SOIL HEALTH IN ORGANIC GRAIN PRODUCTION AS INFLUENCED BY GREEN MANURE MANAGEMENT AND WEED PRESENCE

by

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ABSTRACT

From 2016-2018 two experiments in Bible Hill, Nova Scotia were conducted where GMr of red clover, hairy vetch or common vetch grown two years prior to soybeans with current season presence or absence of weeds (Exp. 1) or one year prior to wheat with different tillage intensities incorporating GMrs (Exp 2). Soil biological, physical and chemical properties were examined following the Cornell Soil Health Assessment (CSHA) protocol. Phospholipid fatty acid analysis (PLFA) measured soil microbial biomass and community composition. GMr or tillage treatments did not affect weed biomass production nor most measures of soil health or microbial community. Non-mycorrhizal weed species withstood tillage practices better. High residue soil input over the last 10+ years may have masked treatment effects. Weed presence improved soil respiration and fungal PLFA, but not overall soil health. Temporal soil sampling resulted in differences in microbial measurements with significantly more fungi observed later in the season.

LIST OF ABBREVIATIONS USED

α	alpha level
AAFC	Agriculture and Agri-Food Canada
ACE Protein	Autoclaved Citrate Extractable Protein
AMF	Arbuscular Mycorrhizal Fungi
AWC	Available Water Capacity
ANOVA	Analysis of Variance
BCA	Biochromatic Assay
C	Carbon
Ca	Calcium
CSHA	Cornell Soil Health Assessment
Cu	Copper
CVO	Common Vetch under-seeded with Oats
DM	Dry Matter
FA, FAMEs	Fatty Acids, Fatty Acid Methyl Esters
Fe	Iron
GC	Gas Chromatography
g	Gram
GMr	Green Manure
ha	Hectare
HVO	Hairy Vetch under-seeded with Oats
K	Potassium
Kg/ha	Kilograms/Hectare
KMnO4	Potassium permanganate
MFA/g	Methylated Fatty Acids Per Gram
MMC	Multiple Means Comparison
nmol	Nanomoles
LOI	Loss on Ignition
LSD	Least Significant Difference
Mn	Manganese
Mg	Magnesium
NW	Non-Weedy
OM	Organic Matter
OSHA	Ontario Soil Health Assessment
N	Nitrogen
P	Phosphorus
PETW	Post-Emergent Tine Weeding
PLFA	Phospholipid Fatty Acid
SAS	Statistical Analysis Software
SE	Standard Error
SMB	Soil Microbial Biomass
SOC, SOM	Soil Organic Carbon, Soil Organic Matter
ST	Spring Till
RC	Red Clover
ug	Microgram
TAG	Triacylycerols
WAS	Wet Aggregate Stability
W	Weedy
Zn	Zinc

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CHAPTER 1- INTRODUCTION

1.1. Soil Health

Soil is composed of air and water pore space, minerals, organic matter and many microorganisms. Some scientists describe the soil as a living organism due to its dynamic characteristics and beneficial ecological roles (Karlen et al. 1997; Bissett et al. 2013). In just 1 teaspoon of healthy soil there are several yards of fungal hyphae, billions of bacteria, many thousands of protozoa, and many nematodes (Lowenfels and Lewis 2010). According to the same source, that could equate to 20-30,000 different species in the soil. The complexity of soil biology currently exceeds that visible and identifiable by the human eye (Van der Heijden and Wagg 2012). Soil is necessary to grow plants with additional roles in ecosystem services, such as biodiversity, climate regulation and water security since soils help to clean our water and remove contaminants (McBratney et al. 2013). If soil is healthy it is more likely performing many of these tasks, so it begs the question; what actually constitutes soil health?

Soil health is defined as the collective term for the condition of biological, physical and chemical properties of soil to promote sustainability (Arias et al. 2005; Idowu et al. 2008). However, soil quality refers more to the ability of a soil to perform a specific purpose, (e.g. crop production), or described as the ability of a soil to function and improve the ecosystem as a whole (Doran and Parkin 1994; Karlen et al. 1997). The two terms are often thought of synonymously but they are not the same. Healthy soil is good quality, but good quality soil is not necessarily healthy. Therefore, to maintain a sustainable ecosystem and a plentiful food supply, healthy soils are at the core; making this to be a very important area of research. Many components of soil health can have direct impacts or associations with each other, so it is important to develop an understanding of these relationships. For example, a positive correlation was found between earthworms or microbial biomass and aggregate stability of the soil (Mader et al. 2002). However, understanding these interactions requires good knowledge of the basic building blocks of soils.

The 5 standard soil-forming factors include topography, climate, parent materials, organisms and time. It is believed that a healthy soil ecosystem is sustainable, and sustainability is related to security. It was proposed that soil security is a better concept to encompass soil health, protection and quality (McBratney et al. 2013). They proposed 5 universal "c" criteria for a secure soil ecosystem include: capability, condition, capital, connectivity and codification. According to this source, the capability refers to the soil being able to function in the environment to reach a particular goal with a large influence from management practices, while condition refers to how it currently works. Capital makes reference to an essential value put on the resources rather than a numerical interpretation. Connectivity considers the soil from a social perspective

with stewardship of the land to build a relationship with the soil. Finally, this source described codification as having policies and frameworks for recognizing the valuable roles soil serves in the ecosystem. Soil security encompasses water, food, biodiversity, energy, and even lessening the negative impacts of climate change (Lal 2004; Koch et al. 2013). For the time being, the terminology of soil health will be used. Therefore, how do organic principles fit into this framework?

1.2. What is Organic Agriculture and its Relationship to Soil Health?

Organic agriculture is a holistic way of cultivating the land to produce crops and raise animals with a focus on organic residues obtained from living matter and humic acids from decomposing material by microorganisms as a source of nutrients. This system has more reliance on biological activity with nutrient release generally slower than conventional fertilizers (Stockdale and Watson 2009). It is very important to maintain soil organic matter, and with the intent of reaching this goal, although quite possible to do in conventional systems, it is suggested that soil organic matter (and thus soil organic carbon (SOC)) is more likely to accumulate in soils when conducting farm practices organically compared to conventionally (Gattinger et al. 2012). The number of weed species in organic systems has been reported higher than those observed in conventional no-till (Pollnac et al. 2009). More crop diversity and longer rotations are often observed in organic compared to conventional agriculture, perhaps to be more competitive with weeds, non-beneficial insects and disease (Barbieri et al. 2017). Actinobacteria and fungi under a wheat crop have been reported as being more prominent following long-term organic crop management in comparison to a conventional regime (Arcand et al. 2016). Organic agriculture does not involve excessive fertility inputs as fertility is being managed through cultural crop practices (Watson et al. 2006). Organic agriculture crops and AMF can help to maintain adequate N and P levels respectively; resulting in lower excess nitrogen and phosphorus; thus contributing to good nutrient management practices (Lynch et al. 2015).

Synthetic fertilizers have a much more concentrated N-P-K content compared to organic sources, with the former interfering with the presence and activity of AMF and some species of arthropods (Lowenfels 2013). *Medicago sativa L.* (alfalfa) has hosted more AMF in organic systems compared to its conventional counterparts (Schneider 2014). Organically managed fields have hosted more earthworms and greater biological activity, in support of better soil health (Mader et al. 2002; Braman et al. 2016). Having higher carbon concentrations and storage in organic soils is likely linked to greater biological activity compared to conventionally-managed soils (Gattinger et al. 2012). Organic agriculture therefore enhances soil quality compared to conventional agriculture (Lynch et al. 2014; Arcand et al. 2016). Cereal production can be more sustainable grown organically compared to conventionally (Manoharan et al. 2017).

In 2015 there were 5,053 organic farm operations registered in Canada, with Quebec, Saskatchewan and Ontario leading ahead of the other provinces (Canadian Organic Trade Association 2016). According to this source, it equates to approximately 983 thousand hectares of production or 1.5% of all land use in the country. Organic agriculture is becoming more popular in Canada, but particularly in the province of Quebec as 4% of all farms are certified to follow organic practices (Halde et al. 2017). In developing organic farming principles, the International Federation of Organic Farming Movements (IFOAM) recognizes 4 categories to promote, including health, ecology, fairness and care of the environment (IFOAM 2006). As described in this source, health encompasses all living components of the ecosystem including humans. Ecology refers to continued interaction between organisms; fairness reflects sound choices for opportunities in support of the environment, and care means conducting practices with precaution to ensure present and future success. A recently published study has analyzed over 200 rotations within 695 different papers from a variety of databases on organic and conventional agricultural crop rotations from across 26 countries globally to make comparisons between the two systems (Barbieri et al. 2017). They found that the composition of crops in any agricultural system is significantly different at a global scale due to variation in climate and soil type to grow particular crops, but overall suggest more diversity and better nitrogen management in the organic systems.

1.3. Green Manures (GMr) in Organic Cropping Systems

Incorporating green manures (GMr) into crop rotations is a good practice as they have many benefits to the soil environment, such as increasing carbon, and especially nitrogen supply if they are legume crops (Lupwayi et al. 1998) and controlling weed populations in organic crop production (Dai 2013). Planting legumes in rotation has reduced nitrate leaching by 40% compared to conventional practices using other fertilizer sources (Tonitto et al. 2006). The timing of GMr crops has an influence on how much N they will contribute to the system (Mirsky et al. 2017). Seeding *Trifolium pratense* L. (red clover) into fall-planted cereals has improved N levels for the main cash crop the next spring (Blaser et al. 2007). Increasing plant diversity is associated with better adaptability to disturbance to maintain sustainable healthy soils (Mader et al. 2002; Lin 2011). Some fields in the U.S. are only managed in 2-year rotations alternating between corn and soybeans with limited variety of plants, so it is practical to study incorporating more crops into the sequence (Plourde et al. 2012). Total SOC has reached an all-time low after 26-31 years in a single crop or short 2-year rotation, indicating the importance of crop rotations (Andrews et al. 2004). Using alfalfa or *Tritium aestivum L.* (wheat) in crop rotations has improved soil health compared to monoculture or 2-year rotations (Congreves et al. 2014).

Choice of GMr and Impact of Residues in Soil

The choice of a certain GMr crop is important, because even though they all tend to increase soil fertility, and prevent erosion through soil cover, they can have different growth habits, root characteristics and interactions with other living organisms in the soil (Berti et al. 2016). Microbes break down the nitrogen in plant residues for biosynthetic production of amino acids (Haney et al. 2018). Some GMr are better to be planted alone vs. in combination with others, depending on their seed size, timing of emergence, space requirements, growth period, competitiveness or companionship. One example is planting *Vicia villosa Roth.* (hairy vetch) underseeded with *Avena sativa* L. (oats), which has resulted in more biomass coverage compared to planting either crop individually (Campiglia et al. 2011). *Lupinus polyphyllus* Lindl. (large-leaved lupin) has produced higher biomass when grown alone, whereas red clover may be a better GMr planted with other crops such as *Hordeum vulgare* L. (barley) to allow for more stabilized biomass over time (Lauringson et al. 2013). More research needs to be done to examine the residual benefits to soil and crops from different GMr in rotation beyond the first year after their incorporation (Lynch et al. 2012).

Comparison of Red Clover and Hairy Vetch

Both hairy vetch and red clover plants are well known high nitrogen-fixers, but they have different physiological characteristics. Red clover is a perennial crop having a taproot, which can reach great depths in the soil of up to 60-90cm (Verhallen et al. 2001). This allows the plant to obtain nutrients from deeper areas in the soil profile. Red clover also has lateral roots extending up to 12cm horizontally, and the allelopathic activity of red clover may have resulted in decreased weed growth (Wyngaarden et al. 2015). There has been a 65% decrease in weed biomass planting red clover after a wheat crop (Blaser et al. 2011). Soil fertility status is higher from incorporating clover species as cover crops, reducing brown mustard (*Brassica juncea* L.) weed biomass by 29%, but by 57% if the fertility status is low (Ross et al. 2001). This is likely because the plants are less competitive with each other in the presence of more available nitrogen compared to an environment with limited amounts. Although in contrast to these findings, brown mustard has been more productive in slow-release fertilizer availability (Sharma et al. 2011) and wild mustard tends to be more competitive in high fertility soils (Warwick et al. 2000).

Hairy vetch is an annual crop with a weak taproot (60-90cm), with a shallow root system (up to 20cm) deep (OMAFRA 2001). According to the same source it grows 90-120cm tall if seeded with a cereal crop. Hairy vetch has excellent cold-hardiness (Wilkie and Snapp 2008), making it a suitable option for fall planting for a soil cover, which may promote good weed suppression in the spring. This crop produces high biomass, with up to roughly 7750 kg ha^{-1} reported in some parts of the USA (Mirsky et al. 2017). Fewer and later-emerging weeds have grown when in direct competition with hairy vetch (Mohler and Teasdale 1993).

Planting hairy vetch paired with reduced tillage has been found to manage weed populations compared to only using GMr or tillage (Campiglia et al. 2011). However, a recent study in Eastern Canada at the first experimental site of focus in this thesis has looked the influence of the type of GMr on N supply and wheat yields; finding that hairy vetch underseeded with oats (HVO) is a good replacement crop for red clover with comparable effectiveness (Alam et al. 2018). There is more literature available on using red clover as a GMr compared to hairy vetch to support the need for more research with this latter crop and weeds on soil health (Talgre et al. 2009; Lauringson et al. 2013; Braman et al. 2016). Ideally, a study examining up to 10 different GMr in the same paper under the same soil type and crop rotation system is beneficial to make a fair comparison of their impact. The role of nitrogen and carbon from GMr is discussed in more detail below.

Nitrogen Contributions from Green Manures

Leguminous GMr in rotation fix nitrogen from the atmosphere, releasing it at the end of their lifecycle when they are incorporated into the soil. These plants work in association with *Rhizobia* bacteria naturally occurring in soil to form nodules on the root systems to provide nitrogen to the plant. It has been suggested that annual legume GMr may meet the N needs of the soil (Sharifi et al. 2014) because long-lived legumes add similar amounts of nutrients, but reduce a year of production to grow another crop for direct income. Wheat roots have added 93kg ha^{-1} of nitrogen across a 0-75cm depth in a 2-year period through rhizodeposition (Munoz-Romero et al. 2013). An Albertan field study has reported *Vicia faba* L. (faba bean) pulse crop to fix 184 kg N ha^{-1}, and contribute over 70% of it to the soil, which is more than the 77 kg Nha^{-1} and 95 kg N ha^{-1} residues from GM faba bean and *Lathyrus sativus* L. (chickling vetch) respectively (Lupwayi and Soon 2015). *Pisum sativum* L. (peas) grown as pulse crop have made most of their residue available 2 to 3 years after planting, while GMr crops have released most of their residues within the first year according to the same source. A similar study has reported more residues from GMr crops in 1 or 3 years later compared to pulse crops (Lupwayi and Soon 2016). The positive role of GMr has been shown with the accumulation of nitrogen 2 years later (Talgre et al. 2009) and even up to 3 years later (Talgre et al. 2012). Therefore due to the varied results in the literature, it is suggested that GMr crops may or may not contribute most of their nutritional benefits to the soil before the timing of analysis, but their use in crop rotation over the long-term has lasting effects for improved soil health.

Carbon Contributions to Soil from Green Manures

Carbon is a key component of biomass from crop residues, including GMr, to support soil nutrient cycling and protein synthesis through biochemical reactions. Green manures add more plant biomass and soil microbial carbon to the soil compared to pulse crop residues (Lupwayi and Soon 2016). Legume GMrs

have contributed 7 tonnes of plant biomass/ha, with roughly 40% from just the root tissue alone (Lauringson et al. 2013). Stable amounts of SOC are also associated more with belowground sources such as roots when compared to crop tissues above the soil surface (Katterer et al. 2011). The SOC below the soil surface is better protected than just left on the soil surface, exposed to more potentially-disturbing environmental factors. The influence of cattle slurries, manure, plant roots and residues have been examined as related to SOC, finding that roots are the best option for maintaining soil carbon. Furthermore, the bulk of SOC is contributed from belowground plant material such as root tissue, undecomposed plant material and weeds (Berti et al. 2016). Over 50 years SOC dynamics have been examined in 4-year rotations with *Beta vulgaris* L. (sugar beet), and winter cereals, finding that weeds comprised a considerable amount of the SOC (Buysse et al. 2012). From a local perspective here in Eastern Canada little research has assessed changes in SOC content in an organically-managed wheat crop; indicating the need for further research in this area; as supported by other literature (Van Eerd et al. 2015). Based on the information available at this time, it is suggested that practices to retain crop residues in the soil environment are beneficial to improving carbon storage over time for better soil health.

1.4. Influence of Weeds on Soil Health

There is limited research on the impact of weeds on soil health outside of crop yield losses. Crop yields are not the only component considered under soil health assessments. What has been developed into managed agricultural ecosystems was once unmanaged landscape inhabited by whatever plants could grow and survive in the environment. Whatever microbes are in the soil build strong associations with the weed communities (Trognitz et al. 2016). As related to agro-ecosystems, weed growth is non-uniform within the same plots and between years of research (Halde et al. 2017). Perhaps a soil with lower yields may be healthier if it hosts a greater diversity of undisturbed plant species. Some research suggests potential for agricultural crop yield improvements and ecological benefits by supporting weed diversity (Smith et al. 2009; Ferrero et al. 2017; Storkey and Neve 2018). Despite the benefits to soil health and quality associated with organic farming, even within the Canadian context (Braman et al. 2016), and the continued increase in organic acreage, there are still challenges in organic agriculture. Aside from potentially adding carbon through plant tissue biomass to the soil to feed soil microbes, weeds are considered to have no immediate direct value to farmers, unless potentially supporting pollinators or enhancing biological pest control. It is clear that weeds compromise crop yields by competing with crops for sunlight, water and nutrients in the soil, starting from early stages of growth (McKenzie-Gopsill et al. 2016). In organically managed systems tillage is often used to control weeds.

Organic farmers do not want to significantly reduce crop yields, but they need methods to control weed growth since they are limited in the types of chemical weed control products they can use. A very common method of weed control is using crop rotation with different types of plants from different plant families and physiological features. Another method is increasing the seeding rate of a crop to a higher density based on seed size, row spacing, nutrient and weed growth dynamics (Shirtliffe and Benaragama 2014). Increasing the seeding rate by 1.5x has been recommended, however, a crop is not to be seeded too thick to prevent intraspecific competition for light and resources between plants of the same crop or foliar diseases (Mohler 2001; Chen et al. 2008). Potentially using green manure (GMr) crops helps improve soil structure, fertility status, biological activity and overall health. Cultural management practices are likely to be the most beneficial to control weeds and still support good soil health. Competitiveness is dependent on the particular plant species of interest, since some are more competitive than others (Teasdale and Mohler 2000). For example, *Sinapis arvensis* L. (wild mustard), non-mycorrhizal weed growth success may be reduced by the presence of a red clover mycorrhizal GMr crop (Conklin et al. 2002; Rinaudo et al. 2010). Plants that form arbuscular mycorrhizal fungi (AMF) associations are known to regulate nutrient accessibility between the soil and plant roots. Soybeans can produce up to 450kg N ha^{-1} through biological nitrogen fixation (BNF), (Peoples and Craswell 1992) accounting for potentially more than 80% of inputs (Burns and Hardy 1975) with root nodulation due to signaling and *Bradyrhizobia* bacterial interactions to promote AMF associations (Antunes 2004; Meena et al. 2018). The AMF concept is further described below with its relationship to weeds.

1.5. Mycorrhizae and Weed Relationships

Mycorrhizae, and more specifically arbuscular mycorrhizal fungi (AMF) are a type of fungus that forms beneficial symbiotic relationships with plant roots, including many weed species. Over 80% of reported vascular plants examined are mycorrhizal (Wang and Qiu 2006), corresponding to over 200,000 species of plants having this symbiosis (Eun-Hwa et al. 2013). These fungi are from the phylum *Glomeromycota* (Schubler et al. 2001). The fungi are attracted to plant roots, infect their tissues and allow for the exchange of nutrients. A direct quote nicely summarizes the infection process as follows: “fungal spore germination, hyphal differentiation, appressorium formation, root penetration, intercellular growth and arbuscule formation” (Giovannetti et al 1994, Abstract). The association occurs between plant roots and AMF enhancing nutrient uptake. Phosphorus (P) is a key nutrient that AMF help plants obtain from the soil, in exchange for carbon as a source of carbohydrates, such that when there is adequate amounts of available soil P, AMF communities have reduced levels of infection on plant roots (Ryan and Tibbett 2008) compared to more abundant or aggressive infection when more P is needed by the plant (Graham and Abbott 2000).

Other benefits of AMF include improving soil structure, better tolerance to heavy metals (lead, copper, mercury, arsenic, cadmium), and protection from disease-causing organisms (Gosling et al. 2006; Sikes et al 2009). Interactions between AMF in diverse plant communities (e.g. crops and weeds) are not understood as well (Klironomos 2003; Vatovec et al. 2005). Mycorrhizal weeds are most beneficial to crops that require mycorrhizal symbiosis to receive nutrients (Stejskalova 1990). Weeds that do not host AMF can have reduced productivity if grown close to AMF in the soil (Jordan et al. 2000). It is possible that plants hosting AMF symbiosis can control plant community structure (Van der Hejden et al. 1998; Veiga et al. 2011). Some common weeds in agricultural fields are non-mycorrhizal (Rinaudo et al. 2010) such as *Amaranthus retroflexus* L. (redroot pigweed), *Chenopodium album* L. (lamb's quarter) and wild mustard (Vatovec et al. 2005). Even though weeds are a nuisance in agricultural systems, particularly those managed organically, they add to the overall plant diversity of the ecosystem, which has been reported to increase the soil microbial activity (Chen et al 2003; Garbeva et al. 2006; Kubota et al. 2015). One concept being considered is the Resource Pool Diversity Hypothesis to regulate weed and crop access in the environment (Smith et al. 2009). Additional research is needed to determine the interactions between weed diversity in organic cropping systems, crop yields and soil health. The AMF are a vital part of healthy soil agroecosystems, especially organic systems that are typically lower in nutrients and have greater reliance on the beneficial role of these symbiotic fungi (Hamel et al. 2006).

In organically-managed corn fields, lower inputs have resulted in more vesicular-arbuscular mycorrhizal fungi (VAM) development and growth compared to higher input conventionally-managed fields (Galvez et al. 2001; Gosling et al. 2010). Having a combination of low nutrient inputs, a good variety of crops in rotation or intercrops, and with less tillage promotes higher populations of microbes including AMF (Nelson and Spaner 2010). Higher plant and microbial biodiversity have improved soil fertility in organic soils compared to conventional farming (Mader et al. 2002). Organic systems tend to have high AMF proliferation due to less fertility inputs through rotation (Hamel et al. 2006; Schneider et al. 2017). Over 21 years up to 50% less amendments have been added to organic land, with only a 20% reduction in the crop yields compared to that obtained from the conventional land.

1.6. Influence of Tillage on Soil Health

Tillage is any practice of working the land to prepare it for agricultural production and it is an important crop management practice used in organic agriculture, particularly for weed control (Teasdale et al. 2007; Carr et al. 2013). The type, shape, weight and size of the equipment with implements, depth of disturbing the soil and intensity or frequency of field passes all influence its effectiveness. A common method is spring tillage followed by tine harrowing 3-5cm deep early in the growing season to control weeds (Gilbert et al.

2009). Tertiary tillage, also called in-crop tillage including the use of rotary hoes, harrows and interrow cultivators has been proposed as a key weed management practice for field crop production by organic standards (Shirtliffe and Benaragama 2014). Rotary hoes are less intensive reaching a soil depth of 2-5cm; harrows have rigid frames and tine-weeders with varying degrees of intensity based on machine settings that can be adjusted for different crop/cropping system; while interrow cultivators operated by a variety of different equipment are used in row crops with best success earlier in the growing season (Cloutier et al. 2007). However, there are both advantages and disadvantages to tillage practices that need to be weighed to evaluate the impacts on the health and quality of soils (Lynch 2014).

Some advantages of tillage may include breaking up hardpan aggregates to make a more homogenous, uniform seedbed while disturbing weed root systems to reduce weed proliferation. There tends to be more moisture belowground; such that tilling plant residues into the soil can generate heat and quicken the rate of their decomposition by microbes (Angers et al. 1997). In different case studies soil chemical health measurements have still been good after tillage practices (Schindelbeck et al. 2008). A proposed rationale for higher chemical scores as exposed to tillage could be better incorporation of crop residues rather than leaving them on the surface of the soil to decompose slower. However, many studies have proven that tillage has negative impacts on soil health (Moebius et al. 2007; Idowu et al. 2009; Nesbitt and Adl 2014) on crop yields compared to crop rotations to control weeds (Anderson 2014) and on mycorrhizal populations or community composition (Nelson and Spaner 2010; Manoharan et al. 2017). Tillage disturbs the natural aggregation of soil, increases compaction and decreases SOC compared to no-till (Puerta et al. 2018).

Soil health is compromised with increased erosion risk through moldboard plowing. Moldboard plowing is a primary form of tillage that has negative impacts on soil health (Van Eerd et al. 2014), as used in some conventional tillage operations (Rasmussen et al. 1993). Other authors have reported disk-tilling and chisel plowing each done twice/year at beginning and end of the season as part of conventional agricultural practices (Mathew et al. 2012). A 12-year study conducted on a silt-loam compared no-till to plow or chisel tillage; such that soil quality was reported to be better under the no-till system by producing stronger water stable aggregates, more microbial activity and carbon supply (Karlen et al. 1994). A 2-year ley period with *Poaceae* (grass) and clover having either intensive or reduced tillage in organic or conventional agriculture has been studied, such that the ley period improves the SOC up to a 20cm depth in both of the organic systems, but not in either of the conventional systems (Puerta et al. 2018). It raises the question as to whether soils can be resilient to the negative impacts of tillage practices on soil health characteristics.

Soil microbial biomass has been reported to be 30% greater, with SOC, earthworm populations, and wet aggregate stability being higher in no-till systems (Mangalassery et al. 2015). In Eastern Canada conventional potato field production has shown similar biological soil features to nearby organic potato fields because of more regular tillage practices in organic production (Nesbitt and Adl 2014) with the challenge of controlling weeds (Teasdale et al. 2007). Using tillage and cover crops may not be effective to control weeds for sustainable agricultural practices because of their destructive and water-depleting implications to soil respectively (Lehnhoff et al. 2017). It is suggested to reduce tillage when possible, and integrate livestock into the ecosystem approach, as supported by other research (Thiessen-Martens and Entz 2011; Mckenzie et al. 2017). There has been limited research conducted on the influence of tillage interactions with plants (crops and weeds), as well as soil microbes (Lynch 2015). The current project will make use of the Cornell Soil Health Assessment (CSHA) manual procedures to measure a series of different indicators of soil health.

1.7. Cornell Soil Health Assessment (CSHA)

Soil health can be measured through various soil properties, such as those outlined in the "Comprehensive Assessment of Soil Health (CHSA): The Cornell Framework Manual" (Moebius-Clune et al. 2016). To establish the CSHA short-list of the most practical soil properties to measure, sampling has been conducted over time at many locations in New York and surrounding states in the US, with a range of different management and production practices (Van Eerd et al. 2015). To help with this selection of indicators, the criteria include: functionality, sensitivity, ability to test it, precision, and cost to analyze (Moebius et al. 2007; Schindelbeck et al. 2008). The most practical indicators suggested to be used in a standard health test include physical features: texture, surface and subsurface hardness, available water capacity (AWC) and wet aggregate stability (WAS); chemical features: (pH, and nutrient analysis for potassium (K), phosphorus (P), zinc (Zn), magnesium (Mg) and iron (Fe)); and biological features: (organic matter (OM), respiration of the microorganisms, autoclaved citrate extractable (ACE) soil protein index, and active carbon with partial oxidation (POXc)) (Moebius-Clune et al. 2016). The physical, chemical and biological soil health indicators are described further below.

1.7.1. Physical Soil Health Indicators

Soil texture is the mineral portion of soil composed of sand, silt and clay. Texture is an inherent soil property that has a direct influence on all the other measures of study. The wet aggregate stability method is a way of trying to mock a natural outdoor setting in a controlled laboratory environment to test the soil's strength against slaking through a sieve when exposed to heavy rainfall over a set period of time. Aggregate stability has been reported to be better in clay-based soils high in organic matter compared to sandy soils (Ekwue

and Stone 1994). Surface or subsurface hardness is a relative measurement of the amount of compaction experienced by the soil from a depth of 0-15cm and up to 46cm respectively. Available water capacity is a measure of the water in the soil that is available to plants. It is a measure of the difference between the water held in the soil at field capacity and at permanent wilting point.

1.7.2. Chemical Soil Health Indicators

The pH is a measure of the acidity or alkalinity of the soil in solution, equal to the negative base log 10 units, based on the concentration of hydrogen ions in solution. Most field crops grow well at an average pH between 5-7. The nutrients measured through the CSHA include available P, K, Mg, Ca, Cu, Mn, Zn, and Fe. A Morgan's (sodium acetate) extraction solution is used according to the Cornell methods (and Barney 2006). Alternatively, the Mehlich 3 nutrient extraction method uses an extract composed of ethylendiaminetetraacetic acid (EDTA), ammonium fluoride, acetate, nitric acid and ammonium nitrate (AgroEcoLab 2016). Acetic acid buffers the pH to 2.5 and preserves calcium, preventing it from being released in a compound with fluoride (Hudak-Wise 2013). This latter method is used in Atlantic Canada because the local soils tend to be more acidic, and it is useful to extract a greater variety of nutrients (Stiles 2018). There is also the Bray-1 and Olsen methods used in Northern Central region of the USA, with either dilute HCl/ammonium fluoride or sodium bicarbonate as the extractant respectively, but the Mehlich 3 has been suggested to be a better universal test (Mallarino 1995). As part of that same study, the Bray-1 method is used for more acidic or neutral soils, while the Olsen test is used for more alkaline soils, as supported by other literature (Ketterings and Barney 2006).

1.7.3. Biological Soil Health Indicators

Organic matter is made of carbon-containing materials such as microorganisms and plants residues that have gone through various degrees of decomposition. More specifically on a chemical level, it is made of humic materials, amino acids, carbohydrates, and lipids (Pribyl et al. 2010). The OM content is less stabilized in sandy soils compared to clay-based soils (Johnston et al. 2009), and it has the benefit of improving soil structure; which in turn improves water-holding capacity. Soil respiration is an oxidation reaction resulting from microbial metabolism. Autoclaved citrate extractable protein (ACE) measures the nitrogen that is organically bound in proteins (Schindelbeck et al. 2016). Cupric (Cu^{+2}) is reduced to the cuprous form (Cu^{+1}) of copper (Thermo-Fisher Scientific 2016). Active carbon (AC), as permanganate oxidizable carbon (POXc), is another oxidation colorimetric reaction to find the portion of the SOC that is easily accessible for the microbes to utilize to meet their metabolic needs (Moebius-Clune et al. 2016). For more specific details or other information about the analytical procedures to conduct each test of the CSHA

indicators, please see the Standard Operating Procedures (SOP) prepared by the Cornell Soil Health lab (Schindelbeck et al. 2016).

1.8. Interpreting Soil Health Results

Within CSHA, soil texture influences soil health indicators and separate scoring functions are often calculated for coarse, medium and fine-textured soils. There are 3 types of responses that can be characterized including "high is better", "low is better" and "optimum" based on the particular indicator of interest, since lower values of some indicators such as surface and subsurface hardness are better scores of soil health (Moebius-Clune et al. 2016). For each individual indicator rating, colored scores are assigned as follows: 0-30 is low (red), 30-70 is medium (yellow) and 70-100 is high (red). An overall score out of 100 is generated for the soil sample as a sum of all the indicators evenly weighted, with constraints and recommendations provided if necessary. According to CSHA, less than 40% is considered very low; between 40-55% is considered low; between 55-70% is medium; 70-85% is high and greater than 85% is very high (Gugino et al. 2007). However, these scoring functions are not as easily adaptable to other regions because they have to take local edaphic agronomic and climate conditions into consideration. Therefore, if scoring functions were generated with adjustable scales for these variable factors, they may be more suitable across larger geographical areas.

