SOC pools after Gmr termination. In this four-year organic grain rotation three Gmr termination methods were compared (no-till crop roller, spring tillage, and fall tillage). A mixture of hairy vetch/oat *(Vicia villosa* Roth and *Avena sativa* L.) was seeded in late May in two trials, the first commencing in 2013 and the second in 2014. SOC was monitored for three years after Gmr termination. In each of two soil depth increments (0-5 cm and 5-15 cm), three pools of SOC were analyzed - total organic carbon (TOC), particulate organic matter carbon (POMc), and permanganate oxidizable carbon (POXc). In Trial 2, TOC (0-15 cm combined) was greater under no-till Gmr termination compared to fall and spring till termination (by 2.4±1.2 Mg C ha^{-1} and 2.3±1.3 Mg C ha^{-1}, respectively) and the effect remained significant in all three years. In Trial 1, POMc was stratified in no-till and concentrated at the soil surface (0-5 cm). Overall, SOC was greater under no-till Gmr termination and the more labile pool of POXc appeared less responsive to termination treatments.

In systems that have relatively high carbon inputs, more labile pools of C (POM and POX) might not make suitable indicators of changes in SOC. Other studies have found too sufficiently greater in POMc compared to SMB and TOC to make a less useful indicator of SOC changes (Ladoni et al., 2015; Simonsson et al., 2014). While some studies have shown POXc to be a sensitive C pool to management (Hurisso et al., 2016; Plaza-Bonilla et al., 2014), that was not found in the present study. This could be due to the high buffering capacity of the smaller, labile C pools created in systems with high organic matter inputs, which is expanded on below.

5.2 Soil Biology Dynamics

In Chapter 3, soil biology dynamics after Gmr termination were analyzed at both sites, but only earthworm data was presented for Carman due to sample loss in transit and issues around sample analysis following a campus fire. Soil microbial biomass (SMB) was higher in plots that were previously in a hairy vetch/oat Gmr compared to clover or reference samples, but the effect was very transient, disappearing after two months. Earthworms appear to be the most sensitive to tillage of the macrofauna studied. They showed the strongest negative response to the tilled termination treatments and took three growing seasons to recover. In this type of organic grain rotation it appears occasional tillage could be performed without causing long-term decreases in the earthworm population, similar to the findings in Nelson et al. (2009) which found earthworm populations recovered after four years following potato cropping on organic farms. No-till termination increased spider captures but had no effect on beetle populations. Response of spiders and beetles to tillage in the no-till Gmr systems appears to be similar to conventional tillage systems.

Analyzing higher tropic level organisms in agricultural systems may give a clearer picture of the effects of disturbance on soil organisms as they are less resilient than smaller-bodies organisms that have shorter life cycles (Woodward et al., 2005). The ability of the earthworm population to recover over a three-year period indicates strong resilience in this population This recovery could be due to the abundance of food provided through the Gmr combined with the lack of tillage in the rotation following Gmr termination.

5.3 Tillage and Mulch Effects on Soil Macrofauna

A closer look at how no-till Gmr influences soil macrofauna was presented in Chapter 4. A factorial study with three levels of tillage (full inversion, no-till, and fallow) and two levels of mulch (present or absent) was conducted in Bible Hill and Carman. Earthworms abundancewas greater under mulch and opilione captures (measured in Carmon only) decreased under mulch. Spider captures increased under mulch in one site year out of three. My research showed that the effects on soil macrofauna appear to be due to the presence of the mulch, as there were no significant effects of tillage. However, the different timing of tillage (late summer vs. fall or spring) may account for the lack of tillage effects in this study compared to the findings in Chapter 3.

The results of Chapter 3 and 4 show that earthworms are the most sensitive to Gmr termination method of the soil organisms considered. The effects of Gmr termination on soil biology varied between different taxa (see summary, Table 5.1). The organisms in these studies are important ecologically for agricultural systems, providing ecosystem services such as decomposition or pest predation. However, the results do not indicate a clear benefit to ecosystem services could be expected from adoption of no-till Gmr termination and further research is needed to draw conclusions regarding ecosystem services provided by soil organisms in no-till Gmr systems.

Table 5.1 Summary of the present studies of no-till Gmr termination effect on populations of soil organisms. Studies were conducted in Nova Scotia and Manitoba in an organic grain rotation that used a full-season Gmr. Positive effects mean there was evidence of increased abundance under no-till Gmr management and negative effects indicate a decrease.

