SOYBEAN PRODUCTION AND SOIL HEALTH RESPONSE TO CROP ROTATION SEQUENCES IN MANITOBA

by

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ABSTRACT

Weerasekara Arachchilage, Chathuri S.W. M.Sc., The University of Manitoba, August 2022. Soybean Production and Soil Health Response to Crop Rotation Sequences in Manitoba. Major Professors; Dr. Yvonne Lawley and Dr. Ivan Oresnik.

Diversity of crop rotations in Manitoba increased after the introduction of soybean [*Glycine max* (L.) Merr.] into the eastern Canadian Prairies. However, the response of including soybean in short rotations with corn or canola and long rotations of wheat-canola-corn-soybean on soybean performance and soil health is less understood in Manitoba compared to Ontario or the mid-west United States. This study was conducted to evaluate the effect of growing soybean continuously vs in rotation with canola, corn, and wheat on soybean performance, biological nitrogen fixation, and biological soil health in the soils of Manitoba.

The experiment was established in 2014 at two locations (Carman and Kelburn) in Manitoba. Crop sequence treatments were continuous soybean (S-S-S-S), canola-soybean-canola-soybean (Ca-S-Ca-S), corn-soybean-corn-soybean (C-S-C-S), and wheat-canola-corn-soybean (W-Ca-C-S). All four treatments had a common soybean test crop in the 4th year (2017) of the study. Soybean production and biological N fixation (BNF) parameters were observed in the soybean test crop in 2017. Soil health analysis were conducted for the surface soils (0-8 cm depth) collected at multiple sampling stages in the 4th and 6th (2019) years of the experiment. After four years, the preceding crop in the sequence had no effect on soybean seed yield. Crop sequence treatments were significant for soybean seed quality, dry matter yield, above ground N uptake, and biological nitrogen fixation. However, the continuous soybean sequence was not consistently different form sequences where soybean was grown in rotation with canola, corn, and wheat. Penalties of continuous cropping when first introducing soybean into the rotations were minimal. Future research is needed to identify long-term impacts of continuous soybean in the soils of Manitoba.

There were inconsistent trends in the activities of β-glucosidase, β-glucosaminidase, and acid phosphatase among sampling stages, crop sequence treatments, locations, and years. However, enzyme activity was frequently greater in the C-S-C-S sequence compared to the S-S-S-S sequence across sampling stages in both years. Active C was also greater in the C-S-C-S in

relative to the S-S-S-S sequence. It is important to include a high residue crop such as corn when growing a low residue crop like soybean in a crop rotation to maintain the soil health. Bacterial population was not different among crop sequences according to principal coordinate analysis. Active C was better at finding differences among sequence treatments. Greater levels of soil enzymes were observed at the beginning and end of the growing season. Bacterial families formed separate clusters at before planting (BP) and full maturity (R8) stages that were distinct from mid-growing season samplings. Therefore, conducting soil health analysis at either of those two sampling stages would be helpful to identify the differences in crop management practices such as crop rotations in the soils of Manitoba.

FOREWORD

References are in the style of the Canadian Journal of Plant Science.

1.0 INTRODUCTION

The acreage of soybean [*Glycine max* (L.) Merr.] production has recently increased in Manitoba. Soybean seeded area in Manitoba increased from 20,200 hectares to 532,900 hectares from 2001–2021 (Soy Canada 2021). Soybeans are adapted to a wide range of soils in Manitoba and the popularity of this crop is increasing due to its low fertilizer requirement, the availability of herbicide resistance cultivars, and its tolerance to excess soil moisture conditions compared to other crops. Crop rotations in Manitoba have shifted with the rapid expansion in soybean acres. Including soybean increases the diversity of crop rotations in the Canadian prairies, which were mainly dominated by canola and wheat (Statistics Canada 2021). Since soybean is a new crop to Manitoba, it is important to identify the opportunities on how to optimize soybean in rotation with other crops, as well as the potential negative impacts of growing soybean frequently in rotations.

Studies have shown that soybean crops produce greater yields when they are grown in rotations compared to continuous soybean (Kelley et al. 2003; Wilhelm and Wortmann 2004; Munkholm et al. 2013; Sindelar et al. 2015; Farmaha et al. 2016). Kelley et al. (2003) reported that soybean yield increased by 16% in rotations with winter wheat or grain sorghum compared to continuous soybean in the eastern Great Plains of the United States. After a 19-year study in Nebraska, soybean yield was greater by 6–36% in soybean-corn rotations when compared to continuous soybean (Sindelar et al. 2015). Most of the studies conducted in the United States corn-belt region have mainly focused on corn-soybean rotations and have well documented the yield benefits. However, little is known about the implications of growing soybean in rotation with canola or long rotations such as wheat-canola-corn-soybean on soybean performance.