1.9. Phospholipid Fatty Acid (PLFA) Analysis

Phospholipid fatty acid analysis (PLFA) is used to examine the microbial community composition and biomass in soil samples (McKinley et al. 2005; Buyer and Sasser 2012). More specifically, it is a measure of the methylated fatty acids per gram (MFA/g) measured in nanomoles per gram (nmol/g) (Willers et al. 2015). The PLFA is not a direct measurement within the CSHA protocols, but it provides additional complementary information about biological soil health (including mycorrhizae) and community composition. Strong healthy soil microbial communities are extremely important to manage ecosystems including those used for agriculture (Lupwayi et al. 1998). There is evidence that both CSHA and PLFA analyses provide a better overall evaluation of soil health when compared to just either method alone (Mann 2017). Phospholipids are made of a polar phosphatidyl head group attached to a glycerol and two acyl side chains each with a non-polar nature in their environment (Chowdhury and Dick 2011). The current work will go beyond crop rotation or tillage practices by also examining what influence weed presence and biomass have in organic cropping of wheat and soybeans on PLFA assessment of soil health. More research is needed to compare PLFA between organic and conventionally-managed field sites.

1.10. CSHA Adaptation to Other Methods

An alternative method to measure soil texture is using a hydrometer, but it takes more time with a 7-hour settling time period between taking measurements (Beretta et al. 2014). The hydrometer method provides more accurate results than the 2-hour settling period in the Cornell method (Owji et al. 2012). A study in Ontario for 14 years has found that CSHA is sensitive to the impacts imposed by crop rotation and tillage practices as a good assessment in that region; and furthermore implementing no-till practices and adding winter wheat into rotation have been recommended to enhance soil health (Van Eerd et al. 2014). Principle Component Analysis (PCA) using selected CSHA indicators and weighted averages of them with eigenvectors is the method for the Ontario Soil Health Assessment (OSHA). It supports being a more sensitive and reliable analysis for evaluating soil health scores since the CSHA uses un-weighted averages of the same indicators (Congreves et al. 2015). Calcium, magnesium, active carbon, silt, organic matter and cation exchange capacity (CEC) form the first component of the PCA, accounting for 44% of the variation. The second component shown contains clay, zinc, and potassium, and they have accounted for 18% of the variation. A trend has shown that the soil health indicators within the same category (biological, physical and chemical) are more likely to be associated with other soil health indicators in the same category. According to OSHA, scores of <44% are poor, 45-54% are fair, and >55% are good. In 3 of their 4 long-term research field experiments, the CSHA and OSHA scores have been higher under no-till management compared to those undergoing conventional tillage practices.

A second method to assess soil quality or soil health includes the Soil Management Assessment Framework (SMAF) (Wienhold et al. 2005; Cherubin et al. 2016) and the Soil Quality Index (SQI) (Karlen et al. 2013). Collectively these indices include the following measurements: electrical conductivity, soil organic carbon, potassium, phosphorus, sodium, pH; bulk density, available water capacity, wet aggregate stability, potentially mineralizable nitrogen, and microbial biomass carbon. However, the interpretation of these indicators is different because SMAF uses stepwise regression with dependent and independent variables (Andrews et al. 2004), while SQI uses General Linear Model (GLM) and transformed means to fit scoring curves. There is third framework for analysis of measuring soil health called the Haney test, which has been used in a recent study on over 21,000 soil samples; finding that many farmers have been fertilizing in excess of the requirements to maintain healthy soils (Haney et al. 2018). The Haney test includes a smaller collection of indicators such as potentially mineralizable phosphorus and nitrogen, microbial respiration and inorganic macronutrients. Both nitrate and ammonium in addition to P, Ca, Zn and K can be extracted with a Haney extractant (Haney et al. 2010). However, the Haney test is not as comprehensive as CSHA, because it does not cover as many biological and physical indicators that have the potential to show some sensitivity.

Recently the Soil Health Institute (SHI) covering North American soils was funded to work with the Nature Conservancy, and the Soil Health Partnership (SHP) to assess a wide variety of soil health indicators classified into either Tier 1 (primary) or Tier 2 (secondary) based on their effectiveness (SHI 2018). Tier 1 indicators have thresholds, are well defined by region in North America, and are influenced by particular control measures; whereas Tier 2 indicators need more validation to hold the same confidence as Tier 1 measures. The SHI covers 31 indicators across the CSHA, the Haney test and SMAF. The SHI classify pH, extractable nutrients, SOC, texture, available water capacity, and penetration resistance as Tier 1 indicators, while they classify active carbon, soil protein index and phospholipid fatty acid (PLFA) as Tier 2 indicators. Generally, they research team put more confidence on the chemical and physical indicators, and less on the biological aspects.

1.11. Rationale of Research Project

There are various projects being conducted on Dalhousie University's Faculty of Agriculture campus to measure soil health through CSHA procedures, but none of them are specifically examining the influence of weed presence and biomass or that of the residual impact of a GMr crop 2-years prior in organic grain rotations. Additionally, there are limited studies examining microbial biomass and vegetative community composition (weeds and crop plants) in combination with these other factors. In Eastern Canada the impact of using different amendments to support potato production was studied; such that potato plants accumulated more nitrogen with a previous GMr of red clover instead of a mix combining oats, peas and vetch (Alam et al. 2016). However, that study only looked at the residual effect 1 year later, and not 2 years later. Therefore, the current study provided some insight into a few longer residual effects.

1.12. Objectives and Hypotheses

Chapter 2-Experiment 1

Objective 1: To examine soil health under organic soybean production as influenced by previous green manure (GMr) grown two years prior in rotation.

Hypothesis 1a: Red clover GMr will promote better soil physical health (greater aggregate stability, and less compaction) due to deeper rooting capabilities compared to GMr of common vetch or hairy vetch underseeded with oats.

Hypothesis 1b: There will be no significant difference in biological or chemical soil health attributes when comparing the different previous GMr treatments.

Objective 2: To examine the influence of weed presence and weed community composition (number of species and contribution of each to total biomass) on soil health parameters and soybean yields.

Hypothesis 2a: The presence of weeds (promoting increased weed biomass and number of species) will improve soil health measurements compared to weed-free soil, but the number of species will be more beneficial than the quantity of weed biomass.

Hypothesis 2b: The presence of weeds will reduce soybean yields.

Chapter 2- Experiment 2

Objective 1: To examine soil health under organic wheat production as influenced by tillage regime of GMr (spring tilled with or without post emergent tine weeding or no till).

Hypothesis 1: The spring tilled GMr treatments will have significantly higher soil respiration, active carbon, ACE protein, and lower wet aggregate stability, and available water capacity compared to no-till GMr treatments.

Objective 2: To examine what relationships exist between weed biomass and number of species on soil health parameters and wheat yields.

Hypothesis 2: The relative weed biomass (based on quadrat sampling sizes) will have a greater negative influence than number of different weed species on soil health properties and wheat crop yields.

Chapter 3- Experiment 1

Objective 1: To examine soil microbial biomass (nmol MFA/g) under soybean production as influenced by the previous green manure (GMr) in rotation.

Hypothesis 1: The treatments with a previous GMr of hairy vetch underseeded with oats (HVO) will result in significantly less soil microbial biomass due to resource competition with weeds.

Objective 2: To examine weed dynamics on contribution to total PLFA from each microbial group under soybean production as influenced by weed presence and weed community composition.

Hypothesis 2a: The treatments with a greater number of weed species will result in the lower %contribution of each microbial group to total PLFA when compared to the treatments with a fewer number of weed species.

Hypothesis 2b: The number of weed species will be more positively correlated with PLFA microbial biomass compared to weed biomass.

Chapter 3- Experiment 2

Objective 1: To examine soil microbial biomass (nmol MFA/g) in wheat plots as influenced by tillage regime of GMr in rotation.

Hypothesis 1: Tillage will reduce soil aggregate structure, nutrient passageways and remove soil carbon, thereby reducing microbial biomass compared to no-till.

Objective 2: To examine weed dynamics on contribution to total PLFA from each microbial group in wheat production as influenced by number of weed species and weed biomass.

Hypothesis 2a: Treatments with a greater number of different weed species in wheat plots will be positively correlated with greater contribution to total PLFA from each microbial group compared to those with fewer weed species.

Hypothesis 2b: Weed biomass will be negatively correlated with the number of weed species.

CHAPTER 2- Influence of green manures, weeds and tillage on soil health in an organic crop rotation

2.1. Introduction

The study of the role of GMr, weeds and tillage on soil health is important for farmers and researchers improve sustainable management practices in organic production. To avoid the use of synthetic fertilizers, pesticides or other products requires reverting to more cultural and physical management strategies such as crop rotations and tillage respectively, to be successful in crop management (Anderson 2015; Barbieri et al. 2017). The sub-topics of incorporating other crops including GMr, contemplating the preservation of some weeds versus tilling the soil are all inter-connected as they influence the overall well-being and interactions of different components within the agro-ecosystem.

Incorporating GMr crops into rotation improves nutrient management, maintains ground cover until being plowed in, and they compete with weeds (Havlin et al. 1990). The variety of different weeds have been shown to be influenced by crop rotation patterns (Dai 2013). GMr crops can prevent loss of nutrients through runoff or erosion, and help to control soil moisture compared to using no GMr (Wyngaarden et al. 2015). Legume GMr are an excellent source of nutrients to improve soil fertility status and crop yields (Tamm et al. 2016). Under-seeding GMr has resulted in residual effects up to 3 years later due to slower release of nutrients compared to a solid seeding of only one crop (Talgre et al. 2009). However, the amounts of N, P and K supplied to the soil have been higher with solid seeding of one GMr compared to being under-seeded with another crop. Hairy vetch taproot systems are not as strong as those of red clover, making the former more preferable for shallower fibrous root growth to support higher root activity and better efficiency of soil nutrient dynamics (Pritchard and Rogers 2000). Depending on the depth of SOC measurements compared, red clover GMr may supply more C and improve aggregation in deeper soil depths, or perhaps more evenly with increasing depth compared to vetches. However, further research is needed to compare the physiological attributes of these legumes to soil health.

Weeds can be considered a contributing factor in assessing soil health, as they compete for and may replenish similar soil nutrients as crops and contribute to SOC. Weeds are a natural part of the ecosystem, even on agricultural land (Altieri et al. 1999), and especially in organic systems (Kubota et al. 2015). Organic practices take a more holistic approach to promote biodiversity including weeds (Hyvonen et al. 2003). There is great interest in maintaining ecologically-sound practices to support soil health, but it is also important to manage weeds effectively to sustain crop yields. The presence of weeds grown in different mixtures of species influences soil microbial communities, in which fewer species actually have increased

fatty acid methyl ester (FAME) extraction (Wortman et al. 2013). An increased amount of FAME's indicates that there is a higher concentration of fatty acids that can be quantified by oxidizing the carbon, identifying corresponding isotope ratios with related signatures and comparing them against a database of known signatures to determine what microbial groupings and concentrations of them are present in the samples (Buyer et al. 2012; Yao et al. 2015). One limitation of the PLFA method is that it does not identify the microbes to the species level. However, having many species may be more beneficial to the soil ecosystem than others, so being able to identify these species could lead to new insights about the role of weeds in agricultural systems. Weed species such as *Persicaria maculosa* S.F.Gray (lady's thumb), *Stellaria media* L. (chickweed), lamb's quarters, *Poa annua* L. (annual bluegrass) and *Senecio vulgaris* L. (common groundsel) have been found to be beneficial in agricultural fields for being low competitors with crops for growth resources (Storkey 2006; Storkey and Westbury 2007). Previous literature is limited on the impact of weeds on soil health, making it an important area of future study (Carr et al. 2013).

Tillage is still a common practice in organic production to control weeds (Teasdale et al. 2007; Cwach et al. 2012; Shirtliffe and Benaragama 2014) and incorporate GMr into the soil (Jordan et al. 2000; Franz and Gunter 2014). Some examples include flex-tines, rolling baskets, and spiked disc blades (Cloutier et al. 2007). However, using tillage alone has not been effective to control weeds, and it is suggested to also incorporate some cultural and biological methods into the overall management strategy (Carr et al. 2013). The trade-off between using tillage or not revolves around whether to protect the fundamental physical structure, biological life, and chemical composition of soil compared to obtaining higher crop yields (Salami et al. 2017). High intensity practices can alter the weed species present in an area, removing some of the plant diversity (van Elsen 1999). The crop species and tillage practices are more influential than organic vs. integrated systems on weed community structure and species (Jastrzebska et al. 2013).

2.2. Objectives and Hypotheses

In the following study two experiments were conducted over the 2016-2018 growing seasons in Bible Hill, Nova Scotia. The overall objective of Experiment 1 was to determine the effect of in-crop weeds in a soybean crop and GMr grown in rotation 2 years prior on soil health and yield of an organic soybean crop. The overall objective of Experiment 2 was to determine the effect of current season tillage regime of GMr and weeds on soil health and yield of an organic wheat crop.

In Experiment 1 it was hypothesized that red clover will promote better physical soil health scores than the vetches, but there will be no significant differences in the biological or chemical soil health indicators between GMr. It was speculated there would be significant differences for the biological and chemical

indicators based on the weed condition, and potentially an interaction between the GMr and weed condition. The relative weed biomass would be more influential than number of weed species present in negatively impacting soil health and soybean crop yields (Experiment 1). Futhermore, in Experiment 2 it was hypothesized the tilled GMr treatments would increase the relative weed biomass (due to the number of non-mycorrhizal species dominating agricultural sites), as well as the biological and chemical soil health indicators, but reduce soil physical soil health responses compared to the no-till treatment.

2.3. Materials and Methods

2.3.1. Experiment Overview

The study comprised two full seasons of fieldwork at two field sites in Bible Hill, Nova Scotia, Canada. In 2016 and 2017 data was collected from Experiment 1. In 2017 and 2018 data was collected for Experiment 2. Information specific to each experiment is described below.

2.3.1.1. Experiment 1 Site Description and Design

This site has geographic coordinates of 45°23′12.71″ N, 63°14′ 20.17″ W. The soil is a Pugwash sandy loam classified as an orthic humo-ferric podzol (Webb et al. 1991). All GMr have been grown for one year only within three-year rotations comprised of a (GMr→Wheat→ Soybean) crop rotation sequence. An additional rotation has no GMr and is just a repeated two-year wheat-soybean rotation. A field map of the cropping system is provided (Appendix 1).

The site is arranged in a split plot randomized complete block design with 3 blocks. This experiment has a 4x2 factorial with 4 levels of the GMr crop [(hairy vetch underseeded with oats (HVO), *Vicia sativa* L. (common vetch) underseeded with oats (CVO)), red clover (RC), and a No GMr control treatment)] as the main plot factor, and 2 levels of the weed condition, as either weedy or non-weedy as the subplot factor. The weedy treatments have been imposed through the split-plot design by dividing each soybean plot into 4 randomly assigned subplots each 4-crop rows wide (91cm). Weeds were removed from each of 2 subplots (designated as non-weedy) and the other 2 left weedy over the whole growing season. There are 8 different treatment combinations/block of the GMr crop and weed condition (Table 1).

Table 1: Treatment combinations green manure (GMr) and weed condition in Experiment 1

Treatment Combo Number	GMr	Weed Condition
1	Red Clover	Weedy
2	Red Clover	Non-Weedy
3	HVO	Weedy
4	HVO	Non-Weedy
5	CVO	Weedy
6	CVO	Non-Weedy
7	No GMr	Weedy
8	No GMr	Non-Weedy

HVO= Hairy Vetch Underseeded with Oats, CVO= Common Vetch Underseeded with Oats

To describe some of this site's cropping history, the site has previously been used to grow forages and *Zea mays* L. (corn) before 2006 (Sharifi et al. 2014). Then from 2006-2010,the Experiment 1 site was dedicated to experiments on organic crop rotations with *Solanum tuberosum* L. (potatoes), *Daucus carota* subsp. sativus (carrots), and 2 or 3-year green manures- oats underseeded with red clover, an oats/pea/hairy vetch mix, *Phaseolus lunatus* L. (lima beans) and *Fagopyrum esculentum* Moench. (buckwheat) (Sharifi et al. 2014; Alam et al. 2016). Data was collected from the subplots and averaged to generate representative data for each treatment. The unit summary for this experiment is outlined below (Table 2).

Table 2: Unit summary for Experiment 1

Component	Experiment 1
Main Plot Units	4 Rotations (GMr of HVO, CVO, RC or No GMr)
Split Plot Units	2 Conditions (Weedy or Non-Weedy Soybeans)
Treatment Combinations/Block	4 main plot x 2 split plot =8
Experimental Units	4 main x 2 split x 3 blocks= 24

Note: HVO= Hairy vetch underseeded with oats, CVO= Common vetch underseeded with oats, RC= Red Clover.

The soybeans (which followed wheat in rotation) were seeded with 30cm spacing while all the other crops have been seeded with 15cm spacing, and the seeding depth for all the crop seed is 1.25 cm (Main 2016). The seeding information and timeline of field tasks for this project has been included below respectively (Table 3 and 4). Some supplementary information about the soybean growth stages is provided, which was considered in scheduling field tasks (Berglund et al. 2015). Oats have been under-seeded at 70kg/ha in mixtures with hairy vetch or common vetch at 30kg-ha^{-1} (Alam 2016). For comparison, if oats had not been

underseeded, but planted as a single crop, they would have been seeded at 160kg/ha (Alam et al. 2016). Between 2011 and 2013 the rotations changed to focus on organic spring wheat, and soybeans as a cash crops, with a GMr of hairy vetch introduced as an added treatment.

Table 3: Seeding dates and rates for crops in 2016 and 2017 growing seasons of Experiment 1

Date of Seeding (DD-MM-YY)	Crop	Variety	Rate
17-May-16	Wheat	Helios	165 kg/ha
02-Jun-16	**Soybean**	**Savannah**	**90 kg/ha**
23-Jun-16	Buckwheat	Not Specified	70 kg/ha
26-May-17	Wheat	Helios	173 kg/ha
30-May-17	**Soybean**	**Savannah**	**91 kg/ha**
31-May-17	Hairy Vetch	Not Specified	30 kg/ha
31-May-17	Common Vetch	Not Specified	30 kg/ha
31-May-17	Oats	Dieter	70 kg/ha
01-Jun-17	Red Clover	Wildcat	12 kg/ha
20-Jun-17	**Re-seed Soybean**	**Savannah**	**91 kg/ha**

Note: Crop of focus is bolded for emphasis.

In 2014, 2015 and 2016 the wheat plots received treatments of Acti-sol pelletized poultry manure as a supplemental nitrogen source (Alam et al. 2018). In more intensive cropping systems growing potatoes, using both GMr crops and compost or dehydrated manure N sources has improved yields (Lynch et al. 2012). Each year plots were tilled with a disc harrow and rolling baskets followed by S-tinning to effectively control weeds and prepare the seedbed at the start of the spring. A moldboard plow has been used on the 2nd year red clover plots (Main 2016). The recorded weather data is shown below for the Debert tracker (Table 5) as the closest location to Truro, Nova Scotia (NS) Canada (Government of Canada 2016-2017).

Table 4: Timeline of field tasks in Experiment 1 for 2016 and 2017 growing seasons

Date (DD-MM-YY)	Number of Days After Seeding (DAS) Soybeans	Task
02-Jun-16		Seeded Soybeans
29-Jun-16	27 days	Imposed Weedy/Non-Weedy Treatments in Soybean Plots
05-Aug-16	63 DAS (9 weeks)	Weed Species ID and Community Composition in Soybean Plots
10-Aug-16	68 DAS (10 weeks)	Soybean Biomass Dry Weights +Elementar
27-Sep-16	115 DAS (16 weeks)	Soil Sampling Soybean Plots for CSHA, and PLFA
06-Oct-16	124 DAS (17 weeks)	Soybean Crop Yield Components Including Grain Yields
20-Jun-17		Seeded Soybeans
26-Jul-17	24 DAS	Imposed Weedy/Non-Weedy Treatments in Soybean Plots
17-Aug-17	57 DAS	Weed Species ID & Community Composition in Soybean Plots
28-Aug-17	68 DAS	Soybean Biomass Dry Weights*
15-Oct-17	115 DAS	Soil Sampling Soybean Plots for CSHA and PLFA
24-Oct-17	124 DAS	Soybean Crop Yield Components
14-Nov-17	145 DAS	Soybean Grain Yields*

Note: *The dry soybean biomass and seed yields were not measured in the second growing season (2017) at Field 206 because of the poor weather conditions in the spring and greater deer browsing damage.

Table 5: Local temperature & rainfall data 2016, 2017 and 2018 growing seasons

Year	Month	Avg. Temp (°C)	Rainfall (mm)
2016	May	10.3	70.8
2016	June	14.6	61.1
2016	July	19	83.3
2016	August	18.4	116
2016	September	14.4	73
2017	May	10.1	135
2017	June	14.6	62
2017	July	18.1	66.1
2017	August	17.6	109
2017	September	15.5	62.7
2018	May	9.5	93.9
2018	June	12.5	175.7
2018	July	20.4	47.4
2018	August	20.1	64.8
2018	September	14	83.3

Source: CBC News Environment Canada weather data tracker for Debert, NS.

2.3.2. Experiment 2 Site Description and Design

This site has geographic coordinates of 5°23′24.72″ N and -63°15′16.15″ W (Wallace 2015). The soil at this site is classified as an orthic-humo ferric podzol class or subgroup (Government of Canada 2013). There are 3 and 4-year crop rotations (GMr → Wheat → Fall Rye → Soybean) depending on the GMr crop; such that those rotations with red clover, which is present for 2 years prior to wheat, do not have ryegrass in them. Experiment 2 for the current project over 2017-2018 is focused on comparing 4 spring tillage treatments to terminate the GMr crops, including spring tilled red clover (STRC)+ post-emergent tine weeding (PETW), spring tilled hairy vetch underseeded with oats (STHVO), spring tilled hairy vetch underseeded with oats+ post emergent tine weeding (STHVO+PETW) and a no-till control (Appendix 2). Soil and weed sampling were done twice during the 2017 season, indicating repeated measures. The field was assessed as a randomized complete block design with 3 experimental blocks. Data was only collected from the wheat plots (n=12). Seeding dates/rates/crop varieties and a timeline of the field tasks are given respectively (Table 6 and 7). A summary of the response variables is also provided (Table 8).

The site was managed until 2013 in 4-year rotations with soybeans, 2 years of red clover and wheat (Marshall and Lynch 2018). From 2013 onwards, all but one of the red clover treatments were switched to HVO as part of a 4 year sequence with rye, wheat and soybeans (GMr → Wheat → Fall Rye → Soybean). While the RC was fall-tilled, three treatments of varying tillage intensity have been applied to the HVO,

including: zero-tillage (only roller crimped), fall rolled + spring tilled, or fall tillage. Commencing in 2016, two of the HVO treatments were fall tilled and one of these also received post emergent tine weeding tillage following wheat seeding emergence the following spring. The remaining HVO treatment involved no-till termination of the HVO GMr. The RC treatment was fall-tilled. The planting schedule for both experiments is provided with the cropping history from the previous 3 years and rotation sequences of focus for this study highlighted (Appendix 3 and 4). This study focused on the impact on soil health (measured by the CSHA framework assessment described below) of these GMr tillage treatments and the influence of weed presence in the wheat crop phase on these responses. All GMr were planted as described for Experiment 1.

Table 6: Seeding dates and rates for crops in 2017 and 2018 growing seasons for Experiment 2

Date of Seeding	Crop	Variety	Rate
26-May-17	**Wheat**	**Helios**	**173 kg/ha**
30-May-17	Soybean	Savannah	91 kg/ha
31-May-17	Hairy Vetch	Not Specified	33 kg/ha
31-May-17	Oats	Dieter	120 kg/ha
01-Jun-17	Red Clover	Wildcat	12 kg/ha
20-Jun-17	Re-seed Soybean	Savannah	91 kg/ha
01-Jun-18	**Wheat**	**Helios**	**173 kg/ha**
02-Jun-18	Soybean	Savannah	91 kg/ha
01-Jun-18	Hairy Vetch	Not Specified	33 kg/ha
01-Jun-18	Oats	Dieter	120 kg/ha
31-May-18	Red Clover	Wildcat	12 kg/ha

Table 7: Timeline of field tasks for Experiment 2 in 2017 and 2018 growing seasons.

Date	Time Period After Seeding	Task
25-May-17	-------	Seeded Wheat
12-Jun-17	12 DAS (1.5 weeks)	Weed and Wheat Density
28-Jun-17	33 DAS (5 weeks)	Soil Sampling Wheat Plots for CSHA, PLFA
07-Jul-17	42 DAS (6 weeks)	Weed Species ID & Community Composition in Wheat Plots
17-Aug-17	82 DAS (12 weeks)	Weed Species ID & Community Composition in Wheat Plots
21-Sep-17	116 DAS (17 weeks)	Wheat Samples Combine Yield for Threshing
27-Sep-17	122 DAS (17 weeks)	Wheat Biomass Dry Weights
02-Oct-17	127 DAS (18 weeks)	Soil Sampling for CSHA, PLFA
01-Jun-18	-------	Seeded Wheat
13-Jun-18	12 DAS	Weed and Wheat Density
27-Jun-18	26 DAS	Weed Species ID & Community Composition in Wheat Plots
28-Jun-18	27 DAS	Soil Sampling Wheat Plots for CSHA and PLFA
23-Jul-18	52 DAS	Weed Species ID & Community Composition in Wheat Plots
24-Aug-18*	81 DAS	Wheat Biomass Dry Weights
07-Sep-18*	95 DAS	Wheat Samples Combine Yield for Threshing
18-Oct-18[1]	134 DAS	Fall Soil Sampling Block 1
19-Oct-18[1]	135 DAS	Fall Soil Sampling Block 2
22-Oct-18[1]	138 DAS	Fall Soil Sampling Block 3

*The original plan has been to keep the same timeframe for each task in 2018 to compliment 2017, but due to a very warm growing season in 2018 with extremely high temperatures, the wheat harvest has been shifted forward a few weeks to keep in line with physiological maturity of the crop. Additionally, due to Canada geese getting into the plots and eating the crop, some yield losses were observed. This may have affected the impact imposed by the tillage treatments by impacting crop yields.

[1]= The fall soil samples collected in October 2018 have not been analyzed as part of this MSc thesis project, but were retained for complimentary analysis to be published later.

Table 8: Summary of measured response variables for both experiments

Component	Experiment 1	Experiment 2
Crop	Soybean Plant Dry Weights, Number of Plants/m, Plant Height, Number of Pods and Pod Weight/Plant, Tissue %N, Grain Yields	Wheat Dry Weight, Tissue %N, Grain Yield
Weed	Community Composition (Number of Species and Biomass)	Plant Density, Community Composition (Number of Species and Biomass)
Soil	CSHA properties, PLFA Microbial Biomass and %total composition by microbial grouping	CSHA properties, PLFA Microbial Biomass and %total composition by microbial grouping

Note: PLFA= Phospholipid fatty acid analysis

2.4. Field Data Collection

2.4.1.1. Experiment 1: Weed Treatments and Characterization

A full list of all field and laboratory equipment is attached (Appendix 5). To control weed seedlings in the non-weedy subplots a light hand-hoeing weed management strategy was used once the soybeans started to emerge from the soil during both growing seasons. In early August 2016 and 2017 aboveground weed biomass sampling was conducted with a ($0.25m^{-2}$ square meter) quadrat (1m by 1/4m) in each weedy soybean subplot to characterize weed biomass, composition and diversity. A total of four (4) quadrats/plot were collected to obtain $1m^2$ of weed data/plot and brought back to campus to dry to constant weight (Barberi et al. 2017).

2.4.1.2. Experiment 2: Weed Characterization

The nature of the weed control treatments from Experiment 1 were not imposed in Experiment 2 because it would not be practical in a crop like wheat, compared to the soybeans row crop. However, in mid-June of 2017 and 2018 the germinated weed and wheat seedlings were counted in a $1/16^{th}$ square meter quadrat area (n=4/plot) to determine plant density. Two weed community composition samplings were completed in both years to see if the weed composition changed within the season. To assess competition between the weed and crop, as influenced by tillage regime, 4 quadrats/plot were sampled in early July and mid-August of each year. Weed biomass sampling was conducted with a $0.25m^{-2}$ quadrant (1/2m by 1/2m) in each wheat subplot (n=4/plot) to characterize weed biomass, the number of species and community composition. Four (4) quadrats/plot were collected to obtain $1m^2$ of biomass samples. A full list of all weed species (scientific and common names) across both experiments is provided (Appendix 6).

2.4.2.1. Experiment 1: Crop Biomass, Nutrient Content and Grain Yield

The vegetative and reproductive growth stages of soybeans are attached (Appendix 7). Soybeans have 7 vegetative stages from April to July in a good growing season, beginning from emergence and cotyledon development. There are 5 reproductive stages to follow from August to October. Having knowledge of the growth and development of soybeans has helped to plan out the field data and sample collection timetable.

In the 2016 growing season sixteen (16) randomized meter strips of soybean plants were flagged in each of the 12-soybean plots (8 within weedy subplots and 8 within non-weedy subplots) for a split-plot design. In mid-August the plants in 8 marked meter strips (4 in weedy and 4 in non-weedy) were cut at the soil surface, collected in cloth bags and temporarily stored in a walk-in cooler at 4°C until being dried to constant weight for 3 days at 55°C (130°F). Once weighed for dry weight biomass, the tissue was ground in a Wiley Mill to 2-mm. The tissue C and N content was determined by dry combustion on an Elementar C and N analyzer (Elementar Americas, Hann Germany).

In early October 2016 the remaining flagged soybean plants in 8-marked meter strips/plot (4 in in weedy and 4 in non-weedy) were cut at the soil surface and brought back to campus in cloth bags. The number of plants/meter, height of the plants and number of pods/plant have been recorded for all sampled areas. The tissue was temporarily stored in a walk-in cooler at 4°C before being air-dried and machine threshed to determine grain yields. This yield determination procedure was planned to be repeated during the 2017 growing season, but due to a wet spring resulting in a late planting date, poor germination, re-seeding and deer browsing after the plants emerged (in spite of an electrical deer fence), the soybean plants were not sampled to determine biomass, nutrient content, or seed yields. However, the number of plants/meter and plant height measurements were still obtained from the soybean plants in the field.

2.4.2.2. Experiment 2: Crop Biomass, Nutrient Content and Grain Yield

There are 10 developmental phases in wheat including germination, seedling growth, tillering, stem elongation, booting, ear emergence, flowering, milk development, dough, and ripening (Appendix 8). The growth season for wheat typically runs from early May to late August-mid September. In late September 2017 and 2018 wheat samples were harvested and threshed with a plot combine, dried down and weighed to determine wheat biomass yields ($t.ha^{-1}$). In addition to that, four $0.25m^2$ quadrats of crop plant tissue were sampled to determine dry weights.

2.4.3.1. Experiment 1: Soil Sampling

In late September of 2016 and 2017, four composite soil samples were collected per plot for soil health determination, with 2 taken from weedy and 2 taken from non-weedy subplots from each of the 12 soybean plots for a total of 48 samples. To collect each subsample a shovel was used to reach a marked 15cm depth to remove a clean slice, and a knife was used to remove and discard the roughly outer 5cm on either end to save the middle section (Moebius-Clune et al. 2016). Within each of the 4 soybean subplots 5 subsamples were collected and mixed to generate a representative composite sample.