Soil Organism	Impact of No-till Gmr Termination Method
Soil Microbial Biomass	No effects, appears more influence by Gmr type
Opilions	Negative effect (only with mulch, no tillage effect found)
Spiders	Positive effect
Beetles	No effect
Earthworms	Positive effect

5.4 Implications of Research Findings

My research showed limited benefits of no-till Gmr termination on SOC and mixed responses of soil biology. Considered with the larger body of research on yield and weed impacts of no-till Gmr that consistently show challenges around achieving adequate mulch biomass to suppress weeds and lower yields caused by late planting and low emergence, this technique still requires more development and research. Benefits to soil health are not evident in the present studies to sufficiently justify the inconsistency of the agronomic response and sometimes depressed yields. Current techniques and Gmr varieties are not sufficient to achieve the desired results - which are similar yields with increased benefits to soil, although there has been some success with rolled cereal rye (*Secale cereal* L.) and soybeans (*Glycine max* L.) (Silva and Delate, 2017). Benefits of no-till Gmr and reduced tillage in general are also likely to vary by climate and soil type (Dang et al., 2015a; Keene et al., 2017; VandenBygaart and Kay, 2004) and should be considered on an individual basis using the available pool of knowledge, both global and local.

The adoption of no-till agriculture by many conventional farmers has led to criticism of tillage frequency in organic systems. However, the findings of my research and that of others points to positive SOC outcomes in organic systems, regardless of tillage. In a meta-analysis of pairwise comparisons of organically and non-organically managed soils organic soils had significantly higher SOC concentrations in surface soil (Gattinger et al., 2012; Lynch, 2014). I think this is an important point to stress, both to organic producers and conventional producers. In addition, the SOC increases seen under conventional no-till management are increasingly being shown to exist in the surface soil only and deeper sampling has shown decreasing SOC in deeper soil layers (Clark et al., 2017; Krauss et al., 2017). Benefits of high carbon inputs on overall SOC may be more influential than the tillage regime. In discussions of conservation agriculture more emphasis needs to be placed on the use of cover crops as it is becoming increasingly evident no-till is not enough to reverse soil degradation.

Use of high organic matter inputs frequently seen in organic production, such as Gmr, may buffer these systems against negative impacts of tillage on SOC. The meta-analysis by Gattinger et al. (2012) found organic systems averaged 1.20 Mg C ha^{-1} y^{-1} of C inputs while conventional systems averaged only 0.29 Mg C ha^{-1} y^{-1}. In comparison, a meta-analysis comparing tilled to no-till crop management found a gain of 0.57 Mg C ha^{-1} yr^{-1} (West and Post, 2002). In the present study, the Gmr delivered up to 2.4 Mg C ha^{-1} more TOC when termination by no-till than tillage, but soil was only measured to 15 cm and I suspect carbon in the tilled treatments was placed below the sampling depth by tillage. Therefore, it could be reasonably assumed a large portion of the C lost to tillage could be replaced by incorporating Gmr or other cover crops within a rotation. Tillage could even facilitate longer-term, more stable SOC storage by placing residues deeper in the soil.

Buffering against soil degradation by tillage though the use of Gmr or other cover crops is also a concept that could be applied in conventionally managed systems. Problems that occur in strict no-till cropping systems include herbicide resistant weeds, increases in diseases propagated by stubble, and depletion of immobile nutrients below the surface soil (Dang et al., 2015b, 2015a). Strategic tillage can be used to mitigate these problems but producers have concerns about undoing the accrued benefits of no-till on soil quality. A study out of Ontario found that one tillage event with a moldboard plow resulted in a loss of over 60% of the SOC accumulated over 22-yr of no-till management in a conventionally managed field in sandy loam soil (VandenBygaart and Kay, 2004). In Germany one tillage event resulted in the total loss of 20-yr of SOC that had accumulated under minimum tillage (Stockfisch et al., 1999). Tilling uncultivated grasslands to put them into cultivation resulted in a 35% decrease in soil aggregation (Grandy et al., 2006). The decreases in SOC and soil aggregation caused by a one-time tillage event highlighted in these studies could be combated by regular use of Gmr and other cover crops in rotation, as they have been shown to increase SOC and soil aggregation (Biederbeck et al., 1998; Puget and Drinkwater, 2001). Combining strategic tillage with increased C inputs through Gmr, other cover crops, or manure has the potential to negate the loss of soil quality with tillage. Use of high organic matter inputs is not limited to organic production and can be incorporated into conventional crop rotations. Indeed, there are innovative producers doing just that all over the globe.