Soybean establishes beneficial relationships with soil bacteria to achieve the plant nutrient requirements. The crop develops symbiotic relationship with bradyrhizobia for biological N fixation (BNF) to acquire majority of the plant's N need (Gentry et al. 2001). However, previous crops in the rotation impact soil nutrients levels and the microbial communities that influence the development of these symbiotic associations (Liebig et al. 2002; Zhu et al. 2014; Hall et al. 2019; Kim et al. 2021).

Crop rotations have impacts on soil health as well. Quantitative and qualitative assessment of soil physical, chemical, and biological indicators are used to identify the changes in soil health under different soil environments and land management systems (Dick and Burns 2011; Poeplau and Don 2013; Bowles et al. 2014; Acosta-Martinez et al. 2018; Jagadamma et al. 2019; Neupane et al. 2021). Biological soil health indictors provide information about the living component of the soil. Soil enzymes and soil microbial communities are sensitive to fluctuations in environmental conditions and agricultural practices. They can therefore be used as indicators for changes in soil properties as a result of management practices such as crop rotation (Dick et al. 1996; Bandick and Dick 1999; Bone et al. 2010; Postma-Blaauw et al. 2010; Cong et al. 2015). Labile organic carbon is another important biological soil health indicator that responds rapidly to the changes of soil management practices (Tirol-Padre and Ladha 2004; Skjemstad et al. 2006; Van Es et al. 2017; Bongiorno et al. 2019). In this project, we selected these three indicators to evaluate the impact of four soybean crop rotation treatments on biological soil health in Manitoba.

A limited number of studies have been conducted in Manitoba to assess the effects of growing soybean continuously vs in rotation with canola, corn, and wheat on soybean performance and soil health. A four-year rotation study was established in 2014 to evaluate the impact of frequency of soybeans in rotation on soybean production at three locations in central Manitoba. The four-year crop sequence treatments were: 1) continuous soybean (S-S-S-S), 2) canola-soybean-canola-soybean (Ca-S-Ca-S), 3) corn-soybean-corn-soybean (C-S-C-S), and 4) wheat-canola-corn-soybean (W-Ca-C-S). The project started its second rotation cycle at two out of three locations in 2018 to evaluate the soybean production and, focus on the impact of soybean in rotation on soil health. Hence, the overall objectives of this project were (1) to evaluate the agronomic impacts of growing soybeans continuously vs every two years or every four years in Manitoban crop rotations and (2) to evaluate the impacts of growing soybeans continuously vs every two years or every four years in crop rotations on biological soil health indicators in the soils of Manitoba.

2.0 LITERATURE REVIEW

2.1 Soybean

Soybean [*Glycine max* (L.) Merr.] is among the largest export crops from Canada, with 6.3 million metric tons of seed yield produced in 2021 (Statistics Canada 2021). The acreage of growing soybean in Manitoba expanded rapidly in the early 2000's. The province reached to its greatest soybean seeded area in 2017 with 929,700 hectares by producing 2.25 million metric tons of seed yield, which was 29% of the national production (Statistics Canada 2017). Canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.) are the major crops grown in the Manitoban crop rotations. Hence, inclusion of soybean has increased the diversity of crop rotations in the province. Although researchers have reported the rotational benefits of soybean in the United States and other soybean growing regions (Brazil, Argentina, China), limited studies have been conducted in the Canadian prairies to evaluate the effects of growing soybean in rotation with canola, corn, and wheat (Sanders 2017; Mohr 2018).

Soybean establishes beneficial relationships with bradyrhizobia for biological N fixation (BNF). Rhizobia is a group of soil bacteria that fix atmospheric N_2 into plant available forms of NH_4^+ by forming symbiotic nitrogen fixing associations with the roots of legumes. *Bradyrhizobium japonicum* is a rhizobia species that facilitates BNF in soybean. When bradyrhizobia attach to the root hair, the plant releases flavonoids that induce the expression of nod gens in the bacteria. It will stimulate the production of enzymes called nod factors that initiate curling of root hairs. The bradyrhizobia are then curled up with the root hair and penetrate the root hair cell with an infection thread. The infection thread grows into the cortex of the root and release bacterial cells into the root cells. The bacterial cells then become bacteroides. Then, root nodules develop from infected cortical cells and bacteroides begin to fix N_2 (Wagner 2011; Peleg-Grossman et al. 2009). Soybean seeds are inoculated with *B. japonicum* strains (e.g. *Bradyrhizobium* USDA 110) before planting to facilitate BNF (Ndakidemi et al. 2006; Hungria et al. 2013). On an average, 50 – 60% of the N demand of soybean is fulfilled by BNF (Salvagiotti et al. 2008).