The soil samples were collected in clear plastic bags and stored in a cooler with ice packs while in the field, and then transferred to a walk-in cooler at 4°C once back to campus prior to Cornell Soil Health Assessment (CSHA) (Moebius-Clune et al. 2016). The analysis for the suite of 15 indicators takes approximately 4-6 weeks to complete (Kurtz 2016). A small amount (50g) from each sample was separated and frozen for PLFA analysis as described in more detail (Chapter 3). The remainder of each soil sample was air-dried, with 50g separated for available water capacity (AWC) and texture determination; 75-100g for soil respiration and autoclaved citrate extractable (ACE) protein; 100g for wet aggregate stability (WAS) and active carbon; and 50g for nutrient analysis (Schindelbeck et al. 2016). The air-dried soil was passed through an 8-mm sieve prior to respiration, texture, ACE protein and WAS analysis. Soil for AWC, nutrient analysis, active carbon and OM has passed through a 2-mm sieve (Moebius-Clune et al. 2016).

A penetrometer was used in both Experiments to collect readings for surface and subsurface hardness during the timing of soil sampling. In Experiment 1, there were 5 readings per subplot collected with 4 subplots totaling 20 readings/soybean plot or 240 readings in the field. In Experiment 2 there was 10 readings/plot collected with 12 plots totaling 120 readings in the field. A depth of 0-15cm was penetrated for surface hardness assessment, and a depth of 15- 46cm was designated for subsurface hardness. These readings are normally collected after a heavy rainfall event when the soil is close to field capacity (Moebius-Clune et al. 2016). The time of the season can influence the rainfall and water holding capacity of the soil. For example, between June and August in both 2016 and 2017, there was almost double the amount of rainfall in the latter month. Although to compare May and September of 2016, rainfall amounts were very similar, but in 2017 there was more than double the rainfall at the start of the season compared to the end of the season, in agreement with weather trends from other studies (Van Eerd et al. 2014). The environmental conditions in each growing season influenced crop and weed growth, soil microbial dynamics. Many agricultural field studies have tracked weather patterns to have good records of these important abiotic factors, as done in other studies (Lounsbury and Weil 2014; Alam et al. 2016).

2.4.3.2. Experiment 2: Soil Sampling

In mid-June of 2017 and 2018, ten soil subsamples/plot were collected for soil health and PLFA analyses with a small hand-held gardening shovel 15cm deep in between the rows of wheat to avoid disturbing the plants. The soil were collected and mixed in a bucket to make a homogenous representative sample for analysis (~2kg/plot). They were collected in clear plastic bags and stored in a cooler with ice packs while in the field, and then transferred to the walk-in cooler at 4°C once back to campus until being processed as described for Experiment 1 in section 2.5.3.1 above. The CSHA indicators were also tested in Experiment 2, with the same procedures as in Experiment 1. About 50g of each sample was also saved for PLFA analysis, as discussed later in this document (Chapter 3).

2.5. Lab Data Collection

2.5.1. Cornell Soil Health Assessment (CSHA)

Soil from both experiments has been analyzed to measure the 15 indicators of the CSHA standard test including various biological, physical and chemical parameters. The CSHA manual and the Standard Operating Procedure (SOP) manual provide a more detailed rationale and instructions for each laboratory test (Moebius-Clune et al. 2016). Parameters to be measured for the CSHA included soil physical features (soil texture, wet aggregate stability, available water capacity), biological features (organic matter, active carbon (POXc)), autoclaved citrate extractable protein, soil respiration), and chemical features (pH and available macro and micronutrients). The specified sieving sizes are as summarized below (Table 9). Also, Mehlich 3 available nutrient extractions have been done in place of the Cornell Morgan's extractant solution. Deviations were made to some of the procedures and these modifications are provided (Appendix 9-14).

Table 9: Sieving specifications for each applicable CSHA indicator test

< 2-mm	0.25-2mm aggregates	8-mm	<250-um*
Available Water Capacity	Wet Aggregate Stability	Respiration	Organic Matter
Active Carbon		ACE Protein	
Nutrients			
Texture			

*Ground on Roller Grinder

2.5.1.1. Physical Soil Health Indicators

2.5.1.1.1. Texture

For this procedure, 14.00g of air-dried soil was measured out for each sample, combined with 42mL of 3% hexametaphosphate soap solution and shaken for 2 hours. Each sample was poured over a 0.053mm sieve into a collection beaker and washed through with water. The sand remaining on the sieve were transferred to labeled aluminum tins and oven-dried at 105ºC to collect the weight. The silt and clay that passed through into the collection beaker was left to sit for 2 hours to separate. The silt sunk to the bottom while the clay dissolved in the water. After 2 hours of settling, the water with clay was decanted, and the silt remaining was collected in another labeled aluminum tin and oven-dried to determine its weight. The percentage of clay was determined through difference once subtracting the silt and sand from the initial soil mass. The step-by-step procedure is provided (Appendix 9).

2.5.1.1.2. Surface and Subsurface Hardness

This is a measure of the level of compaction of a soil. A field penetrometer was pressed into the soil to a depth of 15cm, and up to 46cm to see how far it could go without exceeding 300 units of pressure. If exceeding this value, crop roots are restricted. This test is usually measured 2-3 days after a heavy rainfall event, once rain has had a chance to drain from the field.

2.5.1.1.3. Available Water Capacity

For the soil available water capacity test, two pressurized chambers including 1 and 15 bar pressure plates were used. Roughly 15-20g of soil were used for each sample; enough to fill the rings loosely for the 1 bar plate or tightly for the 15 bar plate. The soil was saturated at set-up before being measured at field capacity (10kPa) and permanent wilting point respectively (1500kPa), to determine the available water capacity. The 1 bar plate takes about 3-4 days to complete, but the 15-bar plate takes closer to a week to complete. The step-by-step procedure is attached (Appendix 10).

2.5.1.1.4. Wet Aggregate Stability

Wet aggregate stability examines the aggregates (250-2000mm) that are able to avoid slaking through a 0.25mm sieve after being exposed to 1.25cm of steady rainfall for up to 5 minutes. As a slight deviation from the CSHA SOP manual, which recommends delivering the 1.25cm of water on the soil in the sieve over a 5-minute period, in this study emphasis was placed on delivering 1.25cm of rainfall instead of exactly 5 minutes of exposure to the water because it proved difficult to do both with the rainfall simulator apparatus available. The step-by-step procedure is attached (Appendix 11).

2.5.1.2. Biological Soil Health Indicators

2.5.1.2.1. Organic Matter

Loss-on-ignition (LOI) is the recommended method of measuring soil organic matter (SOM) in the Cornell Soil Health SOP manual. The soil is exposed to a high temperature at 500°C to combust the organic matter, which the SOM is determined by difference. However, the components of the sample, the temperature and length of combustion all influence the end final sample weight and calculation of SOM (Pribyl 2010). Heating temperature for soil samples affects water content determination of organic soils, with advantages and disadvantages to higher and lower ranges (O'Kelly and Li 2018). If the samples are heated too high, the structured clay soil particles may lose water in heated reactions, called dehydroxylation; but if they are heated too low, all of the organic matter may not be combusted.

A more reliable method of measuring SOC directly is using automated dry combustion through a varioMax CN Elementar from Elementar Americas Inc.© at high temperature (900°C) in the presence of oxygen and carbon dioxide with helium carrier gas to measure the samples through infrared absorption spectroscopy (Van der ven 2016). Therefore, this method and deviation from strictly the Cornell SOP as per Moebius-Clune et al. (2016) was chosen. The soil total C and N content were obtained from the output. The %C was converted to %OM by multiplying each carbon percent value by a conversion factor of 2 (Pribyl 2010).

2.5.1.2.2. Active Carbon (POXc)

Active carbon represents the portion of the SOM that is readily available as a food source for microbes to access (Moebius-Clune et al. 2016). To measure this indicator, soil was mixed with a deep purple solution of potassium permanganate (KMnO4), shaken and allowed to settle in a 10-minute oxidation reaction. The lighter the color the solution becomes (extending from magenta, light pink, pale pink to almost clear as water), the more active carbon that is present in the sample. A cuvette of each sample was run on a spectrophotometer at 550nm wavelength to measure absorbance. The samples are compared to a standard curve made from standard $KMnO_4$ solutions with specified concentrations. The step-by-step procedure is attached (Appendix 12).

2.5.1.2.3. Autoclaved Citrate Extractable (ACE) Protein

This biological indicator is a measure of the nitrogen concentration bound in organic compounds, which is an index of mineralizable soil N. This method involved a 3-part process including autoclaving air-dried 8-mm sieved soil with sodium citrate to sterilize the solution; centrifugation to clarify the suspension, and a colorimetric bicinchoninic acid assay (BCA) with a color change from green to purple to read absorbance on a PowerWave XS2 multiplate spectrophotometer, BioTek Instruments Inc. at 562nm wavelength

(Moebius-Clune et al. 2016; Schindelbeck et al. 2016). Each sample was run in quadruplet (4 wells/sample) to assess for variability or analytical error. The step-by-step procedure is attached (Appendix 13).

2.5.1.2.4. Soil Respiration

This indicator of biological soil health measured the carbon dioxide given off by the microbial life in the samples during a 4-day incubation in mason jars with a small bottle of potassium hydroxide solution to trap the CO_2 respired. The electrical conductivity in microsiemens (uS/cm) of the trap solution was measured before and after the incubation to find the difference after removing the measurements obtained for the blank comprised of just KOH solution (Moebius-Clune et al. 2016; Schindelbeck et al. 2016). Each sample was run in duplicate. The step-by-step procedure is attached (Appendix 14).

2.5.1.3. Chemical Soil Health Indicators

The chemical measurements include the available macro and micronutrients of interest and pH. The Cornell method of determining the plant available soil nutrient content is to use a Morgan extractant solution (Ketterings and Barney 2006). The optimal values for each CSHA chemical indicator are provided below (Table 10) as described (Moebius-Clune et al. 2016).

Table 10: Optimal values for Cornell Soil Health Assessment chemical soil health indicators

Nutrient	Parts per million (ppm)
pH	6.25-7.25
Phosphorus (P)	<25
Potassium (K)	>70
Magnesium (Mg)	>33
Iron (Fe)	<25
Manganese (Mn)	<50
Zinc (Zn)	>0.25

A deviation from Cornell SOP methods was chosen to measure the soil available nutrients through Mehlich 3 extraction, Mehlich 3 extractions have been reported to be affordable, with the ability to measure many nutrients including some that are less common such as lead (Mallarino 1995). They are also the extractant used in Atlantic Canada provincial laboratories due to their suitability for assessing plat available nutrients based on the more acidic soil pH in this region. Their step-by-step procedure is available (Appendix 15). Mehlich 3 extracted filtrates of each soil sample in duplicate were sent to the PEI Analytical Laboratories in Charlottetown PEI where optical emission spectroscopy (OES) determined the nutrient concentrations (Sanders 2017; Brine 2018 Personal Communication). It is an efficient process that allows analyses of a few hundred samples in 3-5 days.

Since the provincial standards and optimal pH for Nova Scotia and PEI was measured in the NS soil laboratory. The pH was also been measured by a 1:1 ratio of soil to deionized water in the Agroecology laboratory at Dalhousie to ensure the readings are in the same range (Kalra, Y. P. 1995; Miller and Kissel 2010). The nutritional content of the soil has been tested at the Nova Scotia Harlow Laboratory in kg/ha^{-1} units for practicality on a larger scale (Appendix 16 and 17).

2.6. Statistical Analysis

Minitab v.19 was used to check for data normality, constant variance and independence of the error terms. The data was confirmed to be normal, so no transformations were needed. In both experiments SAS v.9.4. was used with a Duncan's Multiple Mean Comparison (MMC) for the soil health data when applicable to distinguish significant treatment differences. A Fisher's LSD MMC was used for the wheat crop yield components data. Since the CSHA does not consider relationships between the different soil health indicators measured, Pearson correlations were run in SAS with PROC CORR codes (using 3 levels of significance: $P<0.05$, <0.01, <0.001) to get more specific knowledge of which ones are positively and negatively associated with each other. Both sites and years have been analyzed separately because they are not directly comparable.

Experiment 1 has fixed effects including the GMr crop, weed condition and any interaction between the GMr crop (RC, HVO, CVO, No GMr) and weed condition (weedy or non-weedy). Random effects are any variation by block, and possibly the year of analysis. The data has been analyzed in SAS v.9.4 with ANOVA in Proc General Linear Model (GLM) using $\alpha=0.05$. Other related studies have also used Proc GLM for the analysis (Campiglia et al. 2011; Karlen et al. 2013; Cherubin et al. 2016).

Experiment 2 has a mixed ANOVA with 2 factors including the tillage treatment (ST RC+PETW, ST HVO+PETW, ST HVO, No-Till), and the temporal time-point of soil sampling (June, August, October), assessed with repeated measures, as analyzed in SAS v9.4 with ANOVA in Proc Mixed using $\alpha=0.05$. Pearson correlations have also been tested in this experiment.

2.7. Results -2.7.1. Experiment 1

2.7.1.1. Weed Community Composition and Biomass

There was 12 weed species identified in the first season and 19 identified in the second season respectively (Table 11 and 12). There was an increase in number of weed species from 2016 to 2017, but also a large decrease in the measured weed biomass (t DM ha^{-1}).

Table 11: Proportional contribution of each species to total weed biomass by GMr treatments quantified in August 2016

	Green Manure (GMr) Treatment			
	Red Clover	Hairy Vetch	Common Vetch	No GMr
Weed Biomass ($t.ha^{-1}$)	3.29 ±0.42a	2.20 ±0.05a	2.46 ±0.29a	2.37±0.73a
Amaranthus retroflexus L.	2%	14%	1%	2%
Capsella bursa-pastoris L.	<1%	<1%	<1%	0%
Chenopodium album L.	4%	11%	3%	7%
Cirsium arvense L.	0%	0%	0%	6%
Conyza Canadensis L.	2%	<1%	1%	<1%
Phleum pratense L.	4%	4%	2%	2%
Plantago major L.	3%	5%	6%	3%
Raphanus raphanistrum L.	33%	**28%**	**57%**	**46%**
Rumex crispus L.	12%	11%	7%	5%
Spergula arvensis L.	**39%***	11%	13%	17%
Stelleria media L.	1%	2%	1%	<1%
Taraxacum officinale G.H,Weber ex Wiggers	<1%	14%	9%	12%
Total Number of Weed Species	11	11	11	11

Note: *Bolded values represent weed species/treatment comprising the greatest percentage.

Table 12: Proportional contribution of each species to total weed biomass by GMr treatments quantified in August 2017

	Green Manure (GMr) Treatment			
	Red Clover	Hairy Vetch	Common Vetch	No GMr
Weed Biomass ($t.ha^{-1}$)	0.84 ±0.10a	0.54 ±0.19a	0.79±0.06a	0.59±0.21a
Amaranthus retroflexus L.	2%	20%	7%	9%
Barbarea vulgaris R.Br.	0%	0%	<1%	0%
Capsella bursa-pastoris L.	0%	1%	2%	1%
Chenopodium album L.	7%	16%	19%	3%
Digiteria sanguinalis L.	2%	6%	1%	2%
Echinochloa crus-galli L.	2%	<1%	2%	3%
Elymus repens L.	0%	2%	1%	0%
Erysimum cheiranthoides L.	0%	0%	0%	2%
Glechoma hederacea L.	0%	0%	0%	3%
Jacobaea maritima L.	0%	<1%	<1%	0%
Persicaria maculosa L.	0%	1%	0%	1%
Plantago major L.	0%	0%	5%	0%
Polygonum pensylvanicum L.	0%	0%	1%	0%
Raphanus raphanistrum L.	**45%***	**34%**	**44%**	**45%**
Setaria pumila L.	33%	3%	2%	9%
Spergula arvensis L.	9%	9%	10%	12%
Stellaria media L.	<1%	6%	5%	8%
Tanacetum vulgare L.	0%	0%	0%	2%
Taraxacum officinale G.H.Weber x Wiggers	<1%	2%	1%	0%
Total Number of Weed Species	9	13	15	13

Note: Bolded values represent weed species/treatment comprising the greatest percentage.

2.7.1.2. Soil Health Response to Prior GMr and Weed Presence

In both years of analysis for Experiment 1, most of the measured attributes of soil health showed no significant response based on the influence of the previous GMr in rotation 2 years prior or the weed condition. This was a surprising result, as it was thought that more indicators would be effected; particularly measures such as active carbon and ACE protein as influenced by weeds as small sensitive portions of larger SOM pools. Although some biological indicators, especially soil respiration, have appeared to be quite sensitive to both factors in 2016 (Table 13). This trend has not been consistent for both factors in 2017 since only weeds consistently significantly influenced respiration. In the second year alone WAS and ACE Protein significantly responded to the residual effect of the GMr. Some soybean agronomic parameters were significantly affected by weeds in both years.

Table 13: Summarized significant soil health results in Experiment 1 over 2016 and 2017

Year	Component	Source	F Value	Pr>F
2016	Soil Respiration	GMr	13.81	<0.0001
2016	Soil Respiration	WeedCon	5.83	0.0266
2017	Soil Respiration	WeedCon	9.51	0.0064
2017	ACE Protein	GMr	2.99	0.0471
2017	WAS	GMr	3.9	0.0188

Note: WAS= Wet aggregate stability, GMr= Green manure crop rotation phase, WeedCon= Weed Condition, Mg= Magnesium, P= Probability Greater than F statistic.

The mean and standard error values for all CSHA indicators measured in 2016 of Experiment 1 are provided (Table 14). Soil respiration was influenced by the GMr in rotation and by the weed condition. Red clover produced the highest respiration response, significantly higher than that obtained from either the CVO or No GMr. The weedy soil in both years resulted in significantly higher respiration than the non-weedy treatment. The results for 2017 are also provided (Table 15). The ACE Protein and WAS were significantly influenced by the GMr rotation crop phase, with the most obtained from RC compared to No GMr. However, as found in 2016, only the respiration was affected by the weed condition in 2017, with weedy soil having significantly more compared to the non-weedy treatment. None of the Mehlich 3 extractable nutrients were influenced by the GMr treatments or weed condition in either year (Table 16 and 17).

Table 14: Cornell Soil Health indicators measured in 2016 as influenced by (GMr) treatments in rotation and weed presence in Experiment 1 (Mean ±SE)

GMr Treatment	ACE Protein (mg/g dry soil)	Active Carbon (ppm)	Available Water Capacity (g/g)	Organic Matter (%)	pH (pH units)	Soil Respiration (mg CO2/g dry soil)	Surface Hardness (psi)	Subsurface Hardness (psi)	Wet Aggregate Stability (%)
Red Clover	11.2±0.77a	653±37.1a	0.247±0.012a	3.33±0.06a	6.20±0.05a	3.14±0.16a	176±5.49a	233±3.24a	57.6±2.46a
HVO	9.78±0.58a	685±30.6a	0.289±0.011a	3.24±0.13a	6.63±0.13a	2.98±0.16ab	183±7.03a	237±4.35a	50.2±3.80a
CVO	9.24±0.50a	662±21.2a	0.274±0.010a	3.23±0.11a	6.45±0.07a	2.88±0.07b	177±2.27a	235±2.08a	53.8±2.91a
No GMr	10.1±0.86a	587±27.4a	0.271±0.011a	3.21±0.14a	6.50±0.05a	2.80±0.09b	177±3.08a	231±4.19a	58.4±4.91a
Weedy	10.3±0.26a	634± 40.4a	0.27± 0.009a	3.26±0.06a	6.48± 0.06a	3.09±0.08a	178± 3.67a	233± 2.98a	57.0± 2.63a
Non-Weedy	9.88±0.25a	592±36.6a	0.27±0.008a	3.21±0.05a	6.41± 0.08a	2.81±0.09b	179±3.05a	236±1.85a	53.0±2.50a

Note: GMr= Green Manure, SE= Standard Error, HVO= Hairy Vetch Underseeded with Oats, CVO= Common Vetch Underseeded with Oats. Treatments with the same letter are not significantly different according to Duncan's test at α=0.05.

Table 15: Cornell Soil Health indicators measured in 2017 influenced by GMr treatments in rotation and weed presence in Experiment 1 (Mean ±SE)

GMr Treatment	ACE Protein (mg/g dry soil)	Active Carbon (ppm)	Available Water Capacity (g/g)	Organic Matter (%)	pH (pH units)	Soil Respiration (mg CO2/g dry soil)	Wet Aggregate Stability (%)
Red Clover	10.7±0.70a	1105±42a	0.184±0.015a	3.33±0.16a	5.54±0.14a	2.10±0.04a	60.7±3.86a
HVO	9.8±0.68ab	1099±49a	0.183±0.010a	3.23±0.17a	5.85±0.14a	2.11±0.03a	55.7±3.02ab
CVO	9.2±0.25ab	1087±46a	0.23±0.008a	3.24±0.13a	5.95±0.08a	2.07±0.02a	50.7±2.70ab
No GMr	8.7±0.45b	1158±28a	0.21±0.014a	3.00±0.18a	5.96±0.04a	2.06±0.01a	45.9±2.70b
Weedy	9.7±0.37a	1089±33a	0.197±0.009a	3.23±0.11a	5.87±0.16a	2.12±0.01a	53.43±2.42a
Non-Weedy	9.5±0.48a	1135±24a	0.204±0.010a	3.17±0.12a	5.77±0.19a	2.05±0.01b	53.09±2.91a

Note: GMr= Green manure, SE= Standard Error, HVO= Hairy Vetch Underseeded with Oats, CVO= Common Vetch Underseeded with Oats. Treatments with the same letter are not significantly different according to Duncan's test at α=0.05. Surface and subsurface hardness were not measured due to sharing equipment.

Table 16: Mehlich 3 extractable nutrients in 2016 as influenced by green manure (GMr) treatments in rotation and weed presence in Experiment 1 (Mean ±SE)

GMr Treatment	Phosphorous (P)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)	Boron (B)	Zinc (Zn)	Sulfur (S)	Manganese (Mn)	Iron (Fe)	Copper (Co)
Red Clover	317±23.9a	224±65.5a	883±272a	113±10.2a	5.86±0.35a	14.2±1.22a	31.8±14.6a	57.4±10.3a	206±29.3a	1.61±0.12a
HVO	305±45.0a	181±14.9a	819± 69.9a	161±16.0a	5.40±0.29a	13.7±0.51a	17.7±2.78a	88.3±10.1a	247±15.8a	1.55± 0.17a
CVO	283±43.0a	244±51.8a	611±47.6a	115±10.0a	5.25±0.43a	13.0±1.22a	21.9±4.80a	56.1±6.32a	214±22.5a	1.53±0.15a
No GMr	302±31.5a	206±29.3a	690±33.0a	128±12.7a	5.35±0.32a	13.4±0.77a	26.1±7.90a	61.5±7.29a	229±17.6a	1.63±0.08a
Weedy	303±26.8a	189±23.2a	658±41.9a	123±9.42a	5.19±0.22a	12.8±0.56a	19.1±0.83a	60.8±6.32a	208±13.6a	1.52±0.11a
Non-Weedy	307±17.8a	226±32.4a	775±115a	126±9.92a	5.47±0.23a	13.7±0.70a	26.7±7.21a	64.8±7.21a	223±17.7a	1.62±0.07a

Note: SE= Standard Error, HVO= Hairy Vetch Underseeded with Oats, CVO= Common Vetch Underseeded with Oats. Treatments with the same letter are not significantly different according to Duncan's test at α=0.05.

Table 17: Mehlich 3 extractable nutrients in 2017 as influenced by green manure (GMr) treatments in rotation and weed presence in Experiment 1 (Mean ±SE)

GMr Treatment	Phosphorous (P)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)	Boron (B)	Zinc (Zn)	Sulfur (S)	Manganese (Mn)	Iron (Fe)	Copper (Co)
Red Clover	357±31.6a	1047±392a	882±83.9a	154±29.6a	31.3±7.03a	7.11±0.79a	101±41.8a	84.4±20.0a	285±127a	5.24±1.52a
HVO	306±12.1a	530±162a	1006±66.5a	194±26.3a	47.1±3.41a	5.64±0.60a	105±47.3a	76.3±12.7a	76.3±12.7a	3.52±0.66a
CVO	330±15.4a	512±117a	1028±36.3a	221±9.03a	38.8±5.95a	6.10±0.54a	78.9±38.7a	111±11.8a	111±11.8a	4.48±0.60a
No GMr	317±23.3a	966±167a	945±26.8a	200±8.89a	35.4±7.43a	6.86±0.73a	80.2±16.4a	91.8±9.21a	91.8±9.21a	5.45±1.50a
Weedy	327±16.7a	679±143a	974±40.6a	193±15.5a	39.1±4.11a	6.43±0.45a	84.1±22.2a	92.1±10.5a	236±25.0a	3.73±0.30a
Non-Weedy	328±14.9a	848±199a	956±44.7a	191±16.1a	37.3±4.85a	6.42±0.52a	98.8±28.8a	89±9.75a	287±62.0a	5.60±1.04a

Note: SE= Standard Error, HVO= Hairy Vetch Underseeded with Oats, CVO= Common Vetch Underseeded with Oats. Treatments with the same letter are not significantly different according to a Duncan's test at α=0.05.

2.7.1.3. Soybean Crop Response to Prior GMr and Weed Presence

In both years of Experiment 1 many agronomic components of soybean crop growth were influenced by the weed condition (Table 18). In 2016, the non-weedy treatment had significantly higher soybean plant height, greater dry weight biomass/plant, more pods/plant, higher grain yields, and greater shoot N concentration compared to weedy treatment (Table 19). Grain yield was not measured in 2017 due to a poor crop establishment and persistence (very wet spring leading to late planting and re-seeding, plus deer browsing the crop, etc). The GMr treatment only significantly influenced the soybean height in 2017 as shown. Pearson correlations are provided between the different soil health indicators with weed biomass (t-ha^{-1}), number of weed species, and crop grain yields (kg/ha) in both years of this experiment (Table 20 and 21).

Table 18: Summarized significant agronomic measurements in Experiment 1 over 2016 and 2017

Year	Component	Source	F Value	Pr>F
2016	Soybean Height	WeedCon	16.4	0.0009
2016	Biomass/Plant	WeedCon	12.46	0.0028
2016	Number of Pod/Plant	WeedCon	66.24	<0.001
2016	Grain Yield	WeedCon	118.24	<0.0001
2017	Number of Plant/m	WeedCon	9.56	0.007
2017	Soybean Height	GMr	4.35	0.0201
2017	Soybean Height	WeedCon	7.53	0.0144

Note: GMr= Green Manure, WeedCon= Weed Condition (either weedy or non-weedy).

Table 19: Soybean agronomic measurements in 2016 as influenced by green manure (GMr) treatments in rotation and weed presence of Experiment 1 (Mean ±SE)

GMr Treatment	Number of Soybean Plants/m	Plant Height (cm)	Dry Weight Biomass/Plant (g)	Number of Pods/Plant	Grain Yield (t.ha^{-1})	Shoot %N
Red Clover	13±0.49a	45±2.26a	5.22±1.16a	13±2.32a	0.066±0.012a	3.51±0.15a
HVO	15±1.12a	45±1.86a	4.64±0.48a	12±1.47a	0.064±0.010a	3.67±0.16a
CVO	14±1.01a	46±2.09a	5.15±0.46a	13±1.76a	0.064±0.009a	3.51±0.18a
No GMr	13±0.56a	46±1.99a	6.08±1.06a	13±1.38a	0.067±0.009a	3.33±0.16a
Non-Weedy	14 ±0.62a	49±1.20a	6.45±0.60a	16±0.96a	121.51±2.94a	3.76±0.09a
Weedy	14 ±0.58a	42± 0.73b	4.09±030b	10±0.73b	62.24±3.95b	3.24±0.08b

Note: SE= Standard Error, RC= Red Clover, HVO= Hairy Vetch Underseeded with Oats, CVO= Common Vetch Underseeded with Oats, GMr= Green Manure. Treatments with the same letter are not significantly different according to a Fisher's LSD test at α=0.05.

2.7.1.4. Soil Health Correlations

Table 20: Pearson correlation coefficients between total weed biomass (t ha^{-1}), number of weed species, and soybean grain yields (kg ha^{-1}) with soil health indicators in 2016 Experiment 1

Measurement		1	2	3	4	5	6	7	8	9	10	11	12
AC	1	1	0.299	0.057	0.017	0.513	-0.94	0.151	-0.487	-0.091	0.084	-0.019	0.416
ACE Protein	2	0.299	1	-0.196	-0.163	0.422	-0.47	0.274	-0.123	0.0061	0.21	0.067	0.324
AWC	3	0.057	-0.196	1	0.319	0.348	0.123	-0.149	-0.032	-0.141	-0.234	-0.27	-0.161
Crop Yield	4	0.017	-0.163	0.319	1	-0.485	0.543	-0.28	0.404	-0.785**	-0.084	0.097	-0.428
OM	5	0.513	0.422	0.348	-0.485	1	-0.529	-0.081	-0.546	0.306	0.314	-0.252	0.385
pH	6	-0.094	-0.474	0.123	0.543	-0.529	1	-0.121	0.414	-0.293	-0.601*	0.117	-0.640*
Respiration	7	0.151	0.274	-0.149	-0.28	-0.081	-0.121	1	-0.061	0.197	-0.181	-0.047	0.091
SubHard	8	-0.487	-0.123	-0.032	0.404	-0.546	0.414	-0.061	1	0.066	-0.482	0.231	-0.818**
SurHard	9	-0.091	0.006	-0.141	-0.785**	0.306	-0.293	0.197	0.066	1	-0.354	-0.197	0.006
Weed Bio	10	0.084	0.21	-0.234	-0.084	0.314	-0.601*	-0.181	-0.482	-0.354	1	-0.139	0.449
Weed Spec	11	-0.019	0.067	-0.27	0.097	-0.252	0.117	-0.047	0.231	-0.197	-0.139	1	0.155
WAS	12	0.416	0.324	-0.161	-0.428	0.385	-0.640*	0.091	-0.818**	0.0067	0.449	0.155	1

Note: AC= Active Carbon, ACE= Autoclaved Citrate Extractable, AWC= Available Water Capacity, OM= Organic Matter, SurHard= Surface Hardness, SubHard= Subsurface Hardness, Weed Bio= Weed Biomass, Weed Spec= Number of Weed Species, WAS= Wet Aggregate Stability. Coefficients marked by *, **, *** are represented as significant ($P<0.05$), highly significant ($P<0.01$) or very highly significant ($P<0.001$) respectively.

Table 21: Pearson correlation coefficients between total weed biomass (t ha^{-1}), number of weed species, and soybean grain yields (kg ha^{-1}) with soil health indicators in 2017 Experiment 1

Measurement		1	2	3	4	5	6	7	8	9	10	11	12
AC	1	1	0.376	-0.253	--	0.202	-0.357	0.177	--	--	0.206	-0.433	0.108
ACE Protein	2	0.375	1	-0.477	--	0.851***	-0.680*	0.664*	--	--	0.805**	-0.052	0.863***
AWC	3	-0.253	-0.477	1	--	-0.486	0.377	-0.52	--	--	-0.327	-0.096	-0.249
Crop Yield	4	--	--	--	--	--	--	--	--	--	--	--	--
OM	5	0.202	0.851***	-0.486	--	1	-0.398	0.756**	--	--	0.740**	-0.0064	0.732**
pH	6	-0.357	-0.680*	0.377	--	-0.398	1	-0.315	--	--	-0.49	-0.051	-0.526
Respiration	7	0.177	0.664*	-0.52	--	0.765*	-0.315	1	--	--	0.407	0.046	0.688*
SurHard	8	--	--	--	--	--	--	--	--	--	--	--	--
SubHard	9	--	--	--	--	--	--	--	--	--	--	--	--
Weed Bio	10	0.206	0.805**	-0.327	--	0.740**	-0.49	0.407	--	--	1	0.127	0.633*
Weed Spec	11	-0.433	-0.053	-0.096	--	-0.006	-0.051	0.046	--	--	0.127	1	-0.071
WAS	12	0.108	0.863**	-0.249	--	0.732**	-0.526	0.688*	--	--	0.633*	-0.071	1

Note: AC= Active Carbon, ACE= Autoclaved Citrate Extractable, AWC= Available Water Capacity, OM= Organic Matter, SurHard= Surface Hardness, SubHard= Subsurface Hardness, Weed Bio= Weed Biomass, Weed Spec= Number of Weed Species, WAS= Wet Aggregate Stability. Coefficients marked by *, **, *** are represented as significant ($P<0.05$), highly significant ($P<0.01$) or very highly significant ($P<0.001$) respectively.