There has been promising research using various reduced tillage techniques. European research as part of the TILLMAN-ORG project found that shallow inversion tillage was beneficial in Mediterranean climates (Cooper et al., 2016). In Switzerland yields in an organic production system were not affected by reduced tillage (chisel plow) compared to full inversion tillage (Armengot et al., 2014). Reducing the frequency of tillage or using less intensive forms of tillage, such as strip-till, could present a more realistic scenario for crop production. Research into the impacts of those techniques on the soil ecosystem would further help us understand how tillage affects soil health and better inform the choices of producers.

The ideas presented above are presented in a simplified theoretical model of soil health outcomes (Figure 5.1). Tillage has been well documented to cause soil degradation, and repeated tillage events could reduce the soil's ability to recover from disturbances (Figure 5.1a). While strict no-till management may prevent soil degradation, gains in soil health are not always evident or can be minimal (Figure 5.1b). Research on use of cover crops and Gmr, including the present research, have indicated regular high organic matter inputs could help soils recover from tillage and build resiliency (Figure 5.1c). To further develop this concept, reduced tillage – either through reduced frequency or less disruptive tillage methods, such as strip till or chisel plow – in combination with high organic matter inputs could ultimately increase soil health outcomes (Figure 5.1d)

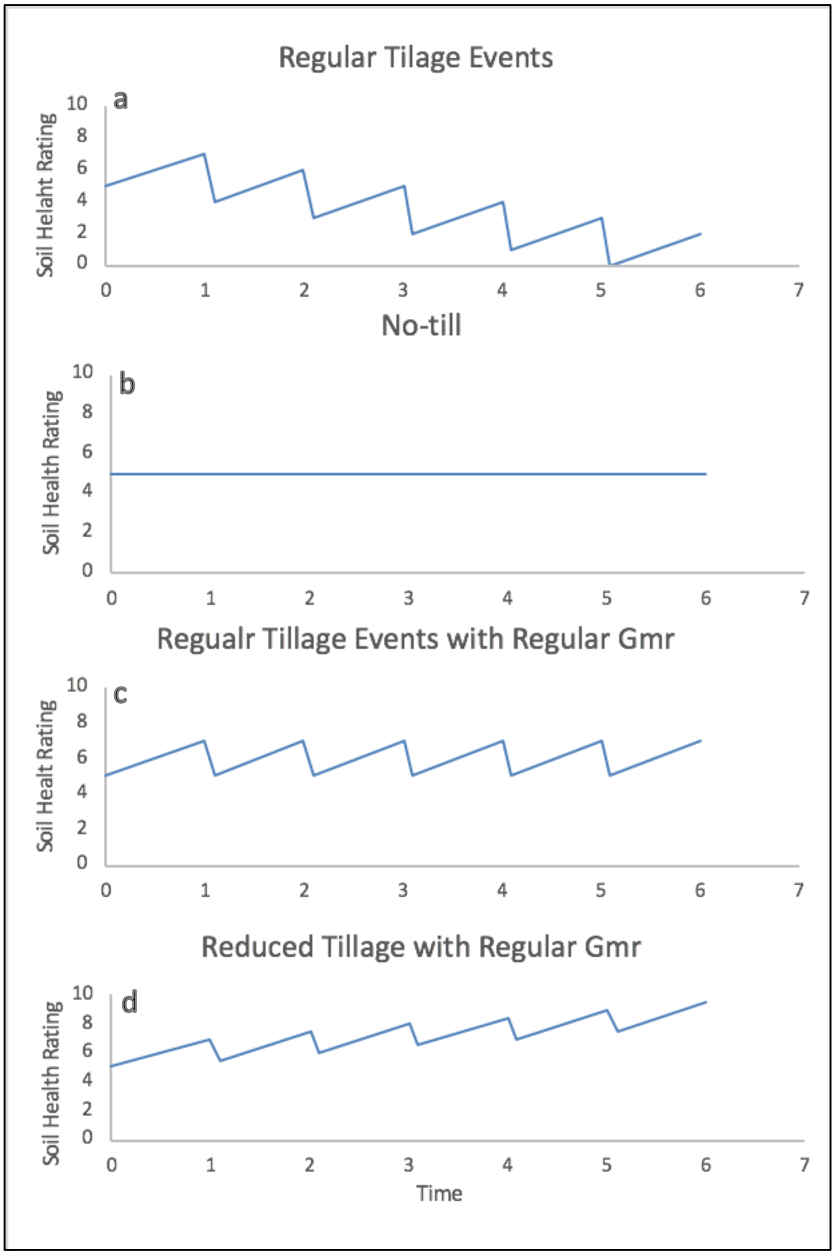

Figure 5.1 Theoretical soil health outcomes under tillage and Gmr management. Four scenarios are presented, (a) regular tillage where soil health rebounds but is unable to fully recover between tillage events, (b) conventional no-till management that prevents soil degradation, (c) regular tillage combined with regular use of Gmr, where the soil is resilient enough to recover between tillage events, and (d) reduced tillage (e.g. chisel plow, strip tillage) combined with Gmr where the overall soil health is able to improve with time.