2.2 Soybean in Crop Rotations

Crop rotation is an agronomic practice where a series of different types of crops are grown in a specified order on the same area in sequential seasons. There are multiple benefits associated with this crop management practice, such as improved nutrient cycling in the soil by incorporating legumes, increased soil moisture retention, improved soil structure, control of insect pests and diseases by breaking their life cycles, help to control weeds, increased soil organic matter content, and increase crop yields (Entz et al. 1995; Tilman et al. 2002, Liu et al. 2010; Davis et al. 2012; Dias et al. 2015; McDaniel et al. 2014). In the United States, studies have shown that soybean provide residual N benefits for the following cash crops in the crop rotations, thereby lowering the cost of fertilizers (Ennin and Clegg 2001; Gentry et al. 2001).

Numerous studies have been conducted to evaluate the effect of including soybean in crop rotations on soybean and other crops yields (Kelley et al. 2003; Wilhelm and Wortmann 2004; Munkholm et al. 2013; Sindelar et al. 2015; Farmaha et al. 2016). Soybean produced greater seed yield (2.7 Mg ha^{-1} on average) when grown in rotations in comparison to continuous soybean productoion (2.4 Mg ha^{-1}) in Nebraska (Peterson and Varvel 1989). In the mid-west United States, soybean and corn yields were reported as 2.57 Mg ha^{-1} and 7.10 Mg ha^{-1}, respectively with rotation compared to the continuous soybean (2.35 Mg ha^{-1}) and continuous corn (5.83 Mg ha^{-1}) practices (Wilhelm and Wortmann 2004). Within the United States Corn Belt region, corn yield was 2-5% greater in soybean-corn rotations relative to continuous corn (Farmaha et al. 2016). The yield advantage of corn grown after soybean, compared to continuous corn has been explained as an increase in soil N levels through symbiotic N_2 fixation by previous soybean, and less N immobilization due to lower C:N ratio of soybean residues compared to the corn residues (Stanger and Lauer 2008; Gentry et al. 2013; Sindelar et al. 2015; Farmaha et al. 2016).

In Manitoba, Manitoba Agricultural Services Corporation (MASC) has summarized the influence of previous crop on subsequent crop yields based on the self-reported crop yields of farmers participating in their crop insurance program when they are grown in rotation (MASC 2022). Based on the percentage of average yield response of crops sown on large fields (>120 acres) from 2011-2020, there was no yield penalty when soybean was grown after canola (100% of average soybean yield). Soybean yield response was above average (104% of average soybean

yield) when the previous crop was spring wheat. Nevertheless, there was a 98% yield penalty when soybean was sown on to corn stubbles. Furthermore, the yield penalty of soybean grown after soybean was 95%. However, the yield penalty of growing two years of the same crop was lower for soybean than for many of the other common crops grown in Manitoba.

Although several studies have shown that soybean yield and quality are influenced by crop rotation (Kelley et al. 2003; Smith et al. 2008; Sindelar et al. 2015; Farmaha et al. 2016), limited literature is available on the influence of crop rotations that include soybean on soil health in the Canadian prairies (McConkey et al. 2003; Bell et al. 2012; Sanders 2017).

2.3 Soil Health

Soil health is defined as *"the capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health"* (Doran 2002, Doran and Zeiss 2000). Soil physical, chemical, and biological properties are the three major factors that affect soil health. Maintaining the balance among these soil health indicators is important in sustainable land management (Larson and Pierce 1994).

Quantitative and qualitative assessment of soil physical, chemical, and biological indicators are used to identify changes in soil health under different soil environments and land management systems. Physical soil health indicators include bulk density, soil structure and macro-pores, aggregate stability, infiltration, soil depth, and water holding capacity (Doran et al 1996; Schoenholtz et al. 2000; Friedman et al. 2001; Bulluck et al. 2002; Andrews et al. 2004; Bünemann et al. 2018; Obrycki et al. 2018). Chemical soil health indicators include soil pH, soil nitrate N, electrical conductivity, cation exchange capacity, reactive C, and extractable P and K (Doran et al. 1996; Schoenholtz et al. 2000; Friedman et al. 2001; Bulluck et al. 2002; Bünemann et al. 2018; Obrycki et al. 2018). Biological soil health indicators include microbial biomass C and N, potentially mineralizable N, particulate organic matter, total organic C, active C, soil enzymes, microbial diversity, and soil respiration (Doran and Parkin 1996; Doran and Zeiss 2000; Friedman et al. 2001; Bulluck et al. 2002; Bünemann et al. 2018; Obrycki et al. 2018).