2.7.2. Experiment 2

2.7.2.1. Weed Density, Community Composition and Biomass

Weed and wheat density counts were quantified (quadrat method) by treatment with up to 3x more weed density compared to wheat stand in both years (Table 22). There were 18 species identified in the first season and 27 identified in the second season respectively (Table 23 and 24).

Table 22: Weed and Wheat Density counts in Experiment 2 June 2017 and 2018

Year	Tillage Treatment	Number of Wheat Plants/0.25m^2	Number of Weed Plants/0.25m^2
2017	ST RC + PETW	12 ±3.26ab	45 ±4.70a
2017	ST HVO + PETW	20 ±1.98a	13 ±3.15b
2017	ST HVO	19 ±1.16ab	32±6.02ab
2017	No- Till	7 ±0.87b	14 ±3.28b
2018	ST RC + PETW	14 ± 1.45a	6 ±1.14a
2018	ST HVO + PETW	16 ±1.88a	21 ±3.12a
2018	ST HVO	13 ±2.22a	26 ±4.41a
2018	No- Till	12 ±1.68a	21 ± 4.17a

Note: ST= Spring Till, RC= Red Clover, PETW= Post Emergent Tine Weeding. Treatments with the same letter are not significantly different according to a Fisher's LSD test at α=0.05. The ST HVO+PETW has shown significantly more wheat plants than No-Till treatment when measured in 2017, while ST RC+PETW has shown significantly more weed plants than ST HVO+PETW and No-Till.

Table 23: Proportional contribution of each species to total weed biomass across tillage treatments quantified in August 2017

	Tillage Treatment			
	STRC+PETW	STHVO+PETW	STHVO	No-Till
Weed Biomass (t-ha^{-1})	2.18 ±0.80a	2.27 ±0.38a	2.37 ±0.04a	2.79 ±1.05a
Articum lappa L.	12%	0%	0%	0%
Bromus inermis Leyss	<1%	0%	0%	0%
Chenopodium album L.	0%	7%	12%	0%
Convolvulus arvensis L.	3%	1%	0%	0%
Digiteria sanguinalis L.	0%	32%	**43%**	0%
Echinochloa crus-galli L.	0%	2%	5%	0%
Elymus repens L.	1%	0%	0%	23%
Conyza canadensis L.	0%	0%	<1%	3%
Erysimum cheiranthoides L.	0%	0%	<1%	0%
Euphorbia maculata L.	0%	0%	<1%	0%
Jacobaea maritima L.	0%	0%	<1%	0%
Persicaria maculosa L.	0%	3%	5%	9%
Raphanus raphanistrum L.	0%	**52%**	23%	20%
Rumex acetosella L.	0%	<1%	1%	<1%
Rumex crispus L.	**15%**	0%	0%	0%
Setaria pumila L.	5%	0%	0%	0%
Sonchus arvensis L.	0%	<1%	0%	0%
Taraxacum officinale G.H. Weber ex Wiggers	14%	1%	9%	**44%**
Trifolium pratense L.	13%	0%	0%	0%
Tussilago farafara L.	3%	0%	0%	0%
Total Number of Weed Species	9	9	11	6

Note: STRC= Spring Tilled Red Clover, STHVO= Spring Tilled Hairy Vetch Underseeded with oats, PETW= Post- Emergent Tine Weeding. The bolded values represent the weed species in each treatment that comprised the greatest percentage of the total community composition.

In August of 2017 the weed species comprising the largest percentage of the total weed biomass varied by tillage treatment. Common vetch dominated in the STRC+PETW; wild radish in STHVO+PETW; *Digitaria sanguinalis* L. (large crabgrass) in STHVO and *Taraxacum officinale* G.H. Weber ex Wiggers (dandelion) in no-till. In 2018 the STHVO and No-Till treatments showed wild radish to comprise the largest amount of weed biomass, while STRC and STHVO+PETW showed quackgrass as the top weed, also as a non-mycorrhizal species.

Table 24: Proportional contribution of each species to total weed biomass across tillage treatments quantified in July 2018

	Tillage Treatment			
	STRC+PETW	STHVO+PETW	STHVO	No-Till
Weed Biomass (t-ha^{-1})	3.51 ±0.88a	1.92 ±0.14a	2.09 ±0.25a	2.00 ±0.52a
Avena sativa L.	0%	<1%	5%	<1%
Capsella bursa-pastoris L.	0%	0%	<1%	<1%
Chenopodium album L.	1%	0%	<1%	1%
Cirsium arvense L.	7%	0%	0%	0%
Convolvulus arvensis L.	0%	<1%	<1%	1%
Conyza canadensis L.	0%	0%	0%	<1%
Daucus carota L.	0%	0%	<1%	0%
Elymus repens L.	**76%**	**42%**	4%	0%
Erigeron annuus L.	0%	0%	0%	<1%
Festuca arundinacea Schreb.	0%	<1%	<1%	0%
Festuca pratensis Huds.	0%	<1%	0%	<1%
Lolium multiflorum Lam.	<1%	0%	0%	<1%
Phalaris arundinacea L.	0%	0%	4%	0%
Phleum pratense L.	0%	0%	0%	2%
Plantago major L.	0%	<1%	0%	2%
Polygonum pensylvanicum L.	<1%	<1%	1%	<1%
Raphanus raphanistrum L.	15%	14%	**40%**	**56%**
Senecio cineraria D.C.	<1%	<1%	<1%	<1%
Setaria pumila L.	0%	1%	9%	<1%
Sonchus arvensis L.	0%	0%	0%	<1%
Spergula arvensis L.	<1%	1%	15%	2%
Taraxacum officinale G.H. Weber ex Wiggers	<1%	31%	18%	25%
Trifolium pratense L.	0%	7%	0%	4%
Tussilago farfara L.	0%	0%	0%	5%
Veronica peregrina L.	0%	0%	0%	<1%
Vicia cracca L.	0%	1%	0%	0%
Vicia villosa Roth.	1%	3%	4%	1%
Total Number of Weed Species	10	15	15	21

Note: STRC= Spring Tilled Red Clover, STHVO= Spring Tilled Hairy Vetch Underseeded with oats, PETW= Post- Emergent Tine Weeding. The bolded values represent the weed species in each treatment that comprised the greatest percentage of the total community composition.

2.7.2.2. Soil Health Response to GMr Tillage Regime

Most soil health indicators were not influenced by the tillage treatments. Subsurface hardness, Mehlich 3 extractable P and Ca were significantly influenced by tillage but only in 1 of 2 years measured (Table 25). The mean and standard error values for all measured indicators including soil health in 2017, 2018 and Mehlich 3 extractions for 2017 are provided (Table 26, 27, 28). In 2017 the STRC +PETW treatment resulted in significantly more subsurface hardness than No-Till. Due to a fire in the research building of the Dalhousie Agricultural Campus on June 20th, 2018, access to the rainfall simulator, pressure plates and compressor was lost before the 2018 samples were analyzed. Therefore, the second set of WAS and AWC tests in 2018 were not conducted on the soil samples from Experiment 2.

Table 25: Summarized significant soil health results due to tillage in Experiment 2

Year	Component	Source	F Value	Pr>F
2017	Subsurface Hardness	Tillage	2.69	0.0401
2017	Phosphorous	Tillage	1.56	0.0099
2017	Calcium	Tillage	5.18	0.0237

Note: Significant (less than α=0.05)

Table 26: Influence of tillage treatment of GMr on weed biomass, species composition and various soil health indicators in 2017 (Mean ± SE)

GMr Treatment	ACE Protein (mg/g dry soil)	Active Carbon (ppm)	Available Water Capacity (g/g)	Organic Matter (%)	pH (pH units)	Soil Respiration (mg CO2/g dry soil)	Surface Hardness (psi)	Subsurface Hardness (psi)	Wet Aggregate Stability (%)
ST RC+ PETW	11.4±1.04a	766±186a	0.22±0.02a	3.41±0.26a	5.36±0.10a	1.17±0.09a	206±11.4a	242±1.03a	53.4±8.09a
ST HVO+PETW	10.8±1.43a	797±178a	0.23±0.01a	3.37±0.30a	5.18± 0.10a	1.02±0.02a	188±4.55a	239±3.01ab	45.1±6.20a
ST HVO	10.6±1.21a	891±165a	0.19±0.03a	3.97±0.26a	5.18±0.13a	1.14±0.10a	185±5.66a	237±3.49ab	45.2±9.10a
No Till	11.2±1.65a	865±167a	0.22±0.03a	4.19±0.27a	5.05±0.12a	1.16±0.12a	192±4.00a	223±4.37b	45.7±6.64a
June	9.53 ±1.03a	1201±23.5a	0.20±0.02a	3.46±0.20b	5.21±0.10a	1.00±0.0006b	193 ±9.74a	235 ±5.87a	39.2±5.21b
October	12.1±0.48a	458±49.5b	0.240±0.01a	4.01±0.19a	5.18±0.06a	1.24±0.06a	202±6.27a	211±5.92a	55.5±3.93a

Key: GMr= Green Manure, SE= Standard Error, ST= Spring Till, RC= Red Clover, PETW= Post- Emergent Tine Weeding, HVO= Hairy Vetch Under-seeded with Oats, ACE= Autoclaved Citrate Extractable. Treatments with the same letter are not significantly different according to a Duncan's test at α=0.05.

Table 27: Influence of treatment tillage of GMr on weed biomass, species composition and soil health indicators in 2018 (Mean ± SE)

Tillage Treatment	ACE Protein (mg/g dry soil)	Active Carbon (ppm)	Organic Matter (%)	Respiration (mg CO2/g dry soil)	pH (pH units)	Surface Hardness (psi)	Subsurface Hardness (psi)
ST RC +PETW	8.33 ±1.05a	109 ±26.02a	3.27 ±0.46a	3.95 ±0.10a	5.38 ±0.05a	50 ±6.5b	180 ±0.54a
ST HVO+PETW	9.97 ±0.66a	164 ±46.49a	3.60 ±0.40a	5.03 ±0.22a	5.62 ±0.03a	105 ±5.6a	208 ±0.58a
ST HVO	10.6 ±1.97a	171 ±50.31a	3.53 ±0.55a	4.52 ±0.07a	5.35 ±0.10a	92 ±5.8a	190 ±0.57a
No-Till	10.6 ±2.17a	203 ±15.96a	3.60 ±0.36a	4.14 ±0.04a	5.48 ±0.05a	108 ±9.3a	224 ±0.30a

Key: GMr Green Manure, SE= Standard Error, ST= Spring Till, RC= Red Clover, PETW= Post- Emergent Tine Weeding, HVO= Hairy Vetch Under-seeded with Oats ACE= Autoclaved Citrate Extractable. Treatments with the same letter are not significantly different according to a Duncan's test at α=0.05.

Table 28: Influence of tillage treatment on Mehlich 3 extractable nutrients (ppm) in 2017 in Experiment 2 (Mean ± SE)

Tillage Treatment	Phosphorus (P)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)	Boron (B)
ST RC+ PETW	94.0 ± 20.4b	177 ±29.4a	636 ±111b	159 ±42.8a	2.35 ±0.52a
ST HVO+PETW	121 ±25.4ab	203 ±86.1a	592 ±75.0b	139 ±29.7a	2.8 ±0.97a
ST HVO	368 ±184a	255 ±127a	594 ±297b	110 ±54.8a	2.97 ±1.48a
No Till	164 ±36.7ab	186 ±57.6a	773 ±183a	145 ±57.1a	2.53 ±0.90a
June	170±22.3a	151±0.54a	876±6.33b	183±0.32a	0.91±0.001a
October	110±13.7a	259±3.03a	421±1.12a	92.9±0.29a	4.42±0.04a
Tillage Treatment	**Copper (Cu)**	**Zinc (Zn)**	**Sulphur (S)**	**Manganese (Mn)**	**Iron (Fe)**
ST RC+ PETW	1.05 ±0.13a	2.08 ±0.08a	23.2 ±6.37a	24.8±2.65a	165 ± 37.80a
ST HVO+PETW	1.12 ±0.18a	2.42 ±0.39a	24.2 ±5.75a	38.2 ±15.02a	180 ±64.99a
ST HVO	0.78 ±0.39a	3.07 ±1.53a	25.7 ±12.8a	26.8 ±13.42a	166 ±82.92a
No Till	1.15 ±0.26a	2.68 ±0.32a	24 ±7.75a	32.3 ±4.95a	175 ±27.40a
June	1.37±3.43a	3.05±2.31a	17.5±6.38a	41.5±2.28a	226±5.11a
October	0.68±0.22a	2.08±1.52b	31±23.2a	19.6±0.83b	117±5.35a

Note: SE= Standard Error, ST HVO= Spring Tilled Hairy Vetch Underseeded with Oats, PETW= Post-Emergent Tine Weeding, STRC= Spring Tilled Red Clover. Treatments with the same letter are not significantly different according to a Duncan's test at α=0.05.

2.7.2.3. Wheat Crop Response to GMr Tillage Regime

Wheat agronomic responses to the GMr tillage treatments were determined in both years of Experiment 2 (Table 29). The ST HVO+PETW treatment resulted in significantly more threshed grain yield after cleaning compared to the other tillage options. The wheat crop tissue dry weight biomass collected from late season quadrats was significantly higher from ST HVO+PETW compared to the other treatments. However the ST RC+PETW and ST HVO did not show significantly differences from each other. In 2018 the dry plant biomass was significantly higher values under ST RC+PETW or ST HVO compared to ST HVO+PETW. Due to the 2018 fire in Cox, access to the Elementar C and N analyzer machine was lost. Therefore, wheat tissue was not analyzed for % shoot N in 2018. Correlation tests were also conducted for the soil health indicators with weed biomass, weed species, and wheat grain yields. The results for 2017 and 2018 of Experiment 2 are provided (Table 30 and 31).

Table 29: Wheat agronomy data as influenced by tillage in Experiment 2

	2017			2018	
Tillage Treatment	Threshed Grain Yield ($t.ha^{-1}$)	Dry Crop Biomass ($t.ha^{-1}$)	%N Shoot Tissue	Threshed Grain Yield ($t.ha^{-1}$)	Dry Crop Biomass ($t.ha^{-1}$)
ST RC + PETW	0.39±0.23b	0.89± 0.17bc	3.60±0.15a	0.22±0.037a	1.67±0.94a
ST HVO + PETW	1.90±1.03a	3.50 ±0.59a	4.22±0.06a	0.93±0.19a	0.096±0.10b
ST HVO	0.55±1.24b	1.89 ±0.62b	3.87±0.14a	0.31±0.076a	1.29±0.11a
No-Till	0.44±1.50b	0.39 ±0.38c	2.80±0.89a	0.10±0.10a	0.71±0.40ab

Note: ST= Spring Till RC= Red Clover HVO= Hairy Vetch Underseeded with Oats. In 2018 plots 2,6, 10 and 12 had no wheat to harvest, as weeds took over. Treatments with the same letter are not significantly different according to a Fisher's LSD test at α=0.05.

2.7.1.4. Soil Health Correlations

Table 30: Pearson correlations between July 2017 weed biomass (t ha^{-1}), number of weed species, wheat grain yields (kg ha^{-1}), and soil health indicators in Experiment 2.

Measurement		1	2	3	4	5	6	7	8	9	10	11	12
AC	1	1	-0.45	-0.590*	0.008	-0.114	-0.319	0.47	-0.105	-0.288	0.318	-0.026	0.157
ACE Protein	2	-0.45	1	0.39	-0.146	-0.269	0.288	-0.464	0.066	0.384	-0.009	0.563	0.106
AWC	3	-0.590*	0.39	1	-0.016	0.252	-0.311	-0.017	0.098	0.524	-0.32	0.174	0.06
Crop Yield	4	0.008	-0.146	-0.016	1	-0.055	-0.005	-0.477	-0.024	-0.551	-0.516	-0.494	-0.15
OM	5	-0.114	-0.269	0.252	-0.055	1	-0.2	0.481	-0.348	0.256	-0.189	-0.568	-0.245
pH	6	-0.319	0.288	-0.311	-0.005	-0.2	1	-0.434	-0.063	-0.22	-0.326	0.19	-0.458
Respiration	7	0.47	-0.464	-0.017	-0.016	0.481	-0.434	1	0.078	0.447	0.384	-0.037	0.164
SubHard	8	-0.105	0.066	0.098	-0.024	-0.348	-0.063	0.078	1	0.306	0.193	0.361	0.42
SurHard	9	-0.288	0.384	0.524	-0.551	0.256	-0.22	0.447	MV	1	0.225	0.337	0.024
Weed Bio	10	0.318	-0.009	-0.32	-0.516	-0.189	-0.326	0.384	0.193	0.225	1	0.138	0.545
Weed Spec	11	-0.026	0.563	0.174	-0.494	-0.568	0.19	-0.037	0.361	0.337	0.138	1	0.335
WAS	12	0.157	0.106	0.06	-0.15	-0.245	-0.458	0.164	0.42	0.024	0.545	0.335	1

Note: MV=Missing Value, AC= Active Carbon, ACE= Autoclaved Citrate Extractable, AWC= Available Water Capacity, OM= Organic Matter, SurHard= Surface Hardness, SubHard= Subsurface Hardness, Weed Bio= Weed Biomass, Weed Spec= Number of Weed Species, WAS= Wet Aggregate Stability.

Table 31: Pearson correlations between June 2018 total weed biomass (t ha^{-1}), number of weed species, wheat grain yields (kg ha^{-1}), and soil health indicators in Experiment 2.

Measurement		1	2	3	4	5	6	7	8	9	10	11	12
AC	1	1	0.692*	--	0.071	0.887***	0.519	0.661*	-0.421	0.281	0.148	0.164	--
ACE Protein	2	0.692*	1	--	0.317	0.867***	0.335	0.336	-0.135	0.146	-0.108	0.494	--
AWC	3	--	--	1	--	--	--	--	--	--	--	--	--
Crop Yield	4	0.071	0.317	--	1	0.266	-0.287	-0.19	-0.167	-0.448	-0.626*	-0.083	--
OM	5	0.887***	0.867***	--	0.266	1	0.464	0.57	-0.49	0.041	-0.019	0.293	--
pH	6	0.519	0.335	--	-0.287	0.464	1	0.363	0.103	0.42	0.589*	0.492	--
Respiration	7	0.661*	0.336	--	-0.19	0.57	0.363	1	-0.42	0.237	0.163	0.202	--
SubHard	8	-0.421	-0.135	--	-0.167	-0.49	0.103	-0.42	1	0.481	0.228	0.381	--
SurHard	9	0.281	0.146	--	-0.448	0.041	0.42	0.237	0.481	1	0.504	0.354	--
Weed Bio	10	0.148	-0.108	--	-0.626*	-0.019	0.589*	0.163	0.228	0.504	1	0.149	--
Weed Spec	11	0.164	0.494	--	-0.083	0.293	0.492	0.202	0.381	0.354	0.149	1	--
WAS	12	--	--	--	--	--	--	--	--	--	--	--	1

Note: AC= Active Carbon, ACE= Autoclaved Citrate Extractable, AWC= Available Water Capacity, OM= Organic Matter, SurHard= Surface Hardness, SubHard= Subsurface Hardness, Weed Bio= Weed Biomass, Weed Spec= Number of Weed Species, WAS= Wet Aggregate Stability. Coefficients marked by *, **, *** are represented as significant ($P<0.05$), highly significant ($P<0.01$) or very highly significant ($P<0.001$) respectively.

2.8. Discussion

2.8.1. Experiment 1:

The GMr treatments in this study did not affect any soybean agronomic responses, including number of plants/m, plant height, dry weight biomass, number of pods/plant, shoot N concentration or grain yields. This may be to due to most of the GMr nitrogen being released and mineralized for plant uptake after the first year, with low residual effects 2 years later when examined in the soybean crop, as supported by other literature (Lupwayi and Soon 2016). Lynch et al. (2012) noted that few studies have examined the residual effect of GMr on crop yields beyond one year after their incorporation. Previous work using the same rotation as the current study examined the impact of the same GMr crops on wheat grain yields and its protein content in the year following the GMr phase, and found that type of GMr significantly influenced the wheat crop response, with HVO resulting in significantly higher yields compared to using a GMr of RC, CVO or soybeans (Alam et al. 2018). However, in the current study, there were no significant differences in protein content of the soybean grain. Related research examining a spring cowpea (*Vigna unguiculata* L.) GMr in rotation directly before seeding winter wheat over a shorter time frame within the same year has found that GMr had no effect on subsequent wheat yields (Kandel et al. 2019). However, in the current study there were also no interaction effects on soil or crop responses between the GMr and weed condition treatments. The main effect of weed presence alone significantly impacted soybean yields.

Previous work elaborated general weed thresholds for different agricultural field crops, but due to high variability across sites, and with unpredictable environmental conditions in addition to the dynamics nature of weeds, the focus shifted to looking at critical periods of weed control (CPWC) (Knezevic et al. 2002). In soybean production the CPWC was assessed to be best between vegetative (V) stage 2 until reproductive (R) stage 3 (Van Acker 1993). For wheat the CPWC was found to be 30-50 days after planting (Chaudhary et al. 2008). Given that the soybean grain yields were 2x lower under weedy versus weeded treatments supports the relevance of using CPWC. In the first 27 days after seeding soybeans, they produced a few trifoliates around the V3 mid-vegetative phase and the weeds in the unweeded treatment made a solid ground cover around the soybeans; such that once the weeds were removed from the subplots designated non-weedy, the soybeans in those areas were more agronomically successful. Controlling weeds for the first 30 days after soybean emergence has been shown to reduce crop yield losses (Van Acker 1993). Therefore, the results of the current study appear to support this recommendation.

The GMr treatments did not significantly affect the weed biomass in either year. However, interestingly, there was a shift in the community composition of weed species based on the previous GMr crop in rotation. In the first of 2 years studied, when the RC treatment was the previous GMr crop in rotation, corn spurry

was the weed species making up the greatest composition of the total weed biomass as assessed in the soybean crop, as compared to either of the vetch GMr (HVO or CVO), which resulted in wild radish as the most abundant weed. In the second year wild radish was most prominent for all GMr treatments, but an important observation was a fairly large amount of yellow foxtail (33% of total weed biomass) coming up in the RC treatment, as not observed following the vetches. These findings seem to indicate that mycorrhizal weed species such as corn spurry were more dominant in response to a RC GMr, while non-mycorrhizal species have been more dominant following vetch GMr. A similar study in Western Canada identified lamb's quarters, and Canada thistle among the top 5 weeds identified in weedy treatments compared to Shephard's purse being very abundant in either weed condition (Kubota et al. 2015). Supporting research has shown that RC GMr can suppress wild mustard growth (Conklin et al. 2002). Also, other research assessing the potential allelopathetic activity of HVO on wild radish has shown that it may not be as effective, particularly when there is additional N in the soil (Inderjit and Asakawa 2001), perhaps in association with AMF.

A key finding of this study was that GMr did not have a strong carry-over effect on soil health when measured two years later. This is important because it indicates that the timing of analysis influences the outcome of the indicators. Only 3 of 15 indicators showed a significant effect, including a biological measure (soil respiration) and two physical soil health measures (WAS and ACE protein) significantly affected by the GMr treatments, and this effect was only observed in one of two years studied. If measured one year later the results may be different. No literature to date has reported on the residual effect of a GMr crop specifically two years later in rotation on these particular soil health indicators. A recent study reported the ACE protein measurement had analytical variability comparable to Mehlich 3 based measurements of available nutrients, indicating very good precision, whereas active carbon had higher variability in line with loss-on ignition method for quantifying carbon (Hurisso et al. 2018). Previous research on the current site of this experiment tested municipal solid food waste compost or paper mill biosolid compost on soil quality under organic potato rotations, finding that potentially mineralizable nitrogen and soil nitrate levels were higher under a RC GMr compared to that of an oats-peas-vetch mixture (Sharifi et al. 2014). In support of good soil health, using GMr and dehydrated manure or compost has also shown to support potato crop yields (Lynch et al. 2012).

A consistent finding of this study was that soil respiration was significantly higher in both years from the weedy treatment compared to the non-weedy treatment. The presence of weeds increases the number of different plant species (both mycorrhizal and non-mycorrhizal species) compared to a non-weedy system, indicating that the very nature of a weedy environment is more likely to host a more active rhizospheric

area. This has the potential for increased mycorrhizal associations and chemical mucilages or exudates released from plants in association with the microbes (Trognitz et al. 2016; Meena et al. 2018). It is speculated that more exudates may have been produced in the weedy treatment, stored and later released during respiration trials for significance to be detected in the weedy treatment. Also, as noted in Chapter 3, the presence of weeds increased overall soil microbial biomass, a labile pool of soil C; which in turn likely contributed to greater measured soil respiration from weedy treatments.

Red clover did not have significantly higher WAS or lower compaction than HVO or CVO, causing hypothesis 1a to be rejected. Hypothesis 1b was also rejected on an overall basis because only soil respiration was affected in 1 of 2 years (having a weak effect), and none of the chemical indicators were significantly influenced. Weed presence did not improve overall soil health in rejection of the first part of hypothesis 2a. The number of weed species was negatively correlated with six SH indicators in June 2016 compared to seven indicators from weed biomass, suggesting that number of weed species was less negatively correlated with overall soil heath, in support of accepting the second part of hypothesis 2a. However, in 2017 of the same experiment, the number of weed species was negatively correlated with six indicators whereas weed biomass was only negatively associated with 2, showing the opposite trend. Neither weed measure (biomass or number of species) was more beneficial to soil health in both years of Experiment 1. Therefore, hypothesis 2a would be inconclusive based on the results of this study, and require further research to reach more sound findings. The presence of weeds reduced soybean yields in half, in support of accepting hypothesis 2b.

2.8.2. Experiment 2

In the first year of this study, STHVO+PETW resulted in significantly higher wheat grain yields compared to the other tillage treatments. However, in the second year there were no significant differences in the wheat crop yields between GMr termination tillage treatments, showing inconsistency of effects between years of analysis. Other studies have also found that post-emergent harrowing had no affect on wheat yields or N content (Gilbert et al. 2009), especially when compared to the comparative effects of till v.s. no-till (Barberi et al. 2000). Contributing factors to these results could perhaps be due to variation in weather patterns each season since there was more than 2x more rainfall in June 2018 compared to June 2017, or interference from Canada geese getting into the fields and damaging the wheat crop stand, which may have reduced final crop yields and partially masked the impact from tillage treatments. The wheat crop plant biomass was significantly influenced by the tillage treatments in both years, but the treatment effects were not consistent, with STHVO+PETW resulting in significantly more crop biomass in the first year, and either STRC+PETW or STHVO resulting in significantly more in the second year. This may be due to inherent

variability of soil at this site, rather than other factors as it is a tile-drained site with a weak slope. In 2018 no-till did not have the lowest grain yields or crop biomass compared to the tillage treatments, in support of other research in Quebec showing that organic no-till practices do not show consistent results over time (Halde et al. 2018). Contradictory research has shown better nutrient use in no-till systems to improve crop yields (Triplett et al. 2008). Other research found some agronomic responses of wheat significantly affected by tillage and others not, depending on the indicator of interest and previous crop residue management (Salami et al. 2017). Wheat shoot N concentrations were not significantly affected by the tillage treatments in the first year, and it was not measured in the second year, resulting in limited data for interpretation.

Tillage treatments affected the number of weed and wheat plant density counts in the wheat crop, but only in the first of two years studied. Using ST HVO+PETW has shown to be the most effective at reducing the weed population while increasing the crop population, with other literature supporting the use of PETW to control weed growth (Pannacci et al. 2017). In the second year there were no significant differences; perhaps because the effectiveness of no-till GMr is variable in organic copping systems and influenced by soil and environmental factors (Marshall 2018; Halde et al. 2018). The tillage treatments had no effect on the weed biomass, even though there were a few dominant weeds identified, which promoted higher biomass of those species. However, there was a shift in the weed community composition by year. In both years dandelion, a simple perennial with a strong taproot, was most common in the no-till treatment, while in the second year quackgrass was more prominent in the most intensive tillage treatments, which included PETW, and a greater amount of wild radish in the tillage treatments without PETW. Non-mycorrhizal species such as quackgrass and wild radish have been better able to survive tillage events compared to mycorhizal ones (Anderson 2014). Redroot pigweed, lamb's quarter and shephard's purse (additional non-mycorrhizal weeds) similarly are known to have higher survival in tilled soil (Bernstein et al. 2014). Lamb's quarter has been found to be a prominent weed in systems with more tillage in other work (Anderson et al. 1998), but made up a very small percentage of the population in the current study. Further research would be useful to assess the role of tillage on weed biomass and species composition in organic field cropping systems, specifically as related to soil health and microbial community composition.

Only a few soil health indicators were significantly influenced by the tillage regime for the GMr, including subsurface hardness, Mehlich 3 extractable P and Ca, but their effect was only observed in the first of 2 years studied. It is possible that the soil is resilient to some disturbance, in which it is able to resist the negative impacts that relative disturbance of each tillage regime imposed, or otherwise demonstrated robustness, in that decreases in some indicators are buffered against over-compensation of others for the soil to recover itself back to a healthy status once the disturbance or stress is removed (Lynch 2015).

Furthermore, the soil may be more compacted around the time of tillage events compared to other times in the growing season, making the time point of sampling important in influencing the physical health status. The available nutrients were less likely to be significantly affected by tillage practices in this study since only one depth was sampled, compared to studies where no-till versus tilled regimes may result in varied nutrient levels at different depths. Previous research on the same site as the current study found significantly higher SOC under no-till systems of GMr crops compared to using tillage practices over a 3- year period, but this effect was not consistent (Marshall and Lynch 2018). A recent BC study growing corn silage with or without tillage consistently for 21 years under conventional management did not pick up significant differences between tillage treatments for most of the same soil health indicators measured; with exceptions including extractable K, Mg, WAS, active carbon, and AWC (Thomas et al. 2019). However their results still suggest following no-till practices improve and contribute to maintaining sustainable land management over time. In contrast to the findings of Thomas et al. (2019), the lack of consistent response in the current study of all soil health indicators to tillage regime is likely significantly attributed to the history of 10+ years of regular incorporation of GMr crops into rotations at this site, and including high residue crops such as fall rye (Wallace 2015; Marshall 2018).

The spring-tilled treatments did not have significantly higher biological soil health indicator values and lower physical values (AWC, WAS) compared to No-Till, therefore rejecting hypothesis 1. The surface hardness measured in 2018 was actually significantly greater under No-Till in 2018 compared to ST RC +PETW, which showed a lot of variability across the field regardless of treatment effects. The weed biomass was slightly more negatively correlated with overall soil health in both years compared to number of weed species, in support of accepting hypothesis 2. This may be due to particular species dominating the quadrats compared to a more even spread of different species comprising the community composition. However, the relationships between the weed measures and crop yields were inconsistent between years of analysis, with weed biomass more negatively associated with crop yields in the first year, but more positively associated with crop yields in the second year of the study, as compared to the correlations with number of weed species.