5.5 Recommendations for Future Research

To better understand the long-term effects of termination of Gmr and the ability of Gmr to ‘buffer’ against tillage, studies looking at the impact of frequency of tillage in a crop rotation are required. The impacts of infrequent (or ‘strategic tillage’) tillage on soils with regular inputs of high organic matter is not well known. A meta-analysis comparing tillage responses in systems with low organic matter inputs compared to those with high inputs would be a starting point and gaps identified in that study could guide further research designs.

A long-term study would provide interesting insights into SOC changes with no-till Gmr management. In Chapter 2, Trial 2 did see an increase in TOC under no-till Gmr termination. If this rotation was maintained, would TOC continue to accumulate and would it be limited to the surface soil? Year-by-year dynamics of SOC pools in no-till versus tilled Gmr studies would increase our understanding of how SOC in these systems responds to disturbance and what is the ultimate fate of the C inputs. Depth of sampling is also a major constraint on SOC studies, and is well acknowledged in the literature as a shortcoming. Despite the extra time and cost required, the knowledge gained on SOC dynamics in soil would be well worth the effort. Ongoing no-till Gmr studies, such as the ROSE project out of Pennsylvania and others that have focused on weed and yield data are candidates for examining changes in SOC at depth over longer time periods and with multiple rotations of no-till management completed.

When interpreting the impact of mulch on macrofauna, only activity-densities were analyzed for beetles, spiders, and opiliones in my research. Thus, I was unable to draw conclusions as to whether the presence of mulch was affecting actual populations of organisms or simply their movement. If mulch deterred movement it would result in lower capture rates. It is possible that beetles preferred the habitat provided by the mulch but that effect was masked by less movement of organisms. This was found in a study that combined pitfall traps with photoeclectors. The two techniques were in agreement in simpler habitats but the pitfall traps underestimated populations when the vegetative habitat was more complex (Lang, 2000). My research showed no effect of Gmr termination on *Harpalus* ssp. but a mark-recapture study found *H. rufipes* DeGeer preferred plots with more vegetative cover (Shearin et al., 2008). I would recommend future studies utilize other techniques to better understand the absolute effect of mulch on macrofauna.

Soil macrofauna could respond to changing resources under no-till Gmr termination, habitat variation, or the disturbance of tillage. To better understand why soil macrofauna respond differently to no-till Gmr termination smaller soil organisms could be sampled. As a food source for larger soil organisms, they could be driving changes in higher tropic levels. Spatial correlation between prey abundance and *Pterostichus* sp. was found in a UK field study (Winder et al., 2005). Combining data on soil macrofauna and mesofauna would increase our understanding of how this management practice affects soil ecosystem services.

If no-till Gmr termination is to become a feasible management practice, development of varieties of Gmr that can provide consistently high levels of biomass to provide weed control need to be developed. Cash crops that are suited to later planting and the cooler, wetter germination environment provided by a no-till Gmr mulch would also be needed. Soybeans are a good example. They can be planted later in the season and have been shown to do well planted into rolled cereal rye (Silva and Delate, 2017). An economic and energetic analysis of no-till Gmr termination would provide another piece of information to allow for well-informed decisions by producers. It is possible that no-till termination would save fuel and energy costs over full-inversion tillage. If yields are agronomically viable and an economic case can be made, soil benefits would be less important. However, currently there is not enough evidence to claim soil benefits to justify use of no-till Gmr termination in the face of often lower yields.