There is no universal way to determine soil health. Hence, commercial soil testing labs provide different soil health assessment packages to evaluate various soil health indicators (Zuber and Kladivko 2018). The Cornell Comprehensive Assessment of Soil Health protocol, Ward lab soil health test package, AGVISE soil tests, USDA-NRCS Soil Management Assessment Framework, and Missouri soil health tests are some of the commercially available soil health testing packages.

Although most of the commercial soil testing packages are widely used to determine soil health in the agricultural fields and research trials in the United States, there aren't any recommended soil health tests for Canadian prairie agricultural soils. We are in a time that scientists are actively researching and adopting methods/ tests that are suited for prairie soil health assessment. This study evaluated soil enzymes activities, microbial community dynamics, and soil active C to assess the impact of including soybean in Manitoban crop rotations on soil health. Those indicators were selected as they are highly responsive and sensitive to the changes that take place within agricultural systems, however, have not been tested in the soybean crop rotation systems in Manitoba (Ekenler and Tabatabai 2002; Dodor and Tabatabai 2005; Li et al. 2010; Jagadamma et al. 2019; Yuan et al. 2021). The remaining of this literature review will focus on those three biological soil health indicators.

2.4 Soil Enzymes

Enzymes are built from proteins that act upon specific substrates to catalyze the biochemical reactions and convert substrate into different molecules (Kandeler 2014). Soil enzymes, produced by microorganisms, increase the rate of organic matter decomposition and release plant available nutrients. These enzymes are responsive to agricultural management practices such as crop rotation, tillage, and fertilization, and are considered as important soil health indicators. Their relationships to soil microbial activities including soil organic matter (SOM) dynamics and nutrient cycling are relatively easy to measure compared to many physical and chemical soil health indicators (Dick et al. 1996; Ndiaye et al. 2000; Acosta-Martinez et al. 2007; Dick and Burns 2011; Acosta-Martinez et al. 2018). Changes in soil enzyme concentrations are rapid compared to other physical or chemical indicators, and they can be used as early indicators of changes in soil health (Dick et al. 1996; Dick 1997). These enzymes hydrolyze different

substrates and catalyze the biochemical reactions that breakdown organic matter and release plant available nutrients (Bandick and Dick 1999; Makoi and Ndakidemi 2008; Stott et al. 2010; Acosta-Martinez et al. 2018).

Soil enzymes can be used as a measure of soil microbial activity (Dick et al. 1996; Dick, 1997; Dick and Burns 2011). The enzymes β-glucosidase, β-glucosaminidase, dehydrogenase, fluorescein diacetate (FDA) hydrolase, phosphomonoesterase, arylsulfatase, amylase, and urease are some of the enzymes that are used as soil health indicators of soil microbial activities (Dick et al. 1996; Bandick and Dick 1999; Acosta-Martinez and Tabatabai 2011; Deng and Popova 2011; Klose et al. 2011).

β-glucosidase is an important soil enzyme that plays a significant role in decomposition of SOM and plant residues (Makoi and Ndakidemi 2008; Stott et al. 2010). This enzyme catalyzes the hydrolysis of polysaccharide cellulose which is a major component of plant cell walls. The hydrolyzed products act as energy sources to the soil microbes by providing monosaccharides or simple sugars (Bandick and Dick 1999; Stott et al. 2010; Deng and Popova 2011). Hence, β-glucosidase is considered to be an index for C cycling in the soil (Acosta-Martinez et al. 2018).

β-glucosaminidase catalyzes the hydrolysis of chitin, one of the most abundant biopolymers found in the exoskeletons of crustaceans, insects, and the cell walls of fungi, and an important pool of organic C and N in the soil. This hydrolysis process is important in N cycling in the soil because chitin is converted to amino sugars, a major source of mineralizable N in the soil (Ekenler and Tabatabai 2002, 2004).

Phosphomonoesterase which includes both acid phosphatase and alkaline phosphatase are two of the most studied phosphatase enzymes and are considered to be indicators of P cycling in the soil (Acosta-Martinez and Tabatabai 2011; Acosta-Martinez et al. 2018). These enzymes hydrolyze different phosphomonoesters such as β-glycerophosphate, β-naphthyl phosphate, phenyl phosphate, and *p*-nitrophenyl phosphate that results in the release of plant available forms of P (Acosta-Martinez and Tabatabai 2011).