CHAPTER 3- Soil microbial biomass and community structure as influenced by green manure management and weeds

3.1. Introduction

Incorporating green manure (GMr) crops into rotation is beneficial to maintain soil health, especially in organic production. The type and quality of GMr grown are suggested to be important factors in affecting soil microbial measurements such as community composition and biomass (Elfstead et al. 2007). Most of the residues from GMr crops have been decomposed in the first year, compared to peas grown as a pulse which have supplied more in the second year (Lupwayi and Soon 2015; Lupwayi and Soon 2016). Soil fertility status has been improved by repeated GMr application (Xiefeng et al. 2014). Incorporating legumes into potato rotations has enhanced microbial ecosystems (Qin et al. 2017). A maize experiment has incorporated different GMr (*Raphanus raphanistrum* subsp. Sativus L., *Vicia sativa* L.,and *Astragalus* spp. L.), which has improved the fertility status, supported soil microbial biomass (SMB), and has helped to maintain sufficient crop yields (Tao et al. 2017). In Saskatchewan, Canada, during various phases of residue decomposition from crops including GMr, actinobacteria and fungi have been more abundant in long-term organic systems compared to conventional systems (Arcand et al. 2016).

Weeds also have an important influence on the soil microbial communities present due to their direct contact and interactions with each other (Wortman et al. 2013). Agro-ecosystems are considered to be more sustainable when having a greater biodiversity of organisms, including weeds (Altieri 1999). Weeds like other plants release mucilage and exudates with nutrients, positively influencing soil microbial communities (Trognitz et al. 2016). Crop yield losses have been lower when weeds and AMF interact to reduce weed pressure (Jordan et al. 2000). In Alberta, Canada, an organic wheat study has found a greater amount of arbuscular mycorrhizal fungi (AMF) in the weedy treatments compared to the less-weedy treatments (Kubota et al. 2015). They also have reported that in the less-weedy treatment, weed biomass has had a positive association with %AMF. A greater number of weed species in a community has raised the amount of SMB including AMF spore counts (Chen et al. 2003). However, there is currently a lack of information on how weeds affect temporal changes in soil microbial communities (Bissett et al. 2013).

Tillage practices can disrupt microbial organisms in the soil environment, but the extent of their impact on SMB and communities is an on-going area of research. Different types of tillage extending up to a 60cm depth have been compared for their effects on SMB and soil organic carbon (SOC); such that they have dropped at greater depths with no or little disturbance, but particularly after 20cm for all types of tillage, in support of implementing no-till (Sun et al. 2011; Mohammadi et al. 2013). Tillage negatively affects soil

fungal biomass including AMF communities by reducing hyphal length and phosphorus accessibility (Kohl et al. 2014; Wetzel et al. 2014). In other research the SMB at spring sampling has been higher under no-till management of the GMr crop (Marshall et al. 2018). Larger quantities of soil bacteria and fungi near the soil surface have been measured under no-till compared to conventional tillage systems (Mathew et al. 2012; Sipila et al. 2012). Some microbial biomarkers may be greatly reduced due to tillage practices (Wortman et al. 2013). Recent studies have shown that terminating GMr by roller crimping and seeding another crop directly into the mulch can be a viable alternative to tillage practices (Boydston and Williams 2014; Lounsbury and Weil 2014; Wallace et al. 2017). Rolling or mowing winter ryegrass has resulted in better weed control than standard tillage practices (Bernstein et al. 2014). From all these results, it is suggested that minimizing soil disturbance with no tillage will improve soil microbial populations.

In the following analysis over 2.5 years, two experimental field sites were used to assess the impact of various agronomic management practices on soil microbial communities through phospholipid fatty acid (PLFA) analyses. The objectives of Experiment 1 were to determine the effect of the previous GMr [*Trifolium pratense* (Red Clover: RC), *Vicia villosa* Roth. (Hairy vetch) underseeded with *Avena sativa* L. (oats) as HVO, *Vicia sativa* L. (Common vetch) underseeded with oats (CVO), or No GMr)] grown and incorporated 2 years prior in rotation, and the current season presence or absence of weeds on soil microbial biomass (nmol MFA/g) and % microbial community composition. The main objectives of Experiment 2 were to assess the impact of different tillage treatments for GMr termination (Spring Tilled Red Clover + Post Emergent Tine Weeding (ST RC+PETW), ST HVO, ST HVO +PETW and No-Till) as well as weed species and biomass ($t\text{-}ha^{-1}$) on SMB (nmol MFA/g) and % microbial community composition. For Experiment 1 it was hypothesized that a previous GMr of HVO will produce the least SMB due to a reduced number of different weed species and weed biomass ($t.ha^{-1}$) in rotations featuring this GMr, in greater competition with weeds. It has been speculated that weedy treatments will have higher SMB compared to non-weedy. In Experiment 2 it was hypothesized that tillage of GMr will reduce SMB compared to no-till GMr treatment. In both experiments it was postulated that weed composition characterized by higher weed biomass of fewer different species would be negatively correlated with SMB.

3.2. Materials and Methods

From each soil sample collected in Experiment 1 and 2 as described in Chapter 2, a small 50g portion was separated and kept frozen in a Falcon tube at -20ºC to preserve for phospholipid fatty acid (PLFA) analysis. Other small 5±0.05g samples were weighed and dried to determine moisture content as the first step of the procedure. The drying temperature depends on the type of soil sample, such that organic soils are dried at lower temperatures than inorganic soils due to the nature of their composition (O'Kelly and Li 2018). The

PLFA analysis was employed in many other microbial biomass and community structure studies (Frostegard et al. 2010; Mathew et al. 2012; Lange et al. 2014; Kubota et al. 2015). The soil sampling dates for each experiment are briefly summarized below (Table 1). Collecting samples at 3 time points was a good method of assessing variation in any measured properties of interest over the growing season (Pollnac et al. 2009).

Table 1: Soil sampling dates for both experiments over 2.5 years

Experiment Number	Sampling Date (Month-Year)
1	Sep-16
1	Oct-17
2	Jun-17
2	Aug-17
2	Oct-17
2	Jun-18

Unfortunately, there was a campus building fire that resulted in severe water damage and loss of power to the freezer storing the frozen soil samples before they were taken to PEI for PLFA analysis. The overall quality of the samples may have been compromised, as warm temperature can have a negative influence on soil quality (Zelles 1999), especially over an extended period of time. Fortunately this exposure was applied to all the samples as a uniform treatment effect. Unsaturated fatty acids are decomposed by exposure to heat over 37ºC or oxygen in the atmosphere (White and Ringelberg 1998). Therefore, as a control measure to test if there has been any negative impact from the fire, some fresh soil samples were collected on June 28, 2018 from both experimental sites a week after the fire to compare with the previously stored samples. All samples from both years and experiments were analyzed at the same time in the summer of 2018.

To provide a brief overview of the PLFA analysis and the response variables obtained, it involves drying the soil, separating the fats into sections (phospholipids, neutral and glycolipids) with a buffer solvent followed by chloroform separately in sequence (Zelles et al. 1997; Quideau et al. 2016). The choice of buffer is important since they can have different properties and interactions with the other chemicals in solution. For example, to obtain more lipid phosphate from organic soil, a citrate buffer is a better choice than a phosphate or acetate buffer (Frostegard et al. 1991). A solution of dichloromethane and methanol to extract fatty acids has proven to be effective and safer than using chloroform (Cequier-Sanchiez et al. 2008). Approximately 10,000 different species of microbes can be inclusive of 50 fatty acids, which speaks to the complexity of the extraction process (Zelles 1999). Methanol has been used in silicic acid column chromatography to obtain phospholipids, which is dried in gas, and dissolved in toluene (Chowdhury and Dick 2011).

Next the samples are centrifuged with the phases separated using a multi-well plate; replacing the side chain of an ester with a side chain of an alcohol through transesterification, before adding a methyl group to the surface of the reaction called methylation. It is recommended to use potassium hydroxide methanol (KOH/MeOH) during the methylation step rather than methanolic hydrochloric acid (HCl/MeOH) (binding an ester and alcohol) to avoid undesired interactions or loss of sample (Chowdhury and Dick 2011). Fatty acid methyl esters (FAME)'s are produced from exposing separated phospholipids to a higher pH solution (Willers et al. 2015). The FAME end-products are analyzed through gas chromatography (GC) (Buyer and Sasser 2012; Quideau et al. 2016). They undergo oxidation or combustion through GC, and the oxidized carbon shows up as peaks of CO_2 measured by a spectrophotometer specified to read isotope ratios (Yao et al. 2015). The isotope ratios are related to particular fatty acid biomarkers or signatures. The computer software analyzes the GC product with output of nmol of the methylated fatty acid material comprising the cell wall tissue of the microorganisms per gram (MFA/g). Therefore, microbial biomass is not a direct measure of the microorganisms, but that information is used to represent the microbial communities living in the soil. The community structure fatty acids are different than those used to determine biomass for particular microbial groups (Zelles 1999). The AMF is a different fatty acid signature from other fungal microorganisms, so that they are detected as two different microbial groups (Mills et al. 2018). A step-by-step flow chart procedure to conduct PLFA in the laboratory is provided (Appendix 18). A more detailed procedure with instructions for making the chemicals and analyzing PLFA data through computer software is attached (Appendix 19).

3.3. Statistical Analysis

Minitab v.18 was used to confirm normality, constant variance and independence of the error terms for the results from both experiments. The experimental design of both experiments overlaps with this chapter, as discussed in Chapter 2. The data was analyzed in SAS v.9.4 separately for both sites with ANOVA in Proc Mixed with Repeated Measures and Tukey's Multiple Means comparison where applicable (α=0.05) (Mills 2018). Fixed and random effects reflect those described in Chapter 2. The only component here specific to Chapter 3 is the temporal influence of soil sampling on microbial measures. For both years of data PROC CORR codes have been used to determine Pearson correlations between weed biomass and number of weed species with the microbial biomass (using 3 levels of significance: $P<0.05$, <0.01, <0.001).

3.4. Results:

3.4.1. Experiment 1: Effect of GMr and weed condition on SMB

In 2016 the average microbial biomass across treatments was 69.25±1.96 nmol MFA/g, and in 2017 it was 28.55±1.51 nmol MFA/g, indicating almost 2.5 times greater biomass in the first year. In both years of analysis, the fungi and AMF respectively showed significantly higher amounts based on the influence of the weedy condition, with various probabilities (Table 2). The most consistent observation found was for fungi and AMF to be significantly increased by the presence of weeds. There were no significant interaction effects detected between the GMr and weed condition.

Table 2: Summarized significant soil microbial biomass (nmol MFA/g) based on the influence of previous GMr in rotation 2 years prior and current season weed condition

Year	Component	Source	F Value	Pr>F
2016	Eukaryotes	GMr	9.1	0.0119
2016	Fungi	WeedCon	12	0.0085
2016	AMF	WeedCon	8.51	0.0194
2017	Fungi	GMr	5.01	0.045
2017	Fungi	WeedCon	10.48	0.0119
2017	AMF	WeedCon	6.23	0.0371

Note: AMF= Arbuscular mycorrhizal fungi, GMr= Green Manure crop rotation phase, WeedCon= Weed Condition

The means and standard error values for each treatment effect are summarized for each microbial group in 2016 (Table 3). The CVO GMr treatment had significantly more eukaryote biomass than RC. The weedy condition showed significantly more AMF and fungi than non-weedy treatment. The results are also shown for 2017 (Table 4). The previous GMr crop in rotation affected actinomycetes, with HVO having significantly more biomass compared to using No GMr.

Table 3: Influence of GMr treatment 2 years prior, and weed presence on soil bacterial and fungal biomass in 2016 (Mean ±SE)

	Microbial Biomass (nmol MFA/g)						
GMr Treatment	**AMF**	**Eukaryotes**	**Fungi**	**GramNeg**	**GramPos**	**Anaerobes**	**Actinomycetes**
RC	4.07±0.52a	1.69±0.33b	1.58±0.21a	30.2±3.76a	21.0±2.74a	0.78±0.10a	3.20±0.40a
HVO	4.94±0.27a	2.44±0.24ab	1.75±0.13a	34.2±1.65a	22.8±1.35a	0.86±0.04a	3.22±0.13a
CVO	4.87±0.16a	3.10±0.27a	1.67±0.08a	34.3±1.00a	23.9±0.74a	0.90±0.02a	3.49±0.089a
No GMr	4.81±0.21a	2.88±0.26a	1.87±0.15a	33.3±1.36a	22.6±0.90a	0.89±0.03a	3.81±0.50a
Weedy	4.99±0.19a	2.58±0.22a	1.92±0.09a	34.0±1.24a	23.2±0.87a	0.89±0.03a	3.33±0.12a
Non-Weedy	4.35±0.27b	2.48±0.23a	1.51±0.10b	32.1±1.88a	22.0±1.40a	0.83±0.05a	3.53±0.32a

Note: HVO= Hairy vetch underseeded with oats, CVO= Common vetch underseeded with oats, RC= Red clover, AMF= Arbuscular mycorrhizal fungi, GramNeg= Gram Negative Bacteria, GramPos= Gram Positive Bacteria. Treatments with the same letter are not significantly different according to a Tukey's test at α=0.05.

Table 4: Influence of GMr treatment 2 years prior, and weed presence on soil bacterial and fungal biomass in 2017 (Mean ± SE)

	Microbial Biomass (nmol MFA/g)						
GMr Treatment	**AMF**	**Eukaryotes**	**Fungi**	**GramNeg**	**GramPos**	**Anaerobes**	**Actinomycetes**
RC	1.81±0.23a	2.67±1.48a	0.72±0.11ab	12.3±1.41a	8.49±0.96a	0.33±0.05a	1.38±0.22ab
HVO	2.68±0.15a	1.14±0.09a	1.11±0.12a	17.6±0.77a	10.2±0.35a	0.40±0.03a	1.67±0.09a
CVO	2.39±0.27a	1.04±0.12a	0.82±0.11ab	15.3±1.78a	8.91±0.86a	0.35±0.04a	1.54±0.18ab
No GMr	1.60±0.16a	0.95±0.08a	0.65±0.06b	10.40±1.10a	6.48±0.53a	0.25±0.03a	0.95±0.13b
Weedy	2.26±0.18a	1.79±0.76a	0.98±0.09a	14.5±1.13a	8.76±0.61a	0.34±0.03a	1.44±0.13a
Non-Weedy	1.98±0.16b	1.10±0.07a	0.68±0.06b	13.4±1.06a	8.29±0.54a	0.32±0.02a	1.33±0.12a

Note: HVO= Hairy vetch underseeded with oats, CVO= Common vetch underseeded with oats, RC= Red clover, AMF= Arbuscular mycorrhizal fungi, GramNeg= Gram Negative Bacteria, GramPos= Gram Positive Bacteria. Treatments with the same letter are not significantly different according to a Tukey's test at α=0.05.

Correlations were determined for the microbial groups with the weed biomass and number of weed species to determine which components of soil health are closely related to each other. These values are provided for 2016 (Table 5) and 2017 (Table 6).

The % total PLFA comprised of particular microbial groups was examined, with significance shown in both years particularly for the fungal microorganisms based on the influence of the GMr crop in rotation or the weed condition (Table 7). The mean and standard error values are provided for 2016 and 2017 (Table 8, 9). In both years non-weedy soil significantly affected fungal composition as well as AMF or gram-positive bacteria respectively.

Table 5: Correlations between weed biomass (t ha^{-1}), number of weed species, and PLFA microbial biomass (nmol MFA/g) in 2016 Experiment 1

Measurement		1	2	3	4	5	6	7	8	9	10
AMF	1	1	0.615*	0.916***	0.313	0.785**	0.974***	0.926***	-0.499	-0.081	-0.338
Actino	2	0.615*	1	0.668*	0.111	0.676*	0.698*	0.638*	-0.039	0.180	-0.305
Anaerobes	3	0.916***	0.668*	1	0.238	0.870***	0.960***	0.958***	-0.430	-0.136	-0.575
Eukary	4	0.313	0.111	0.238	1	0.212	0.188	0.198	-0.565	0.319	0.353
Fungi	5	0.785**	0.676*	0.870***	0.212	1	0.830***	0.788**	-0.512	0.025	-0.585*
GramNeg	6	0.974***	0.698*	0.960***	0.188	0.830***	1	0.967***	-0.408	-0.092	-0.481
GramPos	7	0.926***	0.638*	0.958***	0.198	0.788**	0.967***	1	-0.415	-0.213	-0.460
Weed Bio	8	-0.499	-0.039	-0.430	-0.565	-0.512	-0.408	-0.415	1	-0.140	-0.086
Weed Spec	9	-0.081	0.180	-0.136	0.319	0.025	-0.092	-0.213	-0.140	1	0.097
Yield	10	-0.338	-0.305	0.353	0.353	-0.585*	-0.481	-0.460	-0.086	0.097	1

Note: MFA= Methylated Fatty Acids, AMF= Arbuscular Mycorrhizal Fungi, Actino= Actinomycetes, Eukary= Eukaryotes, GramNeg= Gram Negative Bacteria, GramPos= Gram Positive Bacteria, Weed Bio= Weed Biomass, Weed Spec= Weed Species. Coefficients marked by *, **, *** are represented as significant ($P<0.05$), highly significant ($P<0.01$) or very highly significant ($P<0.001$) respectively.

Table 6: Correlations between weed biomass (t ha^{-1}), number of weed species, and PLFA microbial biomass (nmol MFA/g) in 2017 Experiment 1

Measurement		1	2	3	4	5	6	7	8	9	10
AMF	1	1	0.864***	0.824***	0.072	0.872***	0.977***	0.893***	-0.334	0.014	--
Actino	2	0.864***	1	0.970***	0.418	0.773**	0.917***	0.974***	-0.127	0.203	--
Anaerobe	3	0.824***	0.970***	1	0.413	0.760**	0.90***	0.965***	-0.03	0.217	--
Eukary	4	0.072	0.418	0.413	1	0.220	0.109	0.433	-0.013	0.485	--
Fungi	5	0.872***	0.773**	0.760**	0.220	1	0.868***	0.871***	-0.367	0.061	--
GramNeg	6	0.977***	0.917***	0.906***	0.109	0.868***	1	0.935***	-0.204	0.049	--
GramPos	7	0.893***	0.974***	0.965***	0.433	0.871***	0.935***	1	-0.171	0.156	--
Weed Bio	8	-0.334	-0.127	-0.032	-0.013	-0.367	-0.204	-0.171	1	0.127	--
Weed Spec	9	0.014	0.203	0.217	0.485	0.061	0.049	0.156	0.127	1	--
Yield	10	--	--	--	--	--	--	--	--	--	1

Note: MFA= Methylated Fatty Acids, AMF= Arbuscular Mycorrhizal Fungi, Actino= Actinomycetes, Eukary= Eukaryotes, GramNeg= Gram Negative Bacteria, GramPos= Gram Positive Bacteria, Weed Bio= Weed Biomass, Weed Spec= Weed Species. Coefficients marked by *, **, *** are represented as significant ($P<0.05$), highly significant ($P<0.01$) or very highly significant ($P<0.001$) respectively.

Table 7: Significant soil microbial community composition (%) based on the influence of the previous GMr in rotation two years prior and current season weed condition

Year	Component	Source	F Value	Pr>F
2016	Eukaryotes	GMr	11.14	0.0073
2016	Fungi	WeedCon	20.99	0.0018
2016	AMF	WeedCon	39.08	0.0002
2017	GramPos	WeedCon	6.28	0.0366
2017	Fungi	WeedCon	15.41	0.0044

Note: AMF= Arbuscular mycorrhizal fungi, GramPos= Gram Positive Bacteria, WeedCon= Weed Condition

Table 8: Influence of GMr treatment 2 years prior, and weed presence on microbial community composition in 2016 (Mean ±SE)

	PLFA by Grouping (%)						
GMr Treatment	**AMF**	**Eukaryotes**	**Fungi**	**GramNeg**	**GramPos**	**Anaerobes**	**Actinomycetes**
RC	6.37±0.24a	2.96±0.59b	2.50±0.10a	48.3±0.98a	32.7±1.03a	1.23±0.03a	5.86±0.71a
HVO	7.05±0.17a	3.53±0.45ab	2.52±0.21a	48.9±0.39a	32.2±0.68a	1.23±0.04a	4.63±0.08a
CVO	6.73±0.16a	4.24±0.45a	2.31±0.07a	47.5±0.66a	33.1±0.46a	1.25±0.01a	4.84±0.14a
No GMr	6.85±0.15a	4.11±0.42a	2.64±0.19a	47.5±0.54a	32.2±0.28a	1.26±0.02a	5.44±0.78a
Weedy	7.03±0.09a	3.68±0.38a	2.71±0.10a	47.9±0.39a	32.7±0.35a	1.26±0.01a	4.72±0.10a
Non-Weedy	6.47±0.14b	3.74±0.34a	2.28±0.07b	48.2±0.57a	32.4±0.56a	1.23±0.02a	5.67±0.50a

Note: HVO= Hairy vetch underseeded with oats, CVO= Common vetch underseeded with oats, RC= Red clover, AMF= Arbuscular mycorrhizal fungi, GramNeg= Gram Negative Bacteria, GramPos= Gram Positive Bacteria. Treatments with no similar letter groupings are significantly different according to a Tukey's test at α=0.05.

Table 9: Influence of GMr treatment 2 years prior, and weed presence on microbial community composition in 2017 (Mean ±SE)

	PLFA by Grouping (%)						
GMr Treatment	**AMF**	**Eukaryotes**	**Fungi**	**GramNeg**	**GramPos**	**Anaerobes**	**Actinomycetes**
RC	6.73±0.45a	7.41±2.56a	2.58±0.25a	45.6±1.96a	31.6±1.14a	1.23±0.07a	4.81±0.15a
HVO	7.64±0.30a	3.39±0.39a	3.16±0.37a	50.5±0.74a	29.4±0.43a	1.16±0.06a	4.78±0.20a
CVO	7.86±0.18a	3.58±0.28a	2.61±0.24a	49.8±0.90a	29.8±0.94a	1.13±0.04a	5.21±0.60a
No GMr	7.44±0.14a	4.77±0.55a	3.11±0.26a	48.3±1.10a	31.0±0.96a	1.17±0.07a	4.22±0.34a
Weedy	7.59±0.26a	5.24±1.39a	3.27±0.22a	48.4±1.24a	29.7±0.45b	1.13±0.04a	4.77±0.34a
Non-Weedy	7.25±0.19a	4.34±0.30a	2.46±0.09b	48.7±0.74a	31.3±0.75a	1.21±0.04a	4.74±0.18a

Note: HVO= Hairy vetch underseeded with oats, CVO= Common vetch underseeded with oats, RC= Red clover, AMF= Arbuscular mycorrhizal fungi, GramNeg= Gram Negative Bacteria, GramPos= Gram Positive Bacteria. Treatments with no similar letter groupings are significantly different according to a Tukey's test at α=0.05.

3.4.2. Experiment 2: Effect of tillage and month of sampling on SMB

In 2017 the average microbial biomass across treatments was 39.60±1.22 nmol MFA/g, and in 2018 it was 43.57±1.78 nmol MFA/g. In this first year of analysis the fungal biomass was significantly influenced by the month of soil sampling, and in 2018 the AMF was affected by the tillage treatment (Table 10). The means and standard error values for each treatment effect are summarized for each microbial group biomass in 2017 (Table 11) and in 2018 (Table 12). In 2017 the fungi had significantly less biomass in June compared to August and October. In 2018 ST RC+PETW had significantly less AMF biomass than ST HVO+PETW or No-Till.

Table 10: Significant PLFA microbial biomass (nmol MFA/g) over 2 years of analysis based on influence of month of soil sampling and GMr termination by tillage

Year	Component	Source	F Value	Pr>F
2017	Fungi	Month	9.03	0.0071
2018	AMF	Tillage	6.17	0.029

Note: MFA= Methylated Fatty Acids, GramNeg= Gram Negative Bacteria, AMF= Arbuscular Mycorrhizal Fungi, Month=Month of Soil Sampling.

The correlations for the weed measurements with crop yields and microbial biomass are shown for 2017 and 2018 respectively (Table 13 and 14). An ANOVA summary is provided of the significant response variables that were influenced by the temporal soil sampling effect, (Table 15) while the non-significant results have been shown in the main average ± SE data tables to follow (Table 16 and 17).

Table 11: Influence of GMr termination method by tillage on soil bacterial and fungal biomass in 2017 (Mean ±SE)

	Microbial Biomass (nmol MFA/g)						
GMr Treatment	**AMF**	**Eukaryotes**	**Fungi**	**GramNeg**	**GramPos**	**Anaerobes**	**Actinomycetes**
STRC+PETW	3.01±0.25a	1.61±0.18a	1.41±0.16a	20.0±1.57a	12.2±1.19a	0.49±0.05a	2.44±0.48a
STHVO+PETW	2.47±0.11a	1.59±0.11a	1.22±0.09a	18.2±0.86a	10.7±0.63a	0.46±0.03a	2.20±0.25a
STHVO	2.47±0.11a	1.67±0.14a	1.23±0.14a	19.2±1.18a	12.2±0.14a	0.49±0.03a	2.21±0.15a
No-Till	2.82±0.19a	1.74±0.09a	1.50±0.19a	20.0±1.12a	11.9±0.88a	0.51±0.04a	2.52±0.47a
June	2.63±0.11a	1.75±0.08a	1.13±0.07b	20.6±0.96a	11.8±0.59a	0.50±0.03a	2.46±0.35a
August	2.76±0.17a	1.66±0.11a	1.45±0.12a	19.0±0.85a	13.0±0.70a	0.50±0.03a	2.59±0.38a
October	2.69±0.20a	1.52±0.13a	1.47±0.16a	18.4±1.21a	10.5±0.89a	0.44±0.04a	2.02±0.12a

Note: STHVO= Spring Tilled Hairy Vetch Underseeded with oats, PETW= Post-Emergent Tine Weeding, STRC= Spring Tilled Red Clover, AMF= Arbuscular mycorrhizal fungi, GramNeg= Gram Negative Bacteria, GramPos= Gram Positive Bacteria. Treatments with no similar letter groupings are significantly different according to a Tukey's test at α=0.05.

Table 12: Influence of GMr termination method by tillage on soil bacterial and fungal biomass in June 2018 (Mean ±SE)

	Microbial Biomass (nmol MFA/g)						
GMr Treatment	**AMF**	**Eukaryotes**	**Fungi**	**GramNeg**	**GramPos**	**Anaerobes**	**Actinomycetes**
ST RC+PETW	2.46±0.27b	1.87±0.23a	0.75±0.09a	17.9±1.89a	10.8±0.98a	0.38±0.08a	1.87±0.21a
STHVO+PETW	3.79±0.22a	2.07±0.24a	1.44±0.13a	23.3±0.95a	13.3±0.91a	0.51±0.01a	2.26±0.13a
STHVO	3.11±0.20ab	2.44.±0.22a	1.51±0.25a	21.4±0.77a	12.9±0.54a	0.45±0.02a	2.42±0.10a
No-Till	3.29±0.12a	2.02±0.15a	1.50±0.21a	22.6±0.66a	13.4±0.71a	0.47±0.02a	3.96±1.58a

Note: STHVO= Spring Tilled Hairy Vetch Underseeded with oats, PETW= Post-Emergent Tine Weeding, ST RC= Spring Tilled Red Clover, AMF= Arbuscular mycorrhizal fungi, GramNeg= Gram Negative Bacteria, GramPos= Gram Positive Bacteria. Treatments with no similar letter groupings are significantly different according to a Tukey's test at α=0.05.

Table 13: Correlations between weed biomass (t ha^{-1}), number of weed species, and PLFA microbial biomass (nmol MFA/g) by groupings in 2017 Experiment 2

Measurement		1	2	3	4	5	6	7	8	9	10
AMF	1	1	0.577*	0.639*	0.416	0.161	0.843***	0.745**	-0.252	-0.559	0.164
Actino	2	0.577*	1	0.557	0.353	0.325	0.659*	0.510	0.333	-0.141	-0.00072
Anaerobe	3	0.639*	0.557	1	0.747**	-0.083	0.818***	0.747	0.142	-0.691*	0.314
Eukary	4	0.416	0.352	0.747**	1	-0.134	0.599*	0.545*	0.162	-0.552	0.117
Fungi	5	0.161	0.325	-0.083	-0.134	1	0.253	0.274	-0.057	0.095	0.191
GramNeg	6	0.843***	0.659*	0.818***	0.599*	0.253	1	0.955***	-0.077	-0.735**	0.348
GramPos	7	0.745**	0.510	0.747	0.545*	0.274	0.955***	1	0.142	-0.781**	0.314
Weed Bio	8	-0.252	0.333	0.142	0.162	-0.057	-0.077	-0.146	1	0.138	-0.516
Weed Spec	9	-0.559	-0.141	-0.691*	-0.552	0.095	-0.735**	-0.781**	0.138	1	-0.494
Yield	10	0.164	-0.0007	0.314	0.117	0.191	0.348	0.397	-0.516	-0.494	1

Note: MFA= Methylated Fatty Acids, AMF= Arbuscular Mycorrhizal Fungi, Actino= Actinomycetes, Eukary= Eukaryotes, GramNeg= Gram Negative Bacteria, GramPos= Gram Positive Bacteria, Weed Bio= Weed Biomass, Weed Spec= Weed Species. Coefficients marked by *, **, *** are represented as significant ($P<0.05$), highly significant ($P<0.01$) or very highly significant ($P<0.001$) respectively.

Table 14: Correlations between weed biomass (t ha^{-1}), number of weed species, and PLFA microbial biomass (nmol MFA/g) by grouping in 2018 Experiment 2

Measurement		1	2	3	4	5	6	7	8	9	10
AMF	1	1	-0.056	-0.113	-0.068	0.402	-0.049	-0.325	0.141	-0.181	-0.014
Actino	2	-0.056	1	0.694*	0.849***	0.427	0.765**	0.603*	-0.017	0.470	0.113
Anaerobe	3	-0.113	0.694*	1	0.929***	0.484	0.851***	0.752**	0.154	0.375	0.536
Eukary	4	-0.068	0.849***	0.929***	1	0.425	0.949***	0.735**	0.107	0.597*	0.324
Fungi	5	0.402	0.427	0.484	0.425	1	0.279	0.500	0.030	-0.170	0.326
GramNeg	6	-0.049	0.765**	0.851***	0.949***	0.279	1	0.638*	0.183	0.695*	0.185
GramPos	7	-0.325	0.603*	0.752**	0.735**	0.500	0.638*	1	0.128	0.224	0.409
Weed Bio	8	0.141	-0.017	0.154	0.107	0.030	0.183	0.128	1	0.149	0.634*
Weed Spec	9	-0.181	0.470	0.375	0.597*	-0.170	0.695*	0.224	0.149	1	-0.082
Yield	10	-0.014	0.113	0.536	0.324	0.326	0.185	0.409	0.634*	-0.082	1

Note: MFA= Methylated Fatty Acids, AMF= Arbuscular Mycorrhizal Fungi, Actino= Actinomycetes, Eukary= Eukaryotes, GramNeg= Gram Negative Bacteria, GramPos= Gram Positive Bacteria, Weed Bio= Weed Biomass, Weed Spec= Weed Species. Coefficients marked by *, **, *** are represented as significant ($P<0.05$), highly significant ($P<0.01$) or very highly significant ($P<0.001$) respectively.