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Appendix A

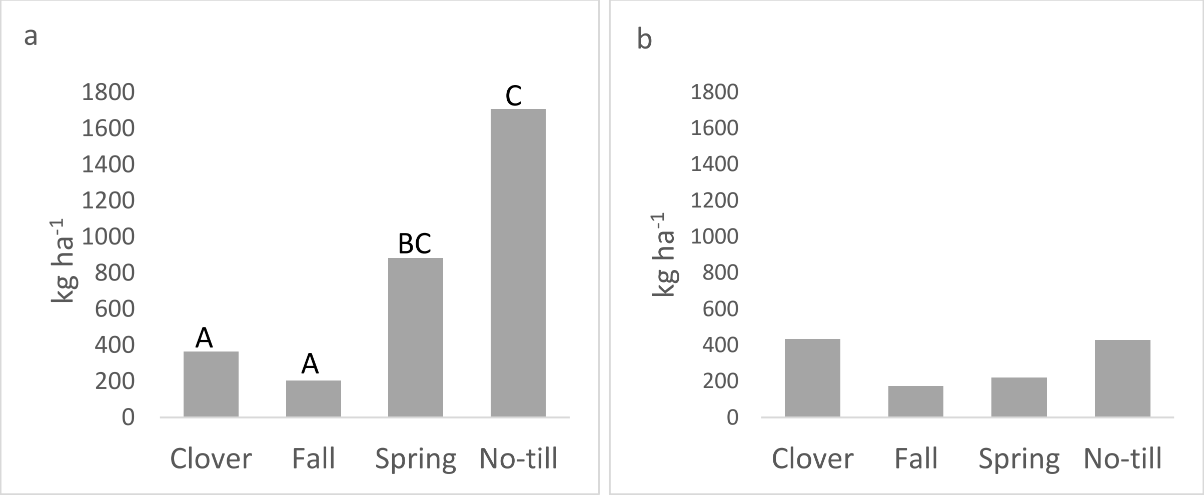

Figure A1. Aboveground weed biomass in kg ha^{-1} during the wheat phase of the rotation. Letters indicate a significant difference in treatments determined by Fisher's LSD test at a 95% confidence level. No letters indicate there was no significant difference in treatmetns at α=0.5. (a) Trial 1 during the wheat phase in 2014 and (b) Trial 2 during the wheat phase in 2015.

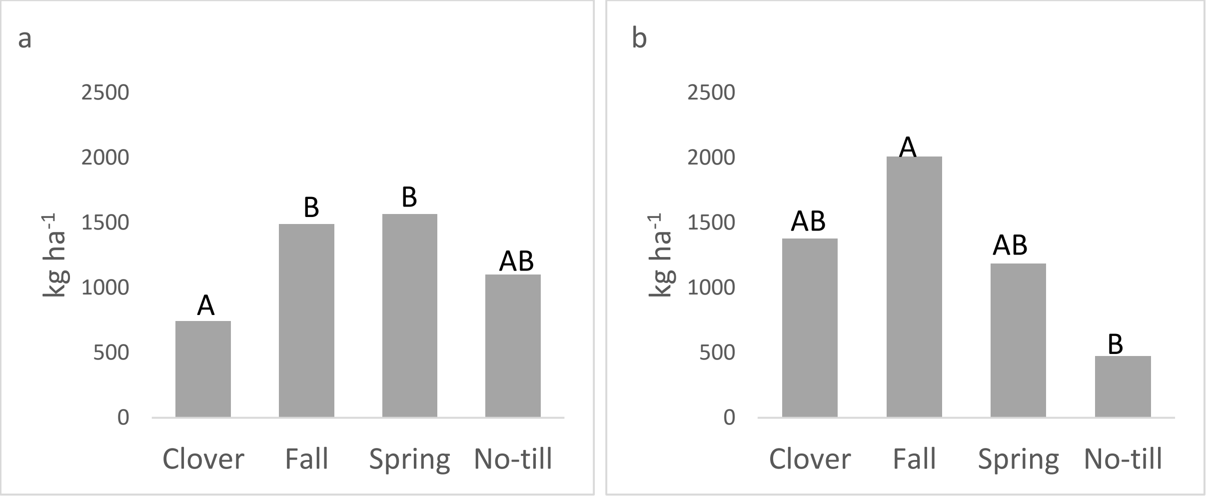

Figure A2. Wheat yield in kg ha^{-1} Letters indicate a significant difference in treatments determined by Fisher's LSD test at a 95% confidence level. (a) Trial 1 in 2014 (b) Trial 2 in 2015.