Table 15: Significant microbial community composition (%) based on influence of tillage treatment and month of soil sampling

Year	Component	Source	F Value	Pr>F
2017	AMF	Month	7.39	0.0241
2017	GramNeg	Month	35.86	0.0005
2017	GamPos	Month	10.21	0.0117

Note: AMF= Arbuscular mycorrhizal fungi, GramNeg= Gram Negative Bacteria, GramPos=Gram Positive Bacteria

Table 16: Influence of GMr termination by tillage on microbial community composition in 2017 (Mean ±SE)

	PLFA by Grouping (%)						
GMr Treatment	**AMF**	**Eukaryotes**	**Fungi**	**GramNeg**	**GramPos**	**Anaerobes**	**Actinomycetes**
ST RC+PETW	7.34±0.23a	4.07±0.48a	3.51±0.42a	48.65±0.55a	29.54±0.99a	1.17±0.04a	5.72±0.80a
ST HVO+PETW	6.71±0.19a	4.30±0.23a	3.39±0.33a	49.39±0.85a	28.91±0.64a	1.23±0.06a	6.07±0.81a
ST HVO	6.33±0.18a	4.21±0.23a	3.16±0.37a	48.67±0.51a	30.77±0.61a	1.25±0.03a	5.59±0.12a
No-Till	6.88±0.26a	4.28±0.20a	3.76±0.51a	48.88±1.03a	28.84±0.86a	1.24±0.06a	6.12±1.00a
June	6.40±0.16b	4.34±0.17a	2.71±0.18b	50.17±0.49a	29.32±0.51b	1.26±0.05a	5.80±0.57a
August	6.79±0.22b	4.09±0.08a	3.64±15.07ab	46.78±8.91b	31.14±0.61a	1.23±0.04a	6.33±0.94a
October	7.26±0.18a	4.22±0.37a	4.01±0.40a	49.74±0.36a	28.09±0.69b	1.19±0.03a	5.49±0.16a

Note: ST HVO= Spring Tilled Hairy Vetch Underseeded with oats, PETW= Post-Emergent Tine Weeding, ST RC= Spring Tilled Red Clover, AMF= Arbuscular mycorrhizal fungi, GramNeg= Gram Negative Bacteria, GramPos= Gram Positive Bacteria. Treatments with no similar letter groupings are significantly different according to a Tukey's test at α=0.05.

Table 17: Influence of GMr termination by tillage on microbial community composition in 2018 (Mean ±SE)

	PLFA by Grouping (%)						
GMr Treatment	**AMF**	**Eukaryotes**	**Fungi**	**GramNeg**	**GramPos**	**Anaerobes**	**Actinomycetes**
ST RC+PETW	6.80±0.09b	5.17±0.19a	2.11±0.24a	49.64±0.50a	30.05±0.34a	1.04±0.10a	5.19±0.16a
ST HVO+PETW	8.12±0.18a	4.47±0.59a	3.08±0.13a	50.00±0.43a	28.38±0.67a	1.10±0.03a	4.86±0.30a
ST HVO	7.03±0.25b	5.49±0.32a	3.41±0.55a	48.33±0.29a	29.23±0.31a	1.03±0.07a	5.47±0.09a
No-Till	6.99±0.38b	4.26±0.14a	3.15±0.32a	48.10±2.25a	28.39±0.21a	0.99±0.07a	8.10±2.78a

Note: ST HVO= Spring Tilled Hairy Vetch Underseeded with oats, PETW= Post-Emergent Tine Weeding, ST RC= Spring Tilled Red Clover, AMF= Arbuscular mycorrhizal fungi, GramNeg= Gram Negative Bacteria, GramPos= Gram Positive Bacteria. Treatments with no similar letter groupings are significantly different according to a Tukey's test at α=0.05.

3.5. Discussion
3.5.1. Experiment 1

There was more than twice as much microbial biomass (nmol MFA/g) obtained in the first year compared to the second year of this study. The average values from the current study were approximately two or four times smaller in 2016 and 2017 respectively, than those obtained from other literature (Buyer et al. 2012) or two times larger in 2016, but similar in 2017 compared to another study (Lange et al. 2014). Alternatively, Kubota et al. (2015) reported higher amounts in the range of 493-496 nmol MFA/g for microbial biomass based on less weedy or weedy treatments. These varied results are supported by criticisms that the interpretation of the data is challenging and the nature of each experimental site is different (Frostegard et al. 2010). The laboratory procedure for PLFA analyses has many possible steps, which can result in compounded errors with respect to the final microbial biomass and community composition obtained. However, proper laboratory techniques were followed using the most suitable extraction and purification chemicals known based on previous studies. Futhermore, the results for blanks and standards used through the multi-step PLFA analyses procedures did not indicate any inherent analytical error, in support of good analytical methods followed. In the initial steps, the type of buffer chosen impacts how well the lipid phosphate are extracted from the sample. Citrate was used in the current study, which has been shown to extract more than phosphate or acetate by comparison (Frostegard et al. 1991). The lipid extraction step has been compared using chloroform or dichloromethane, such that the latter of the two has been reported to extract fatty acids more efficiently due to its chemical composition and nature interacting with the fat molecules (Cequier-Sanchez et al. 2008), and therefore dichloromethane was used in the current study. An additional possible reason for varied microbial biomass values obtained could be due to the choice of chemical compound used for the methylation step. Methanolic HCl has been shown not to detect methyl groups; therefore affecting accurate output of microbial community structure, making KOH a better choice of solution (Chowdhury and Dick 2011). However, methanolic KOH was used in the current study, indicating that it was unlikely a possible source of error. Also the biomarkers related to particular microbial groups can potentially be representative of more than one type of microorganism, which can cause concern with interpretation (Willers et al. 2015).

The GMr treatments in Experiment 1 did not affect most microbial biomass groupings, with only eukaryotes lower under the RC treatment effect in the first year, while fungi and actinomycetes were significantly lower under No GMr in the second year. Perhaps the quality (fatty acid composition) and relative quantity of the decomposing residual GMr residues influenced the

composition of fungal vs. bacterial microorganisms, such that eukaryotes were reported to be more prominent with polyunsaturated FA, while actinomycetes were more prominent with saturated FA (Zelles et al. 1995). While these results were inconsistent with no particular microbial groupings being consistently more affected by a particular GMr grown 2 years previously than others; it suggests that using a GMr may improve fungi and actinomycetes presence, to support soil health. Other work suggests a positive diversity relationship between aboveground plant material and belowground microbial communities, which would support including GMr in rotation (Garbeva et al. 2006). Therefore, including and varying GMrs used in cropping systems has been shown to improve the ecosystem in which for soil biology thrives (Qin et al. 2017).

In both years of this study in Experiment 1, the AMF and fungal biomass were significantly higher in the weedy treatment compared to the non-weedy state. This result is perhaps because the presence of weeds enhances carbon sources for weed-microbial nutrient exchange and decomposition activities (Trognitz et al. 2016; Meena et al. 2018). Also, the contribution of AMF and fungi to the total microbial biomass being significantly higher under the weedy soil condition compared to that of non-weedy, concurred with the results of a similar study in Alberta examining microbial community composition (also using PLFA techniques) in organic wheat with weedy and less-weedy treatments (Kubota et al. 2015). That study found that weed biomass was positively correlated with AMF and gram-negative bacteria in the less-weedy treatment, but that it was negatively correlated with gram-positive bacteria in either weedy treatment.

In Experiment 1 the HVO treatment did not result in significantly less microbial biomass compared to the other GMr options, therefore suggesting rejection of hypothesis 1. Previous research has also shown that using hairy vetch as a GMr increases soil microbial activity by promoting enzyme production to help breakdown plant material and cycle nutrients in biochemical reactions (Kataoka et al. 2017). This result concurs with previous research showing continued use of GMr in cropping systems has promoted increased soil microbial biomass compared to reduced usage of GMr (Xiefeng et al. 2013; Sharifi et al. 2014). Some unusual correlation results were obtained from Experiment 1 data, such as weed biomass being significantly positively correlated with crop yields. It may be explained by other research, suggesting that microbial biomass is not closely enough related to other measures of soil health for those indicators to show direct relationships to each other (Bossio et al. 1998). However, other research has shown that no-till has the potential to increase crop yields due to better use of nutrients and reduced erosion risk (Triplett and Dick 2008). The GMr treatments hosted the same number of weed species in the first year, therefore indicating

that a comparison could not be made to test hypothesis 2a, which speculated that the GMr treatments hosting the greatest number of weed species will result in a lower contribution of each microbial group to total PLFA when compared to the other GMr treatments with a fewer number of weed species. In the second year the CVO treatment hosted the greatest number of weed species in the second year, but it did not contribute significantly less microbial biomass in any groupings to the total PLFA compared to the other GMr treatments with a fewer number of species. Based on the findings of this study, this hypothesis was rejected, or inconclusive until researched further. Weed biomass was negatively correlated with all PLFA microbial groups, while number of weed species was only negatively correlated with half of them in 2016. Also in 2017 weed biomass was negatively associated with all microbial groups, while number of weed species was positively associated with all of them, indicating that overall number of weed species was more positively correlated to soil health, in support and acceptance of hypothesis 2b.

3.5.2. Experiment 2

The average microbial biomass in the first year was slightly higher compared to the second year. One of the main findings of Experiment 2 was that of the fungal microbial biomass being significantly temporally influenced by timing of soil sampling, whereas the AMF was significantly influenced by the tillage treatment. The fungal category in PLFA analyses encompasses all fungi aside from AMF, allowing for a large range of effects compared to AMF as a separate biomarker and a particular type of naturalized fungi particularly negatively influenced by physical soil disturbance. Supporting literature acknowledged that tillage can improve P-uptake efficiency for crop production, but at the expense of reducing AMF activity to supply that and other nutrients to the plant through symbiotic associations (Kohl et al. 2014). In 2018 the temporal influence of soil sampling date resulted in significantly more gram-positive and AMF, as well as significantly less gram-negative bacterial %contribution to total PLFA mid-way through the growing season. In Alberta Kubota et al. (2015) found that the contribution of gram-negative bacteria to total microbial biomass also measured by PLFA significantly decreased later in the season with no effect on the gram-positive bacteria. Both the results of the current study and Kubota et al. (2015) found that AMF and fungal contribution to the total microbial biomass were higher in October compared to earlier in the growing season. Even though both studies were managed organically, this similarity in findings might not have been expected due to differences in the soil types, cropping histories and weather conditions between these study locations.

Soil type has been reported to be a more important factor than management system or time of sampling in influencing soil microbial communities (Bossio et al. 1998). Research has suggested that microbial biomass is not closely enough related to other measures of soil health, perhaps in support of some of the unusual correlation results obtained from the current study such as stronger positive relationships between AMF and anaerobes than AMF with fungi in Experiment 2. However, in the current study correlation analysis showed mostly negative relationships between AMF and the weed measurements, in support of other literature, which found them also to be negatively correlated (Wortman et al. 2013). Tillage has been shown to result in less richness (number of species) of bacteria compared to a no-till system in support of using no-till practices to promote greater soil microbial diversity (Lupwayi et al. 1998).

The tillage treatments in the current study did not result in significantly less microbial biomass compared to the no-till treatment, except for AMF, which was lowest under one of the more intensive treatments (STRC+PETW) compared to no-till, but his effect was only seen in the second year of analysis. Therefore, overall the first hypothesis was rejected since most of the microbial groupings were not significantly affected. Kohl et al. (2014) found that conventional tillage promoted increased overall microbial biomass compared to no-till systems. Other research suggests that no-till is the least harmful practice for sustaining diversified populations of AMF in the soil (Mannoharan et al. 2017), particularly near the soil surface (Sale et al. 2015). In the first year of the current study STHVO had the largest number of weed species, and it did not reflect a significantly greater contribution to total PLFA. In 2018 the no-till treatment had the largest number of weed species, and it also did not reflect a significantly greater contribution from each microbial group to total PLFA. Therefore, based on these findings, hypothesis 2a was rejected. However, in both years the weed biomass and the number of weed species were not negatively correlated with each other in this study, in support of rejecting hypothesis 2b.

The negative bacteria, AMF, gram-positive bacteria and fungi were the four largest groups contributing to the total PLFA compared to the amounts of eukaryotes, actinomycetes and anaerobes. Given that the top four groups were all significantly affected by soil sampling date suggests that the time of soil sampling was a more influential factor than the effects of the tillage treatments on soil microbial composition. To compare the strength of the two main soil health analyses used in this project, the relatively small standard deviations in the PLFA dataset were still observable with some significant differences between treatments when detected with Tukey's MMC, as compared to soil health indicators when detected with Duncan's MMC; particularly

active carbon, wet aggregate stability, surface and subsurface hardness, which had larger relative ranges between the different treatment effects when significant differences were detected, as influenced by GMr or tillage.

CHAPTER 4 -CONCLUSIONS

4.1. Effect of GMr, weed presence, and tillage on soil health including microbial PLFA

In the current project, overall the Cornell Soil Health Assessment (CSHA) did not show an effect on soil health, based on the treatments studied. A few significant differences on soil health indicators were inconsistently detected such as soil respiration based on the influence of the previous GMr treatment grown 2 years prior in rotation in (Experiment 1), i.e. their residual contributions to soil health were similar enough when measured 2 years later. It may have been difficult to detect differences in physical soil health indicators due to a masking effect of GMr used regularly in rotation. However, the weed presence and biomass in the current season of analysis were more influential on soil health, and especially for the biological measures of soil respiration, and with respect to promoting fungal microorganisms when examined through phospholipid fatty acid (PLFA) analysis.

Weed presence did not necessarily improve soil health, depending on the particular indicator of interest, but tillage practices certainly reduced weed growth to improve crop yields. Crop yield is not included as an indicator of soil health in the CSHA, but other tests include it to indicate that healthy soils correlate to healthy plants. There was no clear outcome as to whether weed biomass or number of weed species was more influential on soil health given different results at the two sites, making the results site-specific. Also, both sites hosted a diverse array of different weed species, with a mixture of mycorrhizal and non-mycorrhizal species in various amounts and locations across the fields that likely had many interactions with the soil microbial communities.
Tillage disturbed the microbial life in the soil, but perhaps the species residing there were better able to withstand the physical disturbance to be resilient to it. Bacterial and fungal microbes can be resilient to some environmental stresses, especially if their communities are diversified to support each other. The month of sampling was more influential than the tillage treatments compared in this particular study. The results from the temporal influence are subject to the timing of sampling and analysis. Also, more significant differences were detected in Experiment 2 overall, given the closer time frame of applying the treatments and measuring the soil properties.

The PLFA method was able to detect some significant differences in the biomass and %contribution to total composition of fungal microorganisms based on the weedy treatment in Experiment 1 and for the tillage treatments or time points of sampling in Experiment 2. The PLFA method demonstrated a high level of precision (reproducibility) separately in both experiments; higher than

the CSHA method. To elaborate on that point, when measuring any of the biological measures of soil health through the Cornell index (usually measured in triplicate), if the values obtained deviate from each other by more than 5% then the samples are re-tested according to the regulations in the SOP. However, with the PLFA method, there are no instructions to re-analyze samples unless an obvious mistake is made during the procedure. Therefore, even though both of these methods are examining biological measures, they have different levels of potential error associated with them. I believe that additionally taking the time and effort to conduct PLFA analysis provides beneficial information to get a better understanding of the biological state of soil with more reliable results than the CSHA indicator tests. If there was a way of incorporating PLFA or other weed and crop measurements into the CSHA scorecard, it may allow for a more inclusive study of the whole ecosystem.

4.2. Challenges of Current Research

Even though the two sites in this experiment were rotated with various crops previously as part of other research projects, the specific treatments in this project have only been examined for 2-3 years. Therefore, one challenge for this project is the factor of time, since soil management studies can take many years to see long-term improvements to particular management practices. More specifically, the residual impact of GMr in rotations, tillage practices and the presence or absence of weeds would have to be followed over longer time periods to assess their long-term impacts on soil health, and especially in organic production. Furthermore, different sites are subject to different results. Multiple experiments at different locations showing similar findings are needed to convince a larger scientific community of adopting the same practices. Short-term results can be encouraging in a particular direction, but not as reputable until more experimentation is done. The best interpretation of shorter-term data is to be able to compare the results of each treatment against each other to understand where differences are if they exist at a given time point.

Some scientists question the meaning behind the values obtained for each soil health indicator, and what they actually mean in terms of good long-term management practices for growers. It is difficult to put a value to the results obtained because some change more readily than others over time or as influenced by other environmental factors. I think there is so much to still learn about the soil, given that it contains millions of microorganisms, many yet unidentified and not visible by the human eye. Farmers are less likely to implement changes unless they are guaranteed to see benefits to their land and more profit. How do researchers know the tests are reliable and produce satisfactory results to share with farmers? Since a thorough analysis involves collecting many

samples, another challenge in future work is affordability to generate a good data set. Particularly for PLFA data there are no known scoring functions made for different regions or cropping systems to even use a baseline to know if results obtained from other studies are comparable.

4.3. Recommendations for Future Research

Knowing that soil conditions vary across North America, further work needs to be done to develop a suitable suite of tests and analysis to interpret the results in different regions, particularly for Atlantic Canada as it pertains to this project. Although the CSHA gives a general balanced score for a site, it does not consider the relationships or interactions between the different indicators making up that score. The 3 categories contributing to soil health (biological, physical and chemical) are all integrally associated with each other, but they are not necessarily associated with the same level of influence on each other. Therefore, weighting the indicators, such as that done through the OSHA has been recommended to provide a more accurate evaluation to detect differences between soil health management practices, compared to evenly weighting the indicators through CSHA. However, just because Ontario has had better accuracy with the OSHA as compared to CSHA does not mean there is no value in testing CSHA in Atlantic Canada. Rather than switching to a different type of analysis, I believe the CSHA manual should be calibrated to our weather conditions and production systems, which are different from other parts of Canada and the U.S. In saying that, I think the Mehlich 3 nutrient extractions are a good substitute for the CSHA Morgan's extractant solution to measure the soil available macro and micronutrients, due to applicability in our region. Even within the Atlantic provinces, PEI has very sandy soils, which could require slight variations to the weighing within that province.

For this research project using CSHA methods, soil samples were collected to a depth of 15cm. An interesting area for further research is whether the soil health properties would be significantly different if samples were collected in deeper soil depths for comparison, especially considering differences in GMr characteristics (deeper red clover taproots) in Experiment 1, and the relative placement of GMr residues through tillage regime in Experiment 2. There remains relatively little known regarding the impact of weeds on soil health, as related to agricultural crop production. Determining critical periods of weed control for all major agricultural field crops would be highly recommended moving forward to reduce physical soil disturbance where possible. There is a need to further examine whether weeds are mycorrhizal or non-mycorrhizal, and what implications their characteristics impose, or are influenced by the soil structural integrity, access to nutrients, and biological activity/interactions. The soil health analysis has given me an appreciation of the

complexity of soils, especially at a biological level of study. It is an ever-growing area of research to maintain sustainable soils for many future generations of agriculture. No known literature is published over the longer term on the influence of diverse weed communities on soil health in organic grain production under different tillage systems for weed control and crop rotations incorporating GMr crops all in the same study. In future work, if more strongly contrasting crop rotation systems or conventional agricultural practices were compared to organic ones, perhaps more significant results would be found. More research is needed in this area under organic field crop production.

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Appendices:

			< --- 14 M ---->	<5M>			
			Strip 1		Strip 2		Strip 3
Block 1	∨ 10M ∧	**C1**	**36 a Wheat**		**34b Soybeans**		**35c Y1 Red clover**
		C4	**31 b Wheat**		**33c Soybeans**		**32a Common Vetch/oats**
		C3	**30 a Wheat**		**28c Soybeans**		**29b Fallow**
		C2	**25b Hairy Vetch/oats**		**27a Soybeans**		**26c Wheat**
Block 2		**C4**	**24b Wheat**		**22c Soybeans**		**23a Common vetch/oats**
		C2	**19c Wheat**		**21b Hairy Vetch/oats**		**20a Soybeans**
		C3	**18a Wheat**		**16c Soybeans**		**17b Fallow**
		C1	**13c Y1 Red clover**		**15b Soybeans**		**14a Wheat**
Block 3		**C3**	**12b Fallow**		**10a Wheat**		**11c Soybeans**
		C4	**7a Common Vetch/oats**		**9c Soybeans**		**8b Wheat**
		C2	**6c Wheat**		**4a Soybeans**		**5b Hairy Vetch/oats**
		C1	**1a Wheat**		**3c Y1 Red clover**		**2b Soybeans**

Appendix 1: Experiment 1 organic cropping system for 2017 growing season. Note: ← is the map direction of North. There is 2m between plots E-W. Soybean plots are bolded as the plots of interest in this study. Y1=year 1 Y2=year.

	Block 3				Block 2				Block 1			
	12	11	10	9	8	7	6	5	4	3	2	1
Sub-plot position below:	Spring Till	Fall Till	None	No Till-mulch	Fall Till	None	No Till-mulch	Spring Till	No Till-mulch	Fall Till	Spring Till	None
1	Rye	**Wheat**	Soybean	**Wheat**	**Wheat**	Soybean	Soybean	Rye	Rye	HVO	Soybean	**Wheat**
2	HVO	Rye	Clover Y1	Soybean	HVO	Clover Y2	**Wheat**	**Wheat**	**Wheat**	**Wheat**	HVO	Soybean
3	Soybean	Soybean	Clover Y2	Rye	Rye	**Wheat**	HVO	Soybean	Soybean	Rye	**Wheat**	Clover Y2
4	**Wheat**	HVO	**Wheat**	HVO	Soybean	Clover Y1	Rye	HVO	HVO	Soybean	Rye	Clover Y1
2017 Tillage Treat-ment	Spring Till HVO+ PETW	Spring Till HVO	Spring Till RC + PETW	No-Till	Spring Till HVO+ PETW	Spring Till RC + PETW	No-Till	Spring Till HVO	No-Till	Spring Till HVO	Spring Till HVO + PETW	Spring Till RC + PEWT
Code	3	2	4	1	3	4	1	2	1	2	3	4

Appendix 2: Experiment 2 organic cropping system for 2017 growing season.

Note: There is 5m between plots E-W and 1m between plots running N-S. Wheat plots are bolded as the plots of interest in this study.
Y1=year 1 Y2=year 2
The tillage treatments presented near the top of the figure under the block headings represent the previous tillage practices imposed on the plots.
2017 Tillage Treatments:
1= No-Till; 2= Spring Till Hairy Vetch Underseeded with Oats (HVO); 3= Spring Till Hairy Vetch Underseeded with Oats + Post-Emergent Tine Weeding (ST HVO+ PETW), and 4= Spring Till Red Clover and Post-Emergent Tine Weeding (ST RC+PETW).

	Plot Code	2011	2012	2013	2014	2015	2016	2017
B1	C1 a	Soybean	Red Clover	Wheat	Soybean	Red Clover	Wheat	**Soybean**
	C1 b	Red Clover	Red Clover	Soybean	Red Clover	Wheat	**Soybean**	Buckwheat
	C1 c	Red Clover	Wheat	Red Clover	Wheat	Soybean	Buckwheat	Red Clover
	C4 a	Wheat	Soybean	CVO	Wheat	Soybean	Buckwheat	CVO
	C4 b	Soybean	OPV	Wheat	Soybean	CVO	Wheat	**Soybean**
	C4 c	OPV	Wheat	Soybean	CVO	Wheat	**Soybean**	Buckwheat
	C3 a	Soybean	Wheat	Soybean	Wheat	Soybean	Wheat	**Soybean**
	C3 b	Wheat	Soybean	Fallow	Fallow	Fallow	Fallow	Fallow
	C3 c	Wheat	Soybean	Wheat	Soybean	Wheat	**Soybean**	Buckwheat
	C2 a	Red Clover	Wheat	Soybean	HVO	Wheat	**Soybean**	Buckwheat
	C2 b	Wheat	Soybean	HVO	Wheat	Soybean	Buckwheat	HVO
	C2 c	Soybean	Red Clover	Wheat	Soybean	HVO	Wheat	**Soybean**

Appendix 3: Crop rotation history and planting schedule for block 1 of Exp. 1 from 2011-2017 Note: Y1= Year 1, Y2= Year 2, OPV= Oats/Peas/Vetch, CVO=Common Vetch/Oats, HVO= Hairy Vetch/Oats. Crop rotations of interest to this study are highlighted blue for 2 years prior to each year of analysis, being highlighted yellow.

					Analysis Y1	Analysis Y2
	Plot Code	2014	2015	2016	2017	2018
B1	1a	Soybean	Clover Y1	Clover Y2	**Wheat**	Soybean
	1b	Clover Y1	Clover Y2	Wheat	Soybean	Clover Y1
	1c	Wheat	Soybean	Clover Y1	Clover Y2	**Wheat**
	1d	Clover Y2	Wheat	Soybean	Clover Y1	Clover Y2
	2a	Vetch/oats	Wheat	Fall Rye	Soybean	Vetch/oats
	2b	Wheat	Fall Rye	Soybean	Vetch/oats	**Wheat**
	2c	Fall Rye	Soybean	Vetch/oats	**Wheat**	Fall Rye
	2d	Soybean	Vetch/oats	Wheat	Fall Rye	Soybean
	3a	Wheat	Rye	Soybean	Vetch/oats	**Wheat**
	3b	Fall Rye	Soybean	Vetch/oats	**Wheat**	Fall Rye
	3c	Soybean	Vetch/oats	Wheat	Fall Rye	Soybean
	3d	Vetch/oats	Wheat	Fall Rye	Soybean	Vetch/oats
	4a	Soybean	Vetch/oats	Wheat	Fall Rye	Soybean
	4b	Fall Rye	Soybean	Vetch/oats	**Wheat**	Fall Rye
	4c	Vetch/oats	Wheat	Fall Rye	Soybean	Vetch/oats
	4d	Wheat	Fall Rye	Soybean	Vetch/oats	**Wheat**

Appendix 4: Crop rotation history and planting schedule for block 1 of Exp. 2 from 2014-2017. Crop rotations of interest to this study are highlighted in bold. Crop rotations of interest to this study are highlighted blue for 2 years prior to each year of analysis, being highlighted yellow.

Supplies and Equipment Needed for Field Work:

-Hand Hoe, Knives with Serrated Edges

-Cloth or Garbage Bags, Permanent Marker

-Quadrats (0.25m^2), Colored Flags

-Penetrometer (Dickey-John Part #15585-0003AS1)

Supplies and Equipment Needed for Soil Health Laboratory Work:

-Elementar varioMax C:N analyzer ©

-Roller grinder machine and shaker

-Access to fridge and freezer space

-Large aluminum trays

-Spatula or large spoon

-8-mm, 2-mm and 0.053mm sieves with catch basins

-Balance for weighing samples

-Large weight boats

-Drying oven up to 105ºC

-Beakers (min. 600mL capacity) with wide openings

-1000mL volumetric flasks

-Sink access and squeeze bottles

-Aluminum tins (various sizes)

-Falcon tubes (50mL capacity) with lids

-Rainfall Simulator, Stopwatch or clock

-Filter papers (small and large coffee ground)

-Scoopula with rubber knob

-Large plastic funnels (diameter as wide as a sieve)

-Bottle-top dispenser

- Cole-Parmer pH meter (Model # 05669-20) (Used in Cox Before Fire)

-Oaklon pH Testr 20 © meter and working calibration standards (Used in Haley After Fire)

-10mL,100-1000µL, and 1000-5000µL pippettors and disposable tips

-Stir plate, magnetic stir bar and rod

-Large amber bottle

-Jenway 6505 UV/Vis Spectrophotometer © (Used in Cox Institute Before Fire)

-Bausch & Lomb Spectronic 501© Spectrophotometer (Used in Haley After Fire)

-5 Bar Pressure Plate Extractor Cat. #1600, Soil Moisture Equipment Co.

-15 Bar Ceramic Plate Extractor Model #1500F2, Soil Moisture Equipment Co.

-Pressure Plates Rubber Rings, Chamber and Compressor

-Mason Jars with proper fitting lids

-Colored Tape for Labeling

-Permanent Marker, Clear Tape, Pizza Stools

-Scintillation vials (20mL capacity)

-SympHony B10C Conductivity Meter © (Used in Cox Before Fire)

-Hach sension5 Conductivity Meter © (Used in Haley After Fire)

-Glass Bottles with Caps

-Stratton eppendorf Centrifuge 5415 © (Used in Cox Before Fire)

-Ohaus frontier Centrifuge 5306 © (Used in Haley After Haley)

-Microcentrifuge tubes

-96-well clear flat bottom multiplates

-BioTek PowerWave XS2 Multiplate Reader © (Used in Cox Before Fire)

-Gen5 ACE Protein Software Program © (Used in Cox Before Fire)

-BioTek Synergy HT © Multiplate Reader © (Used in Haley After Fire)

-KC4 ACE Protein Software Program © (Used in Haley After Fire)

Chemicals Needed for Soil Health Laboratory Work:

-Access to distilled and deionized water

-Sodium Hexametaphosphate (3% soap solution)

-Potassium permanganate powder (KMnO4)

-Calcium chloride (CaCl)

-Potassium hydroxide (KOH)

-Hydrochloric acid (HCl)

-Sodium citrate ($NaC_6H_8O_7$) 20mM pH 7.0

-BCA reagents (A and B) and 5 Pre-diluted Standards

-Ammonium fluoride (NH_4F)

-Ammonium nitrate (NH_3NO_3)

-Nitric acid (HNO_3)

-Ethylenediamine tetraacetic acid (EDTA)

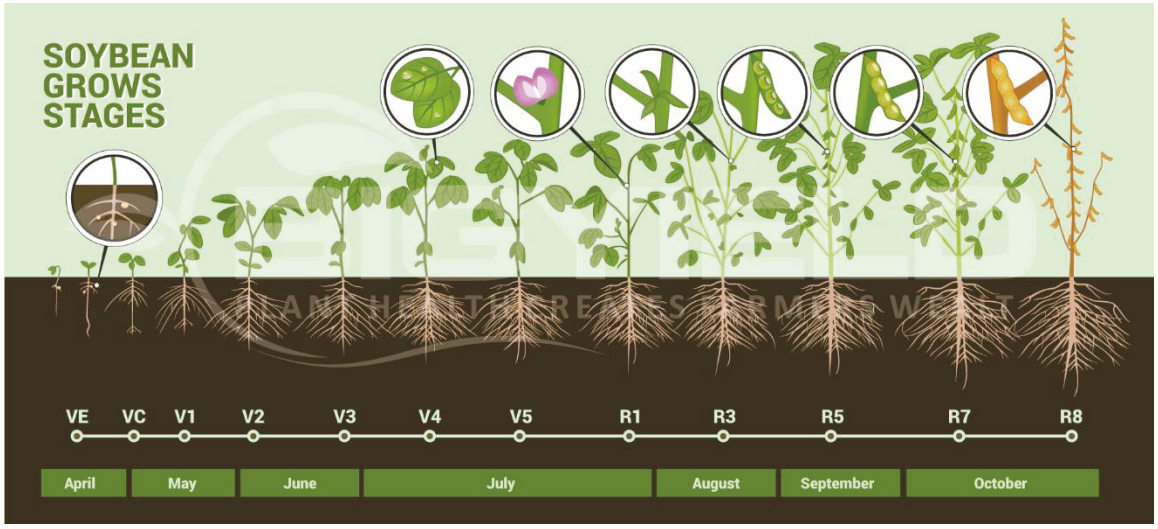
-Acetic acid (CH_3COOH)

Appendix 5: List of all field and laboratory supplies, equipment and chemicals

Appendix 6: Weed species with mycorrhizal status identified in both experiments (scientific and common names)

Scientific Name	Common Name	Mycorrhizal Status	Exp. 1	Exp. 2
Amaranthus retroflexus L.	Redroot Pigweed	Non-Mycorrhizal (Weaver and McWilliams 1980)	*	*
Articum lappa L.	Greater Burdock	Unknown (Gross et al. 1980)		*
Avena sativa L.	Oats	Mycorrhizal (Beckie et al. 2012)	*	*
Barbarea vulgaris R.Br.	Yellow Rocket	Unknown (MacDonald and Carvers 1990)	*	*
Bromus erectus Huds.	Upright Bromegrass	Mycorrhizal (Dostalek et al. 2013)		*
Bromus inermis Leyss	Smooth Bromegrass	Mycorrhizal (Otfinowski et al. 2007)		*
Capsella bursa-pastoris L.	Shephard's Purse	Non-Mycorrhizal (*Brassicaceae*)	*	*
Chenopodium album L.	Lamb's Quarter	Non-Mycorrhizal (Bassett and Crompton 1978)	*	*
Cirsium arvense L.Scop.	Canada Thistle	Non-Mycorrhizal (Moore 1975)	*	*
Convolvous arvensis L.	Bindweed	Unknown (Weaver and Riley 1982)		*
Daucus carota L.	Wild Carrot	Mycorrhizal (Hillis et al. 2008)		*
Digitaria sanguinalis L.	Large Crabgrass	Mycorrhizal (Rinaudo et al. 2010)	*	*
Echinochloa crus-galli L.Beauv.	Barnyard Grass	Non-Mycorrhizal (Maun and Barrett 1986)	*	*
Elymus repens L.	Quackgrass	Mycorrhizal (Vatovec et al. 2005)	*	*
Erigeron annuus L.	Daisy Fleabane	Mycorrhizal (Tawaraya 2011)		*
Conyza canadensis L.	Horseweed	Mycorrhizal (Weaver 2001)	*	
Erysimum cherianthoides L.	Wormseed Mustard	Non-Mycorrhizal (*Brassicaceae*)	*	*
Euphoria maculata L.	Spotted Spurge	Mycorrhizal (Jordan and Huerd 2008)		*
Festuca arundinacea Schreb.	Tall Fescue	Mycorrhizal (Ho 1986; Marks and Clay 1996)		*
Festuca pratensis Huds.	Meadow Fescue	Mycorrhizal (Wang and Qiu 2006)	*	*
Galeopsis tetrahit L.	Hemp Nettle	Unknown (O'Donovan and Sharma 1987)	*	
Glechoma hederacea L.	Ground Ivy	Mycorrhizal (Vannier et al. 2016)	*	*
Jacobaea maritima L.	Dusty Miller	Mycorrhizal (Harley and Harley 1987)	*	*
Linaria vulgaris Mill.	Yellow Toadflax	Mycorrhizal (Saner et al. 1995)	*	
Lolium multiflorum Lam.	Annual Ryegrass	Mycorrhizal (Wang and Qiu 2006)		*
Persicaria maculosa L.	Lady's Thumb	Non-Mycorrhizal (Dhillion 1994)	*	*
Phalaris arundinaceae L.	Reed Canarygrass	Mycorrhizal (Wang and Qiu 2006)		*
Phleum pratense L.	Timothy Grass	Mycorrhizal (Hempel et al. 2009)	*	*
Plantago major L.	Broadleaf Plantain	Non-Mycorrhizal (Hawthorn 1974)	*	*
Poa pratense L.	Kentucky Bluegrass	Mycorrhizal (Vandenkoornhuyse et al. 2003)	*	
Polygonum pensylvanicum L.	Smartweed	Non-Mycorrhizal (*Polygonaceae*)	*	*
Raphanus raphanistrum L.	Wild Radish	Non-Mycorrhizal (Warwick and Francis 2005)	*	*
Rumex acetosella L.	Sheep Sorrel	Non-Mycorrhizal (Stoops et al. 2011)	*	*
Rumex crispus L.	Curled Dock	Non-Mycorrhizal (Vatovec et al. 2005)	*	*
Senecio cineraria D.C.	Dusty Miller	Mycorrhizal (Kumar et al. 2012)		*
Setaria pumila L.	Yellow Foxtail	Mycorrhizal (Sathiyadash et al. 2010)		*

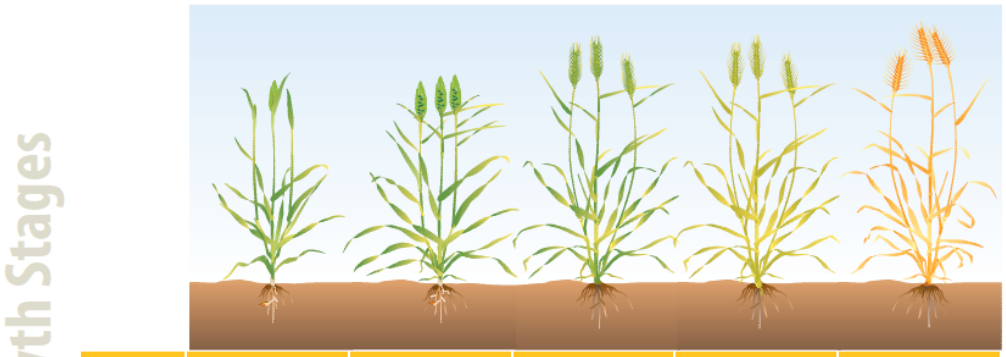
Sonchus arvensis L.	Perennial Sow Thistle	Unknown (Lemna and Messersmith 1990)	*	*
Spergula arvensis L.	Corn Spurry	Unknown (New 1961)	*	*
Stelleria media L.Vill.	Chickweed	Unknown (Turkington et al. 1980)	*	*
Tanacetum vulgare L.	Tansy	Mycorrhizal (Betekhtina and Veselkin 2010)	*	*
Taraxacum officinale G.H.Weber x Wiggers	Dandelion	Mycorrhizal (Stewart-Wade et al. 2002)	*	*
Trifolium pratense L.	Red Clover	Mycorrhizal (Arines et al. 1988)	*	*
Tussilago farafara L.	Coltsfoot	Mycorrhizal (Pawlowska et al. 1996)	*	*
Veronica peregrina L.	Pursulane Speedwell	Mycorrhizal (Wang and Qiu 2006)	*	*
Vicia cracca L.	Tufted Vetch	Mycorrhizal (Wang and Qiu 2006)	*	*
Vicia sativa L.	Common Vetch	Mycorrhizal (Wang and Qiu 2006)		*
Vicia villosa Roth.	Hairy Vetch	Mycorrhizal (Wang and Qiu 2006)	*	*



Appendix 7: Vegetative and reproductive growth stages of soybeans (*Glycine max.)*

Note: Source of Image: http://mashastudio.net/portfolio/soybean-grows-stages/

Zadoks Growth Stage	GS 00 - 09	GS10 - 19	GS20 - 29	GS30 - 39	GS40 - 49
Development phase	Germination	Seedling growth	Tillering	Stem elongation	Booting



Zadoks Growth Stage	GS 50 - 59	GS60 - 69	GS70 - 79	GS80 - 89	GS90 - 99
Development phase	Ear emergence	Flowering	Milk Development (grain fill period)	Dough Development (grain fill period)	Ripening

Growth Stages

Appendix 8: Developmental stages of wheat (*Triticum aestivum*) crop

Note: Source for Image: https://www.agric.wa.gov.au/soil-nutrients/critical-tissue-nitrogen-concentrations-diagnosis-nitrogen-deficiency-wheat

Cornell Rapid Texture Method *(CSHA Test)*
Cornell Soil Health Laboratory 2016
Modified: July 2017; Jackson, G. McNeil, M.O. and Lynch, D. H.

Materials:

- 2-mm Sieved Soil (20.00g/sample)
- 3% Sodium Hexametaphosphate solution
- Snap Cap Bottles
- 75ml and 200ml Aluminum Weighing Tins
- Top Loading Balance
- Wash Bottle with Distilled Water
- 53µm Sieve with Catch Pan
- Spatula
- 1000ml Beakers
- Drying Ovens

Procedure:

1. Prepare sample ID-labeled snap cap bottles with 65mL of 3% HMP soap solution.
2. Sieve 2 tablespoons (TBS) of air-dried soil through a 2-mm sieve.
3. Weigh out 20g (+/- 0.3g) of sieved soil into their corresponding snap cap bottles.
4. Shake the bottles for 2 hours at 150osc/min to properly separate the soil particles.
5. Label, record ID, and weigh a 75ml and a 200ml aluminum tin that corresponds to each snap cap bottled sample; each sample will have a 75ml tin for sand and a 200ml tin for silt.
6. Have prepared a 53µm sieve with pan underneath, this sieve will separate the sand and POM from the silt and clay which will pass through the sieve.
7. Take sample in snap cap bottle and shake to re-suspend the sample. Open snap bottle and pour out the contents onto 53µm sieve and using wash bottle filled with distilled water to wash out all sample onto sieve.
8. Using wash bottle, spray all silt and clay particles through the mesh of the sieve until only clear water is passed through the sieve.
9. Collect sand into its corresponding labeled and pre-weighed aluminum tin using a spatula. To collect remaining sand grains and POM, use wash bottle to wash and pour remaining grains into sand tin.
10. Decant sieved contents of the pan into a 1000ml beaker while using the wash bottle to spray out the settled silt into the beaker as well. Note the time of the decanting and allow the mixture to settle for 2 hours. This will allow the silt to settle at the bottom of the beaker and the clay to remain suspended in the water.
11. After the 2 hour settling period, decant and dispose of the clay water mixture which lies on top of the silt. Using the wash bottle, spray and pour the silt into its corresponding labeled and pre-weighed aluminum tin.
12. Allow the sand and silt to air dry in their respective tins. Then, put the sand and tin into an oven at 65ºC for 24h and the silt and tin in a different oven at 100ºC for 24h.
13. Reweigh the samples in their tins and record their mass on data sheet.

Appendix 9: Cornell Soil Health Assessment- Rapid Soil Texture Procedure

Cornell Available Water Capacity Method *(CSHA Test)*
Cornell Soil Health Laboratory 2016
Modified: November 2016; McNeil, M.O.

Materials and Equipment:

- Air-dried 2-mm soil (Roughly 20g/sample)
- Pressure plates
- Rubber chamber ring and plastic rings
- Pressure chamber
- Compressor
- Pressure regulation system
- Balance
- Spatula
- Wash bottle

Procedure:

1. Saturate 1 and 15 bar plates (100 and 1500kPa respectively) with a wash bottle. **Note: Often both plates could not run at the same time due to high pressure usage, so one was run after the other. Neither is required to be run before the other, but it is good to keep in mind that the 15-bar plate takes a little longer to run (closer to a week compared to 4-5 days for the 1-bar plate).

2.In the 1 bar plate place up to 14 small filter papers on the plate, followed by rubber rings. Fill each ring loosely with sample (roughly 15-20g). For the 15 bar plate, do the same, but pack the soil into the rings for a tight fit to prepare for higher pressure exposure.

3. Add water to sides of rings to help saturate the soil without adding water directly over the soil in the rings.
4. Stack the plates into the appropriate chambers using holding clamps and spacers between plates.
5. Connect outflow tubes between plates and pressure chambers.
6. Put ends of outlet tubes into a beaker.
7. Tighten lids with bolts.
8. Gradually add pressure to the chambers by rotating the dials above the machine until it is running smoothly/efficiently.
9. If possible, check on the plates everyday for up to a week until no more water drips from the tubes into the collection beaker.
10. When complete, turn pressure dials back to zero and turn off power button.
11. Unscrew bolts, remove lid and disassemble the chamber to expose the plates.
12. Transfer each soil sample into its own labeled weighed aluminum tin and oven-dry them until fully dried.
13. Do calculations to determine the available water capacity (%).

Calculations for each sample:

Theta M = ((wt. wet soil + can) - (wt. dry soil + can)) / ((wt. dry soil + can) - wt. of can)
For sample X
$AWC_{\text{sample x}} = \text{Theta M}_{0.1\ \text{bar}} - \text{Theta M}_{15\text{bar}}$

Appendix 10: Cornell Soil Health Assessment- Available Water Capacity Procedure

Cornell Wet Aggregate Stability Method *(CSHA Test)*
Cornell Soil Health Laboratory 2016
Modified: November 2016; McNeil, M.O.

Materials:

- Air-dried 8-mm sieved soil (20.00g/sample)
- Reverse osmosis water system
- 0.25mm Soil Sieves (At least 8)
- Balance, Soil Sieve Brush
- Large plastic funnels
- Large coffee filters
- Aluminum Tins
- Permanent Marker
- Sink access with water and squeeze bottles
- Cornell Rainfall Simulator
- Large plastic storage container
- Stopwatch
- Drying Oven

Procedure:

1. Fill rainfall simulator with water, monitor it until full and record initial water level from ruler.
2. Number sieves depending on how many samples to process in each round.
3. Weigh and record dry sieve weights, and corresponding filter paper weights.
4. Label, weigh and record two aluminum tins for each sample: 1 designated as sand tin and 1 as soil taco tin.
5. Place a coffee filter in a large plastic funnel, and then place sieve over top of the coffee filter. Add weighed soil sample.
6. Place this assembly into the stand 0.5m below the rainfall simulator.
7. Open and close rubber stopper and large paperclip from tubing on top of the simulator to release pressure and allow water to start dripping.
8. Start timer for 5 minutes and allow 1.25cm of water to hit the sieve. Over this period rotate the sieve to help deliver an adequate amount of velocity to the system.

****It is challenging to deliver exactly 1.25cm of water over a -5 minute period. It can take a few practice rounds to get the calibration right.** If experiencing difficulty with this, more emphasis was placed on releasing 1.25cm than covering a full 5 -minute period of raining.

9. After a 5-minute period record the final water level off the ruler.
10. Determine the difference between the first and second water level readings.
11. Collect wet soil taco in its corresponding tin.
12. Wash sand on sieve into sand tin. Use soil sieve brush as necessary not to miss any of the sample.
13. Transfer all tins to the drying oven at 105ºC until completely dry (minimum 24 hours).
14. Re-weight and record dry weights.
15. Do calculations from CSHA manual to determine % wet aggregate stability.
16. Repeat steps 1-15 for every other sample to be analyzed. ****Note: Sieves must be full dried in oven between samples so that moisture does not affect the weights in the calculations.**

Appendix 11: Cornell Soil Health Assessment-Wet Aggregate Stability Procedure

Cornell Active Carbon Analysis (*CSHA Test)*
Cornell Soil Health Laboratory 2016
Date Modified: August 2018 Modified By: Alam, M.Z.

Materials and Equipment:

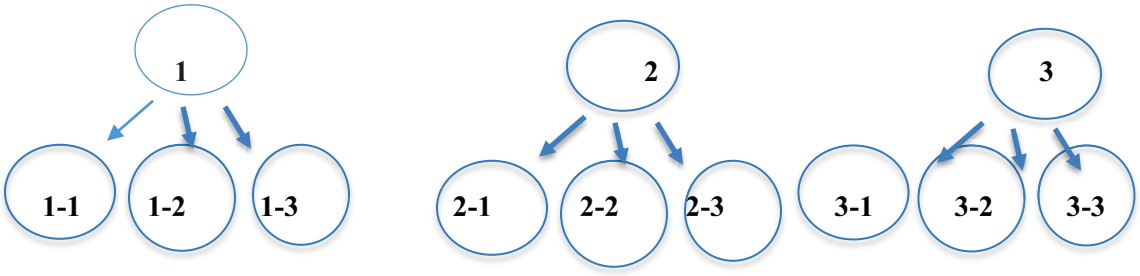
- Air-dried 2-mm soil (2.5g/sample)
- Access to distilled water
- Volumetric flask (1000mL)
- Stir plate and stir bar with magnetic rod
- Calcium chloride (CaCl)
- Potassium permanganate (KMnO4)
- Brown paper bag
- Large amber bottle
- Working pH meter and fresh standards
- Small quantities of HCl and KOH
- Falcon tubes (50mL)
- 11.Spectrophotometer (550nm wavelength)
- Disposable lab gloves
- Clear cuvettes
- Pipettes and tips (100-1000uL and 1000-5000uL)

Preparation of 0.2M $KMnO_4$ stock solution: **Important: Make fresh if possible. Best results with newer stock. Preferred not to store solution 3-6 months before using. It is very important to wear lab gloves to prevent the spread of any oils from hands on/in the samples.

1. Dissolve 11.09g $CaCl_2$ in ~750ml distilled deionized in a beaker. Dissolve completely, using stir plate with stir bar (reaching final concentration of 0.1M).
2. Add 31.61 g KMnO4 to the solution and a further ~200 ml of distilled water. Allow to dissolve completely (about one hour), covering solution and stir plate with a paper bag.
3. Adjust the pH to 7.2 using 0.1N HCl or KOH as necessary.
4. Pour solution into a volumetric flask and bring to 1000ml with distilled deionized water. The solution is light sensitive, and therefore it should be stored in an amber bottle.

Standard curve:

1. Ensure that the colorimeter is set to 550 nm and zero with distilled deionized water.
2. Dispense 45 ml distilled deionized water into each of three Falcon tubes.
3. Add additional distilled deionized water to the tubes in the following volumes: tube 1→ 3.75 ml; tube 2, → 2.50 ml; tube 3 → 0.0 ml.
4. Add 0.2M KMnO4 to the tubes in the following volumes: tube 1→ 1.25 ml; tube 2→ 2.50 ml; tube 3 →5.00 ml. Final concentrations of 50 ml KMnO4 solutions are now 0.005M, 0.01M, 0.02M. Cap and shake for 10 seconds.
5. Dispense 49.5 ml distilled water into 9 Falcon tubes – three for each standard solution.
6. Add 0.5 ml of each standard to each respective triplicate set. Cap and shake for 10 seconds.
7. Read and record the absorbance of each triplicate standard, rinsing the cuvette with one volume of standard and cleaning the outside with a KimWipe to remove any liquid or smudges before each reading.



To operate the spectrophotometer:

- Turn on the switch and let the machine set. It will show the maximum wavelength it can measure (e.g. 490 nm).
- Set the wavelength 550 nm using the key pad and press 'Second Function' then press 'Go to λ'.
- Then pour some deionized water in a quvate and '0' it. To '0' it, place the quvate in the spectrophotometer reading position and press 'Second Function' then press 'Zero A'.

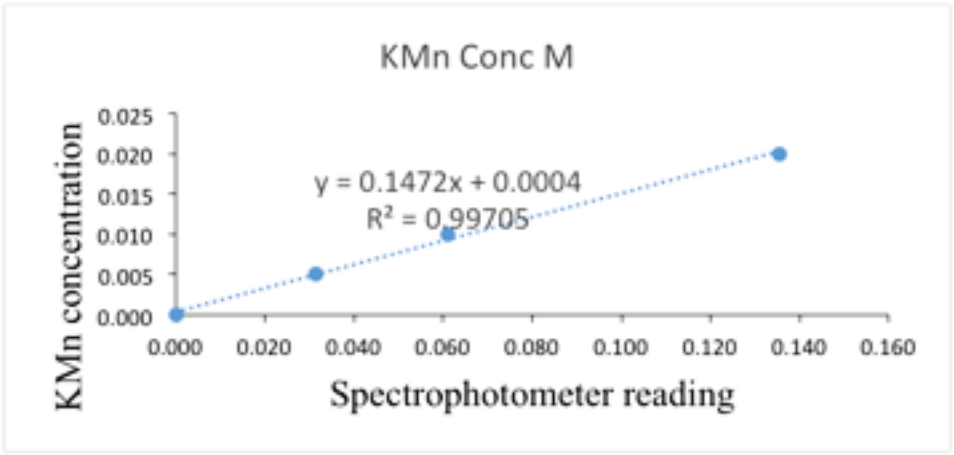


Figure 1: Standard curve for Active C. August 2, 2018

Measuring Active Carbon in Soil Samples

(Each soil sample is run in duplicate and are generally run in groups 10 soil samples)

1. Weigh out 2.5 g of air-dried soil samples in each of the two 50 mL falcon tubes.
2. Add 18 mL of DD water in each.
3. Add 2ml of 0.2M KMnO4 to each tube and cap tightly.
4. Shake the tubes on the shaker at 120 rpm for 2 minutes.
5. After 2 minutes (do not stop stopwatch), remove samples from the shaker and 'slosh' solution in tubes to ensure that soil is not stuck to the cap or top of the tube.
6. Uncap tubes and allow (on the bench-top) settling and reaction to continue for a further eight minutes.
7. After 10 minutes of total reaction time, remove 0.5 ml from each reaction tube and transfer to a Falcon tube with 49.5 ml DD water.
8. Cap diluted sample tubes and shake by hand for 10 seconds.
9. Read and record absorbance as described above, in the same sequences as KMnO4 solution was added to the samples, taking care to keep all reactions as close to 10 minutes as possible.

Notes:

- KMnO4 solution should be kept covered with an opaque container to block light.
- Repeat duplicates with a difference in absorbance greater than 5%.
- Repeat samples when duplicate sample absorbance readings fall outside the values of the standard curve, adjusting weight of sample used in reaction if necessary.

Calculations:

The bleaching (loss of purple color; reduction in absorbance) of the $KMnO_4$ is proportional to the amount of oxidizable C in the soil sample. It is assumed that 1 mol MnO_4 is consumed (reduced from Mn^{7+} to Mn^{2+}) in the oxidation of 0.75 mol (9000 mg) of C.

0.02 mol/L is the initial solution concentration; 9000 is the mg of C (0.75 mol) oxidized by 1 mol of MnO4 changing from Mn^{7+} to Mn^{2+}; 0.02 L is the volume of $KMnO_4$ solution reacted, and 0.0025 is the kg of soil used.

Standard curve:

Concentration = a + b * (absorbance). Determine the slope (b) and y-intercept (a) of a linear regression equation with concentration as the dependent variable (y) and absorbance as the independent variable (x).

- Where a is the intercept and b is the slope of the standard curve.

Active Carbon Calculation:

Active C (mg/kg) = [0.02 mol/L - (a + b * absorbance)] * (9000 mg C/mol) * (0.02 L solution/0.0025 kg soil).

References:

Weil, R., Islam, K. R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E. Estimating active carbon for soil quality assessment: A simple method for laboratory and field use. Amer. J. Alternative Agric. 18(1):3-17 (2003).

Stiles, Cynthia A., et al. "Validation testing of a portable kit for measuring an active soil carbon fraction." Soil Science Society of America Journal 75.6 (2011): 2330-2340.

Appendix 12: Cornell Soil Health Assessment- Active Carbon Procedure

Autoclaved Citrate Extractable (ACE) Protein Method *(CSHA Test)*
Cornell Soil Health Laboratory 2016
Modified: November 2016; McNeil, M.O.

Materials and Equipment:

- Air-dried 8-mm soil (3.00g/sample)
- Shaker bottles
- Balance
- Spoon
- Sodium citrate extraction buffer (20mM pH 7.0)
- Autoclave
- Microcentrifuge tubes
- Pipettors (1000uL) and tips (20uL, 200uL, 1000uL)
- 96-well clear flat bottom multiplate
- BCA reagents (A and B)
- Falcon tube (50mL)
- Pre-diluted Standards (0, 125, 250, 500, 750, 1000, 1500, 2000 ug/mL BSA)

Procedure:

Part 1: Extraction

1. Weight out 3.00g of each soil sample and transfer them to labeled glass bottles.
2. Make sodium citrate solution to pH 7.0 and adjust with HCl if needed.

Note: Mole Wt. of Sodium Citrate Dihydrate ($Na_3C_6H_5O_7.2H_2O$) is 294.10. So, 294.10 g in 1 L H_2O = 1 M solution. 0.2941 g in 1 H_2O = 1 mM solution. 0.2941 X 20 = 5.882 g in 1 L H_2O = 20 mM solution.

3. Check the pH and adjust to 7.0 with diluted HCl.
4. Use a bottle-top dispenser to add 24.00mL of sodium citrate solution to each tube.
5. Cap and shake for 5 minutes.
6. Remove caps and cover bottles with aluminum foil.
7. Autoclave samples for 30 minutes according to the instructions of the particular machine.
8. After autoclaving remove samples, and let them fully cool before handling them.

Part 2: Clarification

9. Label 2.2mL microcentrifge tubes for each sample.
10. Transfer 1.75mL of solution to each microcentrifuge tube and microcentrifuge them for 3 minutes at 10,000xg (relative centrifugal force).
11. Preheat a hot plate to 60ºC.
12. Label another microcentrifuge tube for each sample.
13. Transfer 1mL of cleared extract to another labeled microcentrifuge tube (always store in the refrigerator if not using the same day).

Part 3: Quantification

14. Make 50:1 working reagent with 1mL reagent B (blue copper sulfate) and 50mL of reagent A (clear) in a Falcon tube. When mixed it should change color to green.
15. Prepare a template for the placement of the samples in the well-plate (Figure 1). See below.
16. Add 10uL of each sample to the plate (running 4 for each sample to conduct good statistics) covering columns 1-8. Leave column 9 wells empty for spacer, and add 10uL of standards to columns 10-12 running horizontally (3 of each standard for good quality control).
17. Add 200uL of green BCA working reagent to each well for all the samples and standards. Within a few minutes the color of the liquid in the wells should change from green to purple.

18. Once the wells are filled, place the multi-well plate on the hot plate for 1 hour to help mix the contents well through conductive motion.
19. After an hour turn off the machine and let the plate cool for 10 minutes.
20. Put the plate in the spectrophotometer machine designed to read 96-well plates with the Gen5 software at a 562nm wavelength.
21. Follow the Excel calculations provided in the CSHA manual to determine the ACE protein content (mg/g soil).

	1	2	3	4	5	6	7	8	9	10	11	12
A	1-1	1-2	1-3	1-4	2-1	2-2	2-3	2-4	Empty	0	0	0
B	3-1	3-2	3-3	3-4	4-1	4-2	4-3	4-4	Empty	125	125	125
C	5-1	5-2	5-3	5-4	6-1	6-2	6-3	6-4	Empty	250	250	250
D	7-1	7-2	7-3	7-4	8-1	8-2	8-3	8-4	Empty	500	500	500
E	9-1	9-2	9-3	9-4	10-1	10-2	10-3	10-4	Empty	750	750	750
F	11-1	11-2	11-3	11-4	12-1	12-2	12-3	12-4	Empty	1000	1000	1000
G	13-1	13-2	13-3	13-4	14-1	14-2	14-3	14-4	Empty	1500	1500	1500
H	15-1	15-2	15-3	15-4	16-1	16-2	16-3	16-4	Empty	2000	2000	2000

Note: In columns 1-8 the samples are run in sets of 4. In column 10-12 are the standards run in triplicate. In column 9 it is empty to separate the samples from standards.

Figure 1: Placement of samples and standards in the 96 multi-well plate for ACE protein analysis.

References:

Keen N.T. and Legrand M. 1980. Surface glycoproteins - evidence that they may function as the race specific phytoalexin elicitors of *Phytophthora megasperma* f. sp. glycinea. Physiological Plant Pathology 17: 175-192.

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Appendix 13: Cornell Soil Health Assessment- Autoclaved Citrate Extractable (ACE) Protein

Soil Respiration Method *(CSHA Test)*
Cornell Soil Health Laboratory 2016
Modified: November 2016; McNeil, M.O.

Materials and Equipment:

- Air-dired 8-mm soil (20.00g/sample)
- Mason Jars with lids (medium-sized)
- Labeling tape and permanent marker
- Small Filter papers
- Balance
- Pre-perforated aluminum tins
- Pizza stools
- Scintillation vials (20mL)
- Potassium hydroxide (KOH) (0.5M)
- 19mL pipettor and tips
- Electrical Conductivity meter
- KimWipes

Procedure:

1. Label Mason jars (in duplicate for each sample).
2. Add a filter papers to each jar.
3. Weigh 20.00g of each soil sample and add them to each respective tin.
4. Tape scintillation vials to pizza stools.
5. Place soil tins in Mason jars and add pizza stool apparatuses to fit into edges of tins.
6. Make potassium hydroxide solution and measure the electrical conductivity of it.
7. Add 9.00mL KOH to each scintillation vial.
8. Add 7.5mL distilled water to each Mason jar along the inside edges to avoid wetting the soil directly.
9. Seal jars well to start the 4-day incubation period.
10. After 4 days, use the Electrical Conductivity meter to measure the conductivity of the KOH trap solution.
11. Use these results with the excel calculations from the CSHA manual to determine the % soil respiration for each sample.

    Theoretically, 1 mol of KOH can absorb 0.5mol CO_2, so 9.00mL of KOH can absorb 99.025mg CO_2 if fully saturated:
    0.009 L * 0.25 mol/L * 44.01 g/mol * 1000 mg/g = 99.025 mg CO_2

    The following calculation is used to determine the EC for the samples.
    $((EC_{raw} - EC_{sample})/(EC_{raw} - EC_{sat}))=P$

Appendix 14: Cornell Soil Health Assessment- Soil Respiration Procedure

Mehlich 3 Extraction Protocol

Description
Mehlich 3 (M3) estimated plant available micro- and macro-nutrients on soils. It correlated well with crop response to fertilizer P. During the extraction, P is solubilized by several different mechanisms. (1) nitric and acetic acid increases the solubility of Fe and Al- phosphates and extracts a portion of calcium phosphates if present. (2) Acetic acid buffers the solution below pH 2.9 to prevent calcium fluoride from precipitating. (3) Fl will complex Al2+ that potentially bind with P. (4) NH4+ exchanges with potassium, calcium and magnesium and EDTA chelates iron,manganese, zinc, and copper P and cations can be determined by ICP-AES instrumentation simultaneously. P content in solution can also be determined spectrophotometrically at an acidity of 0.20M H2SO4 (Rodriguez et al., 1994) by reacting with ammonium molybdate using ascorbic acid as a reductant in the presence of antimony (Murphy and Riley, 1962).

Reagents
1. Ammonium nitrate (NH3NO3), fw = 80.05, CAS# 6484-52-2
2. Ammonium fluoride (NH4F), fw = 37.04, CAS# 12125-01-8
3. Nitric acid (HNO3), 68-70%, fw = 63.02, 15.5N, CAS# 7697-37-2
4. Ethylenediamine tetraacetic acid (EDTA), (HOOCCH2)2NCH2CH2N(CH2COOH)2, fw = 292.25, CAS# 60-00-4
5. Acetic acid, glacial [CH3COOH] fw = 60.05, CAS# 64-19-7
Mehlich 3 stock solution (5000 samples)
Ammonium fluoride-EDTA stock solution (3.75M NH4F, 0.25M EDTA)

Note: The recipes were adjusted to make enough product for 500 samples rather than 5000 samples since fewer samples were needed for analysis.

Steps
1. Dissolve138.9g of NH4F in 600 mL of deionized water
2. Add 73.06 g EDTA (or 93.06 g of Na2-EDTA•2H2O) and mix thoroughly.
3. Bring to 1000 mL final volume.
Mehlich 3 extracting solution (4L)
0.2 N acetic acid, 0.25N ammonium nitrate, 0.015N ammonium fluoride, 0.013N nitric acid, and 0.001M EDTA at pH 0.25 ± 0.05.
1. Dissolve 80.05g NH3NO3 in 3L of DI water.
2. Add 16.0 mL of 3.75M NH4F, 0.25M EDTA stock solution and mix well.
3. Add 46 mL of concentrated glacial CH3COOH.
4. Add 3.3 mL of concentrated HNO3.
5. Bring to 4L final volume and check pH.
6. Adjust pH if necessary to 2.50 ± 0.05.

Extraction
1. Weigh 2.0 ± 0.05 g of air-dried, ground soil into a 50 mL centrifuge tube.
2. Add 20.0 mL of Mehlich 3 extracting solution. Make sure to include blanks and reps.
3. Place centrifuge tubes on their sides on the shaker table for 5 minutes.
4. Immediately after shaking, filter the soil suspension through a #41 whatman filter paper into 23 mL plastic sample bottles. If the samples are not analyzed right away, store them in the fridge.

Analysis
Run for ortho-phosphate on LACHAT QuikChem 8000 series by spectrophotometrically at an acidity of 0.20M H2SO4 (Rodriguez et al., 1994) by reacting with ammonium molybdate using ascorbic acid as a reductant in the presence of antimony (Murphy and Riley, 1962).

Calculations
Soil mass (mg/kg)
Report M3 extractable macronutrients to the nearest 0.1 mg/kg and micronutrients to the nearest 0.01 mg/kg
Soil nutrients mg/kg = (mg/L in extract – blank) x 10
Soil pool (kg/ha)
Soil nutrients kg/ha (assuming 15 cm depth and bulk density of 1.3 g/cm3
Soil nutrients kg/ha = soil nutrients mg/kg * 195

References

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Appendix 15: Mehlich 3 protocol for nutrient analysis of soils.