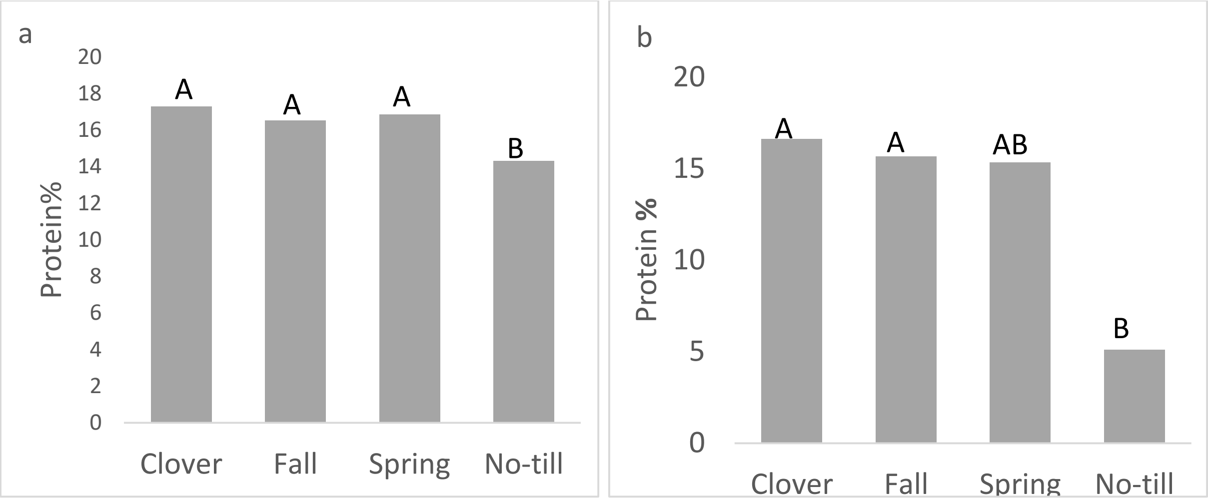

Figure A3. Wheat grain protein content as a perctange determined by NIR analysis. Letters indicate a significant difference in treatments determined by Fisher's LSD test at a 95% confidence level. (a) Trial in 2014 and (b) Trial 2 in 2015.

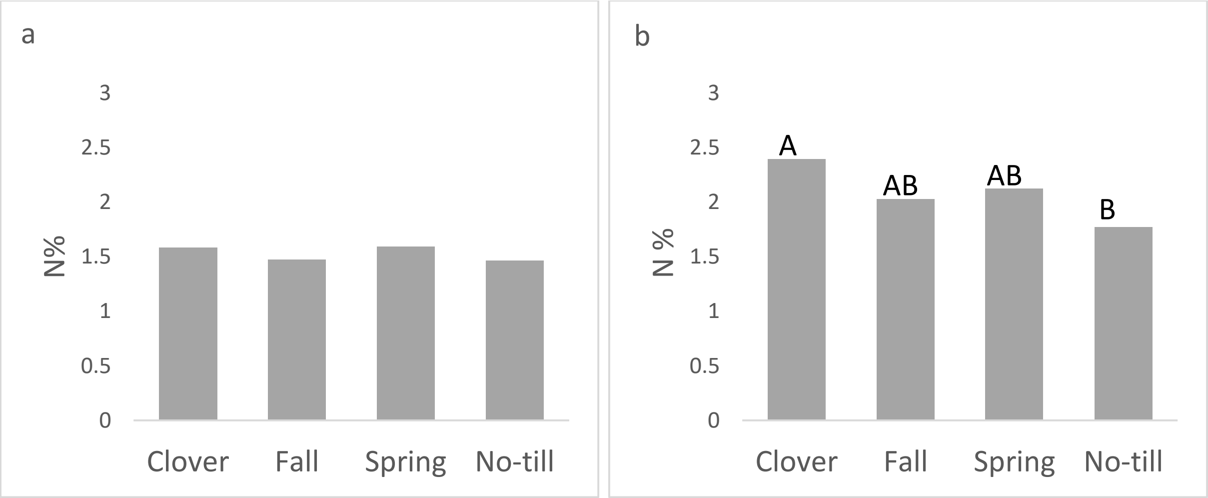

Figure A4. Wheat plant N content as a percent of tissue sampled at soft dough stage. Letters indicate a significant difference in treatments determined by Fisher's LSD test at a 95% confidence level. No letters indicate there was no significant difference in treatmetns at α=0.5. (a) Trial 1 during the wheat phase in 2014 and (b) Trial 2 during the wheat phase in 2015.

Table A1. Bulk density values (gcm^{-3}) by termination method and depth for both trials. Termination methods are fall tilled (full inversion tillage in the fall), spring tillage (full inversion tillage in the spring), and no-till (rolled with a crop roller only). P-values are from an ANOVA analysis of bulk density.