Appendix 16: Soil nutrient content from Experiment 1 (Field 206) in 2016 and 2017 through NS Harlow Laboratory

Field 206	2016			2017		
	Block 1	Block 2	Block 3	Block 1	Block 2	Block 3
pH	6.03	6.13	6.13	6.04	6.09	5.98
Organic Matter (%OM)	2.7	2.9	2.8	2.9	3	2.7
P2O5 (kg/ha)	793 (E)	907 (E)	1105 (E)	716	894	1091
K20 (kg/ha)	311 (H)	261 (H-)	258 (H-)	231	322	242
Calcium (kg/ha)	2029 (M-)	2304 (M-)	2280 (M-)	1719	2240	1966
Magnesium (kg/ha)	320 (M+)	344 (M+)	332 (M+)	283	344	285
Sodium (kg/ha)	16	<16	<16	<16	<16	<16
Sulfur (kg/ha)	26	24	23	19	21	20
Aluminum (ppm)	1468	1450	1553	1319	1465	1531
Boron (ppm)	< 0.50	0.51	< 0.50	<0.50	<0.50	<0.50
Copper (ppm)	1.84	1.91	1.63	1.08	1.47	1.37
Iron (ppm)	233	184	168	236	199	169
Manganese (ppm)	53	47	46	48	52	45
Zinc (ppm)	1.44	1.75	2.14	1.09	1.64	2.08
Required N (kg/ha)	170	0	40	N.P.	N.P.	N.P.
Required P2O5 (kg/ha)	170	0	55	N.P.	N.P.	N.P.
Required K2O (kg/ha)	170	0	55	N.P.	N.P.	N.P.

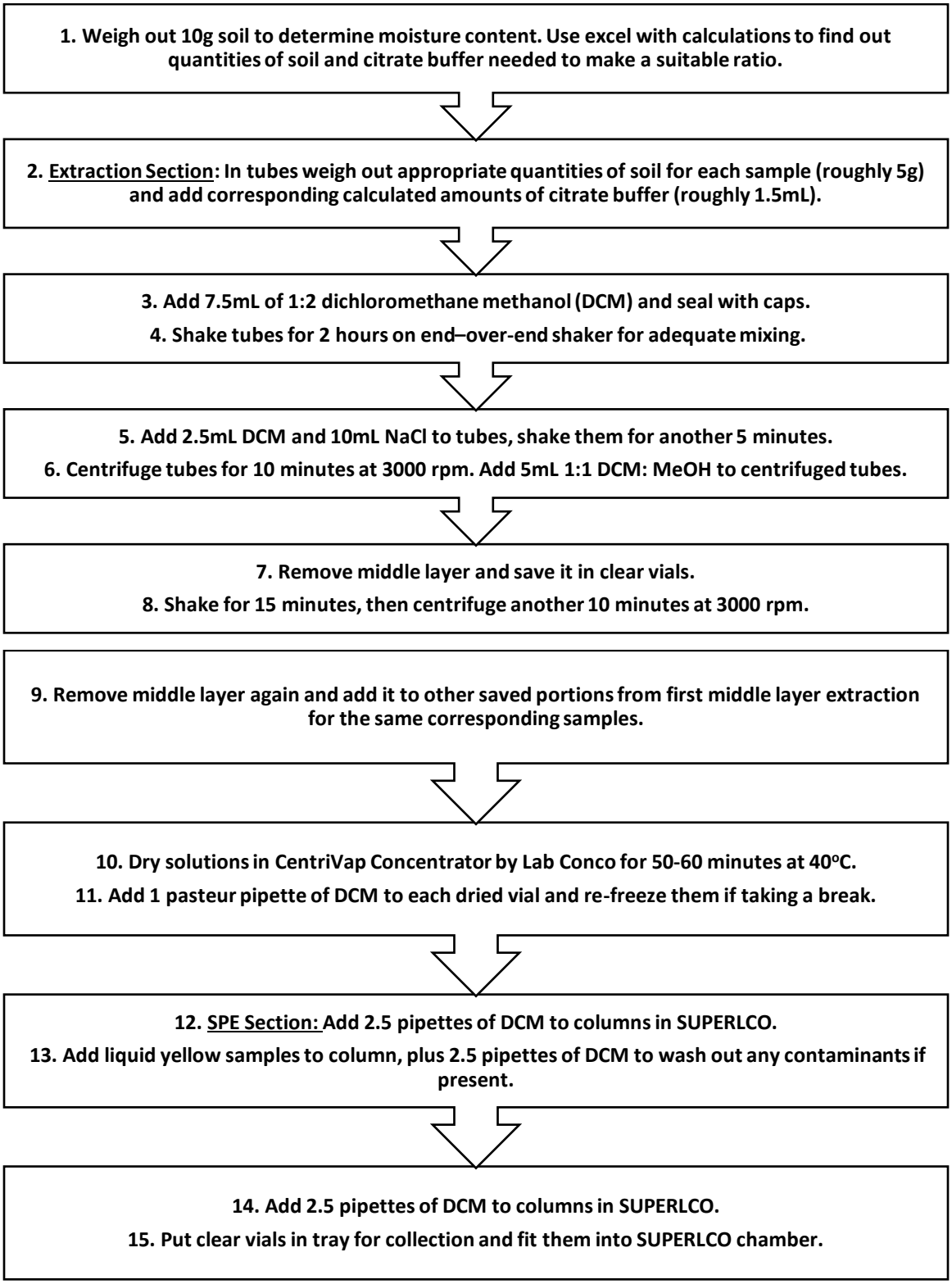
Note: N.P. =Not Provided. E= Excessive, H=High, M= Moderate, +=High, -- =Low

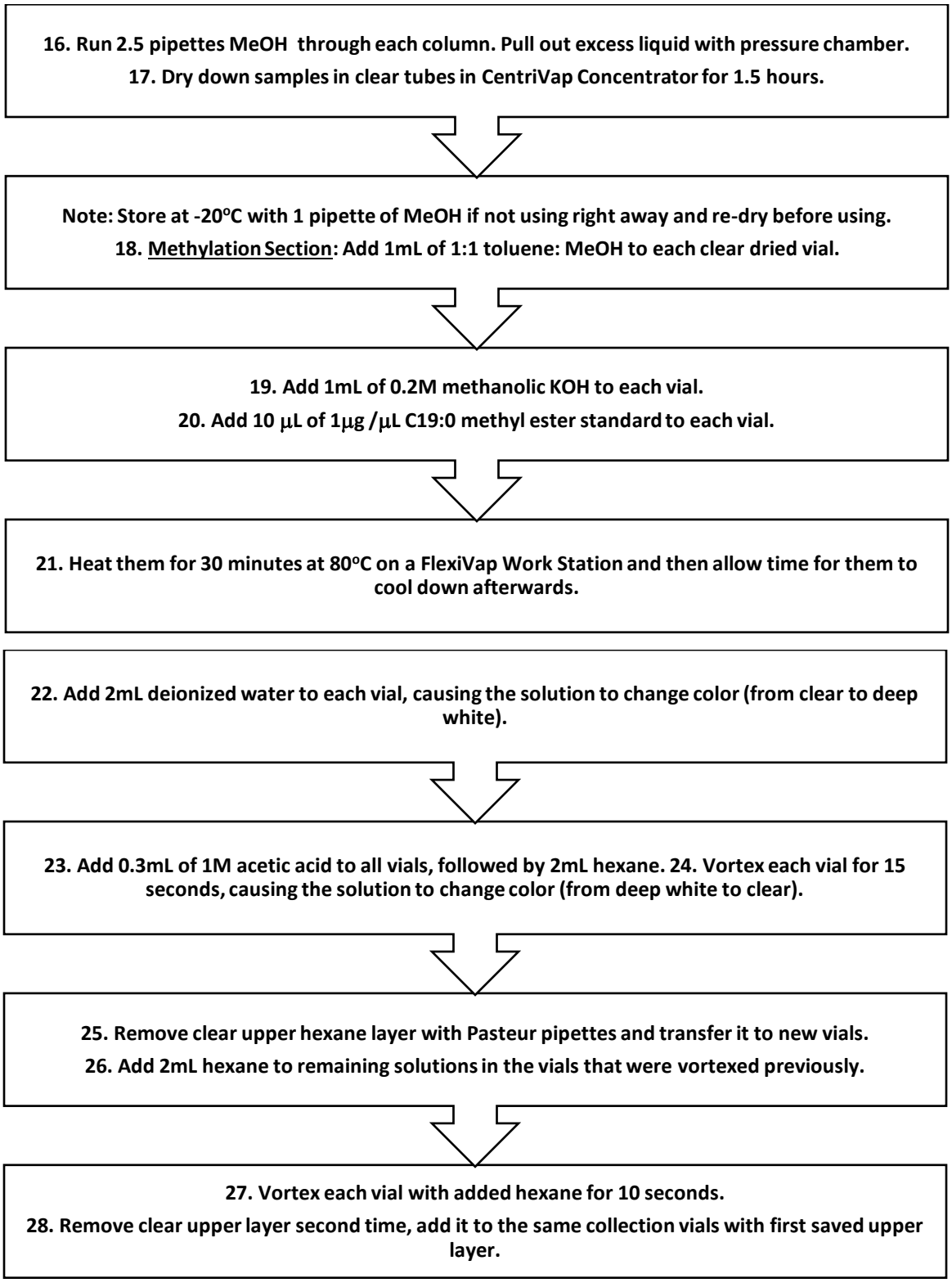
Appendix 17: Soil nutrient content from Experiment 2 (Brookside) in 2017 and 2018 through NS Harlow Laboratory

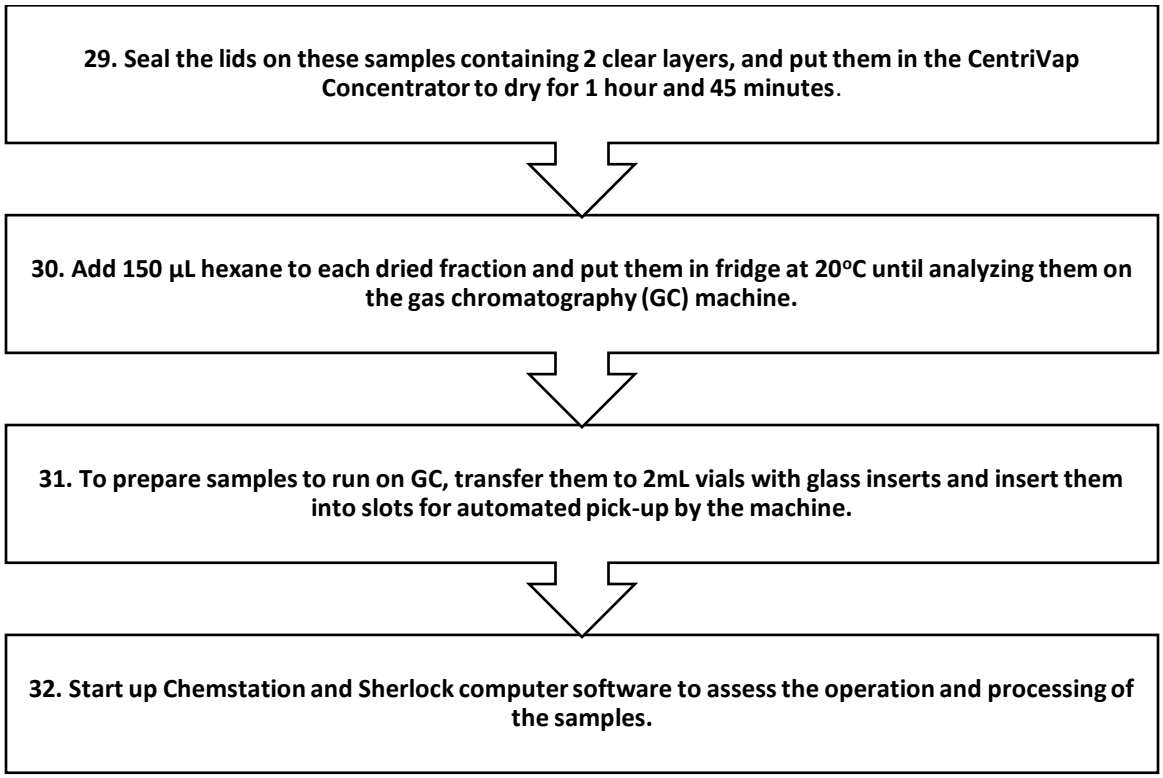
Brookside	2017			2018		
	Block 1	Block 2	Block 3	Block 1	Block 2	Block 3
pH	5.67	5.82	5.71	5.79	5.88	5.71
Organic Matter (%OM)	3.8	3.3	2.7	2.8	2.8	3.7
P2O5 (kg/ha)	287	265	499	535 (E)	262 (H-)	273 (H)
K20 (kg/ha)	253	160	126	134 (M-)	168 (M)	188 (M)
Calcium (kg/ha)	1601	1979	1547	1801 (L+)	2187 (M-)	1773 (L+)
Magnesium (kg/ha)	226	513	266	305 (M+)	588 (H-)	239 (M)
Sodium (kg/ha)	<16	<16	<16	<16	<16	<16
Sulfur (kg/ha)	25	23	23	25	21	28
Aluminum (ppm)	1574	1245	1286	1396	1223	1618
Boron (ppm)	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
Copper (ppm)	0.73	0.56	0.56	2.03	1.75	1.23
Iron (ppm)	168	257	189	168	255	145
Manganese (ppm)	31	28	34	29	35	26
Zinc (ppm)	0.76	0.71	1.01	1.43	0.88	0.88

E= Excessive, H=High, M= Moderate, +=High, -- =Low

PLFA flowchart


1. Weigh out 10g soil to determine moisture content. Use excel with calculations to find out quantities of soil and citrate buffer needed to make a suitable ratio.
2. Extraction Section: In tubes weigh out appropriate quantities of soil for each sample (roughly 5g) and add corresponding calculated amounts of citrate buffer (roughly 1.5mL).
3. Add 7.5mL of 1:2 dichloromethane methanol (DCM) and seal with caps.
4. Shake tubes for 2 hours on end–over-end shaker for adequate mixing.
5. Add 2.5mL DCM and 10mL NaCl to tubes, shake them for another 5 minutes.
6. Centrifuge tubes for 10 minutes at 3000 rpm. Add 5mL 1:1 DCM: MeOH to centrifuged tubes.
7. Remove middle layer and save it in clear vials.
8. Shake for 15 minutes, then centrifuge another 10 minutes at 3000 rpm.
9. Remove middle layer again and add it to other saved portions from first middle layer extraction for the same corresponding samples.
10. Dry solutions in CentriVap Concentrator by Lab Conco for 50-60 minutes at 40°C.
11. Add 1 pasteur pipette of DCM to each dried vial and re-freeze them if taking a break.
12. SPE Section: Add 2.5 pipettes of DCM to columns in SUPERLCO.
13. Add liquid yellow samples to column, plus 2.5 pipettes of DCM to wash out any contaminants if present.
14. Add 2.5 pipettes of DCM to columns in SUPERLCO.
15. Put clear vials in tray for collection and fit them into SUPERLCO chamber.


16. Run 2.5 pipettes MeOH through each column. Pull out excess liquid with pressure chamber.
17. Dry down samples in clear tubes in CentriVap Concentrator for 1.5 hours.
Note: Store at -20°C with 1 pipette of MeOH if not using right away and re-dry before using.
18. Methylation Section: Add 1mL of 1:1 toluene: MeOH to each clear dried vial.
19. Add 1mL of 0.2M methanolic KOH to each vial.
20. Add 10 μL of 1μg /μL C19:0 methyl ester standard to each vial.
21. Heat them for 30 minutes at 80°C on a FlexiVap Work Station and then allow time for them to cool down afterwards.
22. Add 2mL deionized water to each vial, causing the solution to change color (from clear to deep white).
23. Add 0.3mL of 1M acetic acid to all vials, followed by 2mL hexane. 24. Vortex each vial for 15 seconds, causing the solution to change color (from deep white to clear).
25. Remove clear upper hexane layer with Pasteur pipettes and transfer it to new vials.
26. Add 2mL hexane to remaining solutions in the vials that were vortexed previously.
27. Vortex each vial with added hexane for 10 seconds.
28. Remove clear upper layer second time, add it to the same collection vials with first saved upper layer.



Appendix 18: Flow chart for PLFA procedure step-by-step process

Agriculture and Agri-Food Canada Analytical Chemistry Lab SOP for Extraction, Purification, and Analyzing PLFA from Soil

Revision: #2
Revision Date: November 28, 2017

Date last modified and by: Christian Gallant Date created: August 10, 2015

Samples

1. **Scope of application**

- To extract, purify, and analyze phospholipid fatty acids from soil samples

2. **Summary of method**
3. **Interferences**
4. **Apparatus and Materials**

4.1. Extraction

- Soil sample
- Hot plate with magnetic stirrer (CORNING Hot Plate Stirrer PC-351)
- Stainless Steele Spatulas or Spoons
- glass reagent bottles (250mL-1L)
- Glass Beakers of all sizes
- 50mL Teflon centrifuge tubes with Teflon lined lids
- 13x100MM glass culture vials with Teflon lined lids to hold samples
- Repeating Glass pipettes (5mL and 10mL are helpful)
- Gyratory Shaker
- Fisher Brand $5^{3/4}$" disposable Pasteur pipettes and pipette bulbs
- Sorvall Legend T Centrifuge
- Flexivap work station by Glas-Col
- Nitrogen Gas (N_2)

4.2. SPE

- Glass beakers of all sizes
- SUPELCO Visiprep DL
- Strata Si-1 silica SPE cartridges (55µm)
- Disposable Flow Control Valve Liners for the SUPELCO Visiprep DL
- Fisher Brand $5^{3/4}$" Disposable Pasteur Pipettes and pipette bulbs
- 13x100MM glass culture vials with Teflon lined lids to collect samples
- Nitrogen Gas (N_2)
- Flexivap work station by Glas-Col

4.3. Methylation

- Glass beakers of all sizes
- 13x100MM glass culture vials with Teflon lined lids
- Repeating Glass Pipettes (1mL, 2mL are useful)
- Pipettors with disposable tips (100µL, 1mL, and 5mL are useful)
- Nitrogen Gas (N_2)

- Flexivap work station by Glas-Col
- Agilent 2ml screw top write-on
- Agilent vial insert, 100µl glass with polymer feet
- Agilent screw type caps blue in color

5. Reagents- When working with reagents pour useful amounts into clean beakers or bottles to avoid contamination! **Note: smaller amounts of these reagents can be made, just take care to ensure that the correct concentrations are being made.

5.1. Extraction

- **Citrate Buffer**

Solution A- 3.152g citric acid (A-104) made up to 100mL with deionized water in a 100mL volumetric flask

Solution B- 4.412g trisodium citrate (S-279) made up to 100mL with deionized water in a 100mL volumetric flask

-Using a Thermo Scientific Orion 5 Star pH meter; pour solution A into a clean beaker and place a stirbar in the solution, place the beaker containing solution A on a stir plate and gradually add solution B to solution A until the mixed solution in pH 4.0. Pour the solution into a clean glass bottle and store in fridge at 4°C.

- **Saturated NaCl Solution**

Add 250g of NaCl (S-7653) into 750mL deionized water in a 2L flask with a stirbar, stir over high heat until all crystals are dissolved, allow solution to cool to room temperature before use. If there are crystals still forming in the bottom of the flask, add more water and stir until dissolved again. Solution should be stored in a glass bottle with a Teflon lined screw top lid at room temperature.

- **1:1 DCM: Methanol (v/vol) for 1L**

Mix 500mL of Dichloromethane (DCM) with 500ML of Methanol (MeOH). Store in glass bottle with Teflon lined screw top lid at room temperature. (More or less of this solution can be made for the amount of samples being processed. Ensure that the amount of DCM and MeOH are equal.)

- **1:2 DCM: Methanol (v/vol) for 975mL**

Mix 325mL of DCM with 650mL of MeOH. Store in a glass bottle with Teflon lined screw top lid at room temperature. (More or less of this solution can be made for the amount of samples being processed. Ensure that the amount of MeOH is 2times the amount of DCM.)

- **DCM**

Pour a useful amount of DCM into a clean beaker

5.2. SPE

- Dichloromethane (DCM)
- Acetone
- Methanol (MeOH)

(Pour useful amounts of solvents into beakers for use with SPE)

5.3. Methylation

- **1:1(v/v) MeOH: Toluene for 500mL**

250mL MeOH in 250mL Toluene. Store in glass bottle with Teflon lined screw top lid at room temperature.

- **0.2M Methanolic KOH (prepared fresh!!)**

Weigh 1.12g of KOH and add to 100mL MeOH in a beaker with a stir bar. Use a magnetic stirrer and set to low speed, until KOH is dissolved.

- **1M Acetic Acid (Always add Acid to Water!!) for 1L**

58mL of Glacial Acetic Acid (A-38212) into 800mL deionized water. Top up solution to 1L with deionized water. Store in a glass bottle with a Teflon lined screw cap lid. (Should be kept under the fume hood with the other acids.)

- **Deionized Water**

Pour a useful amount into a glass beaker

- **Hexane (H-303-4)**

Pour a useful amount into a glass beaker

- **1µg/µL C19:0 Methyl Ester Standard (Sigma cat. #N5377)** standard is kept in freezer number 28 on the first floor

weigh out 0.0030g of nonadecanoic methyl ester standard using a glass weigh boat and place in a 4mL amber vial, add exactly 3mL of hexane and vortex until dissolved store at 4°C

6. Procedure

6.1. Extraction-This step takes about 4.5 hours to complete for 18 samples (Each soil sample should be done X3)

- Soil should be sub-sampled to determine % moisture if not already done so. For moisture determination weigh approximately 10g of soil in a moisture tin of known weight, record the weight and place into a 105°C oven overnight. Record the weight of the dried sample and calculate % moisture of soil. $MC\% = \frac{W2-W3}{W3-W1} \times 100$ **. Where W1 is the weight of the tin (g), W2 is the weight of the moist soil + tin, and W3 is the weight of the dry soil + tin. (If soil moisture is 15% and you are using 4g of oven dried equivalent moist soil then 0.15x4=0.6 so you would need 4.6g of moist soil).**

- Using Lab tape, label 50mL Teflon tubes with the sample number
- Rinse the 50mL Teflon tubes with DCM before adding soil to the tubes
- Weigh out correct amount of moist soil as per soil moisture calculations into 50mL Teflon tubes
- Add the required amount of citrate buffer (can use plastic pipette tips) for the amounts of soil you have: 2mL for 2-4g dry weight soil

(To maintain the proper extract ratio, be sure the amount of soil moisture is taken into account when adding citrate buffer. As in the above example 0.6g of moisture is present in the sample therefore the amount of citrate buffer to add is 2mL-0.6mL=1.4mL).

- Add 7.5mL of 1:2 DCM:MeOH to each tube, put covers on Teflon tubes

- Place tubes in glass bottles and place on shaker for 2 hours at 200 RPM
- After shaking is complete add 2.5mL of DCM to each tube
- Add 10mL of the saturated NaCl solution to each tube
- Place on shaker for 5 minutes
- Centrifuge for 10 minutes at 3000rpm (weigh each of the centrifuge buckets with your samples in them, weights should be all roughly the same to ensure that the centrifuge is balanced correctly)
- After centrifugation carefully remove the middle layer (the layer between the dirt and the aqueous layer) using a Pasteur pipette and place in a new 13x100MM glass culture vial
- Add 5mL of 1:1 DCM:MeOH to the Teflon tube to wash the aqueous phase, replace covers and vortex briefly
- Place on shaker for 15 minutes
- After shaking is complete centrifuge for 10 minutes at 3000rpm (weigh each of the centrifuge buckets with your samples in them, weights should be all roughly the same to ensure that the centrifuge is balanced correctly)
- After centrifugation is complete carefully remove the middle layer and add to the 13x100MM glass culture vial containing the previously removed layer
- Dry the solution in the 13x100MM glass culture vial under N_2 using the Flexivap work station by Glas-Col. Dissolve the dried fraction in 1 Pasteur pipette full of DCM and store at -20°C if SPE step is not to occur immediately

6.2. SPE- This step takes about 1.5 hours for 18 samples

- Place the Strata Si-1 SPE cartridges on the SUPELCO Visiprep DL
- Add 2.5 Pasteur pipettes of DCM to the columns to condition them
- Load each sample into the appropriate columns
- Add 2.5 Pasteur pipettes of DCM to each column. This acts as a wash for the column
- Add 5 Pasteur pipettes of Acetone to the columns, allow the acetone to run completely through each column. The columns will look dry
- Place new 13x100MM glass culture vials in the SUPELCO Visiprep DL to collect the MeOH
- Add 2.5 pipettes of MeOH to the columns
- When all the MeOH has gone through the columns, close chamber vent to pull any remaining liquid out of the columns.
- Remove the glass vials and place in the Flexivap work station by Glas-Col and dry under N_2
- If methylation is to occur the next day the fractions can be left dry, if the samples will not be worked with for multiple days dissolve the fraction in 1 pipette of MeOH and store at -20°C if methylation step is not to occur immediately

6.3. Methylation- This step takes about 2 hours to complete for 18 samples

- (Prepare 0.2M Methanolic KOH and turn on heating block (80°C) before starting)
- Take the fraction from the end of the SPE step to dryness under N_2 Flexivap work station by Glas-Col if not already done
- Dissolve the dried fraction in 1mL of 1:1 MeOH:Toluene **(<u>Must use a glass pipette for this step!</u>)**

- Add 1mL of 0.2M Methanolic KOH to each tube (pipettor with disposable tips may be used for this step)
- Add 10µL of a 1µg/µL C19:0 methyl Ester Standard to each vial (pipettor with disposable tips may be used for this step)
- Place covers on the vials and incubate at 80°C for 30 minutes using the Flexivap work station by Glas-Col heat block
- Remove the vials after 30 minutes and allow to cool to room temperature
- Add 2mL of deionized water to each vial (**Note: after adding the deionized water the solution will turn white)(pipettor with disposable tips may be used for this step)
- Add 0.3mL of 1M acetic acid to each vial (pipettor with disposable tips may be used for this step)
- Add 2mL of hexane to each vial (pipettor with disposable tips may be used for this step)
- Vortex for roughly 15 seconds (you will see the sample go from a very white color to almost clear)
- Using a Pasteur pipette remove the upper (hexane) layer and place in a new 13x100MM glass culture vial
- Add 2mL of hexane to the aqueous solution in each vial to wash (pipettor with disposable tips may be used for this step)
- Vortex for roughly 10 seconds
- Using a Pasteur pipette remove the upper (hexane) layer and place with the previously removed layer
- Take the fractions to dryness under N_2 using the Flexivap work station by Glas-Col
- Dissolve the dried fraction in 150µL of hexane and using a Pasteur pipette transfer to a 2 mL GC vial containing a glass insert
- Samples that are not going to be run immediately on the GC should be stored at 20°C until needed

7. **Quality Control**
8. **Method Performance**
9. **References and Tables**

Seasonal and long-term resource-related variations in soil microbial communities in wheat-based rotation of the Canadian prairie
Hamel, C., Hanson, K., Selles, F., Cruz, A.F., Lemke, R., McConkey, B., and Zentner, R. 2006

****Note: If you have never used this software before you will need to open the Chemstation (online) software first. Once opened it can be closed and then the Sherlock software can be used.**

Running Samples on GC

- MIDI GC PLFA Calibration Standard:
  - (Follow instructions on procedure provided with Cal. Std. or see below)
  - Open the ampoule (must be at room temp!) by using the opener as indicated. Add 2mL ± 0.2mL of hexane (HPLC grade, ACS certified) to an empty GC vial. Remove some hexane with a Pasteur pipette and flush interior of ampoule neck several times, and then transfer this to ampoule body. Place rest of hexane in ampoule body and flush/rinse repeatedly to mix well. Transfer 100µL of this calibration standard solution to GC vials (approx. 20) containing inserts. Cap vials and store in freezer (-20ºC)
  - ****Note: Every GC run needs a vial of the calibration mix**

- Open Chemstation (online) software first to bring GC out of shutdown mode. Once GC is 'Ready' you may exit the Chemstation program
- Open **Sherlock Sample Processor**
- If the table has samples logged that have already been run select **Table** and **Clear if Done**. This will clear all samples except the calibration standard
- Click **Add Samples**. The sample name should include the year, trial name, sampling period, plot number, and the soil weight in the form of (G= ex: (G=4.8
- When you are done adding samples click on **Done Adding**
- Click on **Lock Table** this will ensure that no samples get changed or added accidently
- **(ensure that the shutdown instrument at end of batch option is checked in the Sherlock sample processor this will allow you to run the GC overnight or into the weekend as the GC will automatically shut down after the last sample is run)**
- Click on start batch. This will open the **ChemStation GC software**, a dialog box will appear press 'OK' (no username or password required) and the samples will begin to run automatically when the GC has reached all parameters for the PLFAD1 method
- When GC run is finished proceed to transforming MIDI GC Data

Transforming MIDI GC Data

- Open **Transform Samps (**<u>Note</u>: If this is not on the desktop open the C drive, Sherlock, EXE and the transform samps file will be located there. A shortcut can be placed on the desktop)**
- Open volume **DATA7**
- Select your data file (data file name can be found in C-drive/Sherlock/Results, and look for the most recent date)
- Select **Set Transform Parameters**
- Select **PLFAD1** for **GC Method** and **Category Method**
- Select **PLFAMole3.txt** for the **Category File**
- Change **File Suffix** to **WGT** if not already done
- Click ok
- Click **Generate Weighted Samples** to create a weight file of the data
- Open volume **DATA7** once again
- Select the weight file you just created (will have the same name as the original file only with **.WGT** at the end)
- Select **Set Transform Parameters**
- Click on **Category Transform**
- Select **BACTYPE2** for the **Category Method**
- Select **PLFAD1Soil2.txt** for the **Category File**
- Change the **File Suffix** to **BAC** and click ok
- Click on **Generate Categorized Samples** to create the **.BAC** file
- Still using the weight file select **Set Transform Parameters** again
- Change the **Category Method** to **FATYPE2**
- Select **PLFAD1FA2.txt** for the **Category File**
- Change the **File Suffix** to **FAT** and click ok
- Click on **Generate Categorized Samples** to create the **.FAT** file

- Still using the weight file select **Set Transform Parameters** again
- Change the **Category Method** to **RATIO3**
- Select **RATIO3.txt** for the **category file**
- Change the **File Suffix** to **RAT** and click ok
- Click on **Generate Categorized Samples** to create the **.RAT** file
- Close **Transform Samps**

Creating a Database of Transformed Data

- Open **Sherlock Command Center**
- Click on **Utilities** on the left side
- Click on **Data Export**
- Under the **Selection Criteria** tab select **BACTYPE2** for the **Calculation Method**
- Choose the **Files and Samples** tab and open **DATA7** choose the **.BAC** file you created in Transform samps
- Choose the **Methods** tab and ensure that **BACTYPE2** is clicked under **Select Methods**
- Click **Update Profile List** in the bottom right corner
- Choose **Database Export** from the top
- Name the file with the following standardized format: Year_TrialName_SamplingPeriod/date_FileSuffix (e.g. 2016_ECODA_Mid-Season_BAC) and press ok
- Access database containing the **BACTYPE2** data will open
- Choose to save the database as and save as the same file name under the correct folder for that trial
- Follow the same steps to create a database for the **FATYPE2** file and **RATIO3** file
- Copy data from database files and place in Excel spreadsheet for that year and under the correct sheet tab for that trial: 'Year – PLFA Data, MasterSheet'.

Appendix 19: Procedure for phospholipid fatty acid analysis and computer software programming