	Trial 1		***Trial 2***	
	0-5cm	5-15cm	0-5cm	5-15cm
Fall Tillage	1.2±0.06	1.21±0.04	1.34±0.10	1.29±0.06
Spring Tillage	1.23±0.06	1.24±0.04	1.32±0.08	1.22±0.06
No-till	1.24±0.05	1.24±0.04	1.26±0.08	1.20±0.07
p-values				
Termination	0.359		0.580	
Depth	0.847		0.283	

Appendix B

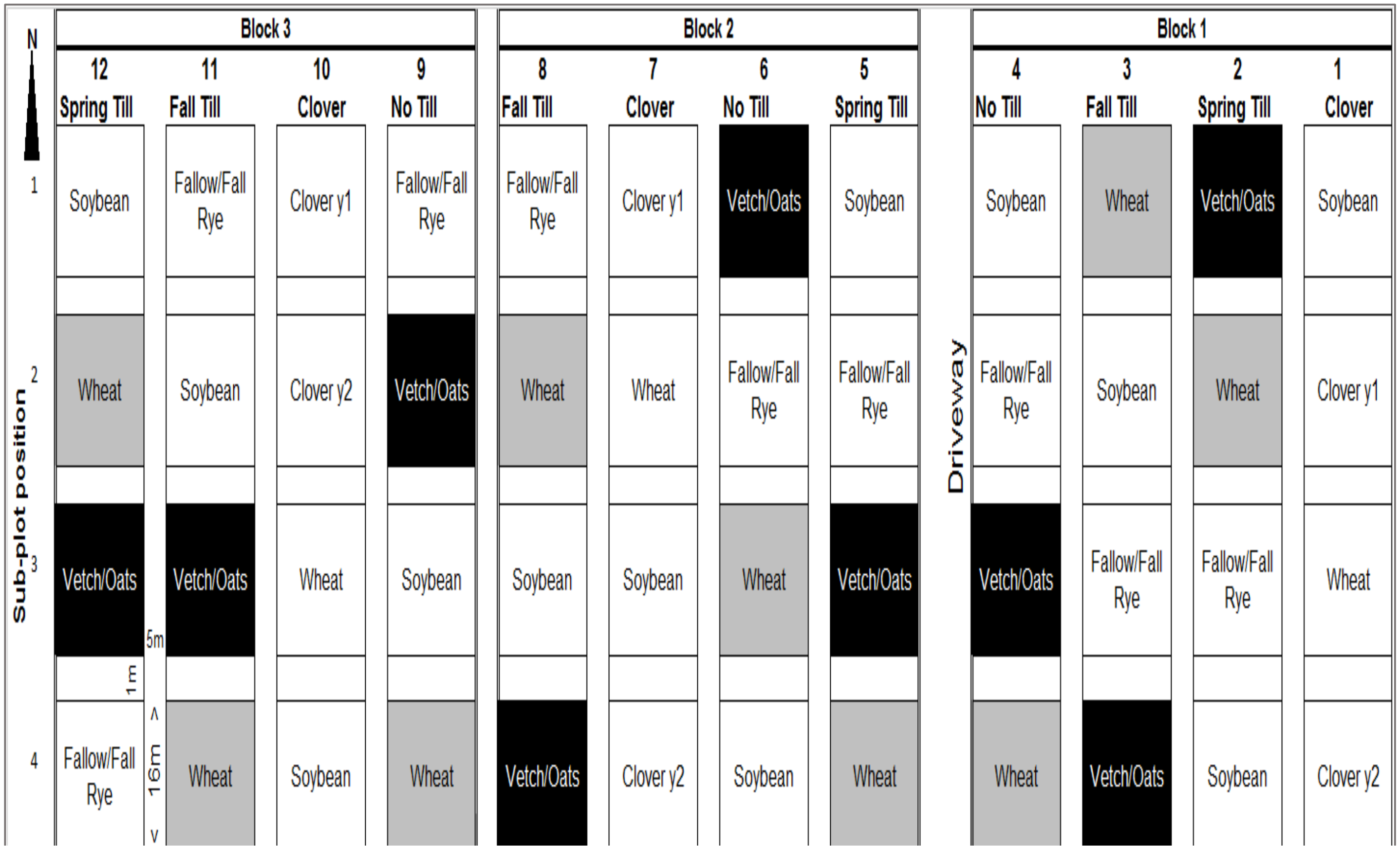

Figure B1. Plot layout from Truro, NS rotations trials as it was in 2014. Grey plots are for Trial 1, which starte in 2013 and was in the wheat phase in 2014. The black plots are for Trial 2, which started in 2014 with the green manure phase. Numbers along the top of the plots are plot numbers and subplot numbers are given or the left side of the image.

Table B1. Common name, species name, variety, and target seeding rate used in the Bible Hill and Carman sites. Seeding rate is for viable seeds and was adjusted to account for seed germination rate.

Common name	**Species name**	**Variety**	**Target seeding rate (kg ha^{-1})**
Red Clover	*Trifolium pretense L.*	AC Endure	12
Hairy Vetch	*Vicia villosa Roth*	Common	30/40†
Barley	*Hordeum vulgare* L.	Cowboy	60
Oat	*Avena sativa L.*	Triple Crown/ Leggatt†	70
Forage pea	*Pisum sativum* L.)	40-10	135
Wheat	*Triticum aestivum L.*	Helios/Cardale†	180/125
Fall Rye	*Secale cereal L*	Common	150
Soybean	*Glycine max (L.) Merr.*	Savannah	90

†When two entries are given, the first is for Bible Hill and the second is for Carman.

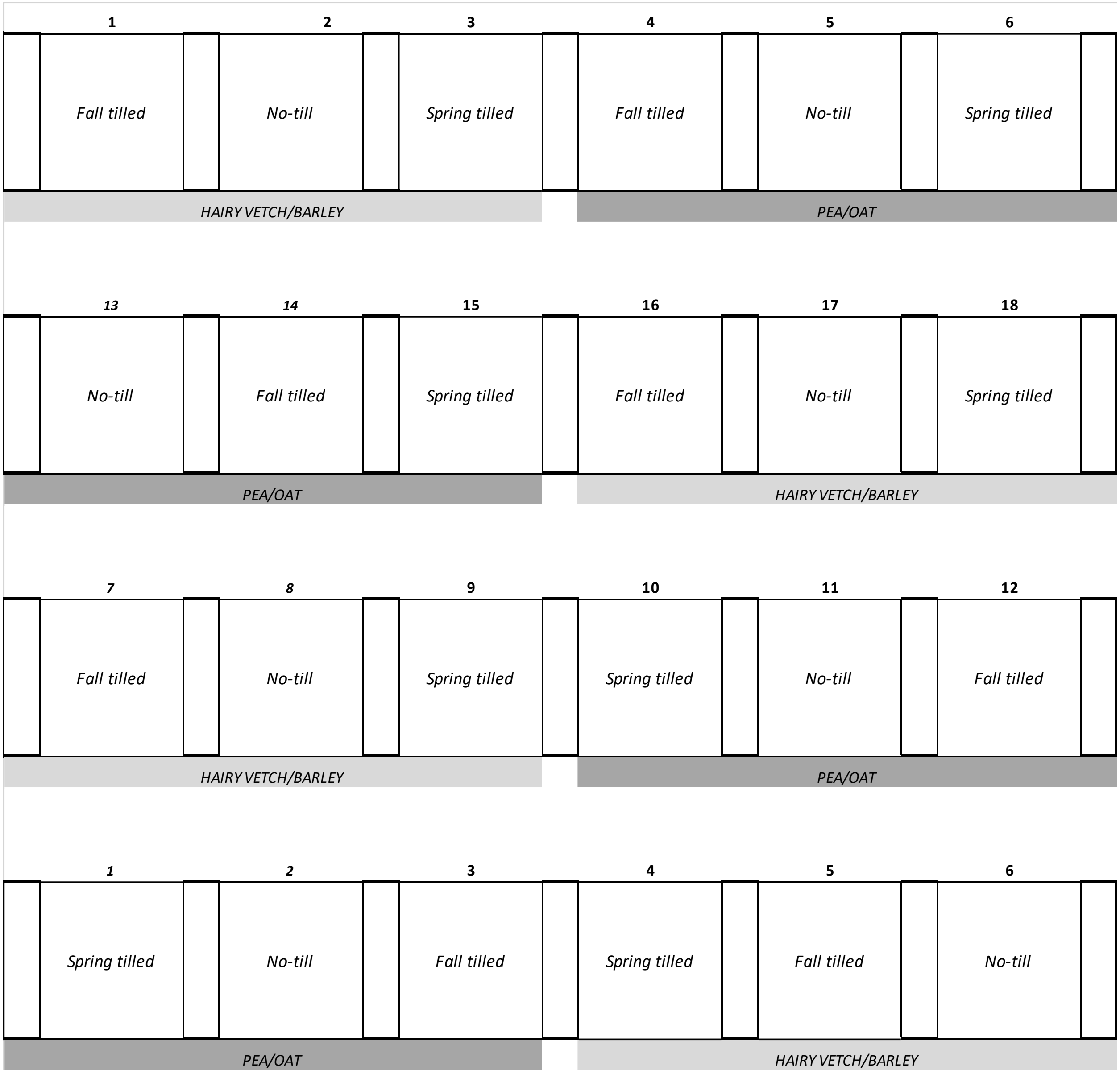

Figure B2. The plot layout of the first trial (established 2013) at Carman, MB. The other trial (established 2014) was identical except for the random assignment of green manure crop and tillage treatment within the green manure.

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