Since these enzymes catalyze the hydrolysis of different substrates in the soil, the results of these enzyme assays can be used to determine changes in soil properties which are affected by agronomic management practices. The actual concentration of soil enzymes is not measured in these assays. Instead, the activity of a specific enzyme-catalyzed reaction in one soil relative to another soil is measured in the laboratory using standard biochemical assays (Dick 2011).

2.4.1 Effect of Crop Rotations on Soil Enzymes

Soil enzyme activities were found to be greater under diverse cropping systems compared to continuous cropping (Dodor and Tabatabai 2003a, 2003b; Ekenler and Tabatabai 2002; Balota et al. 2004; Dodor and Tabatabai 2005). According to a rotation experiment conducted in southern Brazil, the activities of amylase, cellulase, arylsulfatase, acid phosphatase, and alkaline phosphatase were significantly greater in the soils under corn-wheat rotation compared to soybean-wheat and cotton-wheat rotations (Balota et al. 2004). They further explain that greater biomass production by corn which provides greater amounts of substrate for microbial growth and enzyme production may be the reason for greater soil enzyme activity for crop rotations that include corn. β-glucosidase and arylsulfatase activities were significantly greater in rice-rice-rye, rice-rice-milk vetch, and rice-rice-rape rotations than rice-rice-winter fallow rotation in a study conducted in China (Hai-Ming et al. 2014). They suggest that returning the residues of winter cover crops to the soil increases the amount of substrate for microbes, which improves both soil enzyme activities and microbial populations.

In a 17-year long-term rotation study conducted in Iowa, the highest β-glucosaminidase activity was observed under the corn-corn-oats-meadow rotation while continuous corn exhibited the lowest enzyme activity (Ekenler and Tabatabai 2002). The authors explain that greater β-glucosaminidase activity in the soils under diverse crop rotations may be due to the improved soil structure, stabilized microclimate, and greater root density compared to continuous cropping systems (Ekenler and Tabatabai 2002). At the same location, Dodor and Tabatabai (2003a, 2005) found that acid phosphatase and β-glucosidase activities were significantly greater in a corn – soybean – corn – soybean rotation compared to continuous soybean.

Differences in the quantity, quality, and distribution of crop residues in contrasting crop rotation treatments cause changes to the soil microbial activities thus, affecting the levels of soil enzymes

(Ekenler and Tabatabai 2002). Apart from differences in the amount of residues, the rate of decomposition of those materials also varies depending on the amounts of C, N, S, lignin, and other carbohydrates in the residues. This also influences the amount of enzymes secretions by soil microorganisms (Klose and Tabatabai 2000).

2.5 Soil Microbial Communities

Soil biota consist of many types of organisms including bacteria, fungi, algae, protozoa, nematodes, mites, insects, and earthworms. These organisms bring more diversity to soil than aboveground plant and animal communities (USDA-NRCS 2001). The majority of soil biota are made up of the smallest organisms, bacteria, and fungi. These organisms play a significant role in the functioning of soil because, they are a major part of organic matter decomposition and soil structure and aggregate formation (Barrios 2007; Dias et al. 2015; Usman et al. 2016; Bünemann et al. 2018). Soil organisms can also contribute to nutrient cycling (C, N, and P cycles), promote plant growth by producing plant growth regulators (e.g., production of plant hormones such as auxin, cytokinin, gibberellin, and ethylene), and provide protection to plants from pests and diseases (Riggs et al. 2001; Lendzemo et al. 2005; Heijden et al. 2006; Heijden et al. 2008; Raaijmakers et al. 2009; Montañez et al. 2012; Dias et al. 2015).

Microbial community analysis provides a good indication of soil health since these microorganisms are highly responsive to soil management practices. Different techniques can be used to characterize the diversity of microorganisms in the soil ecosystems (Hartmann et al. 2015). Molecular methods focusing on DNA and RNA gene sequencing are faster and more informative compared to the conventional methods such as phospholipid fatty acid (PLFA) profiles and fatty acid methyl ester (FAME) profiles (Bouchez et al. 2016; Hermans et al. 2016). In the recent studies, bacterial 16S rDNA sequencing and fungal nuclear ribosomal internal transcribed spacer (ITS) sequencing are used to analyze the relative abundance of bacterial and fungal communities in the soil (Goodrich et al. 2014; Cong et al. 2015; Smets et al. 2016; Schöler et al. 2017; Liu et al. 2018).

2.5.1 Effect of Crop Rotations on Microbial Communities

Microbial communities are sensitive to crop and soil management practices (Xuan et al. 2012; Chamberlain et al. 2020; Neupane et al. 2021; Yuan et al. 2021). Crop rotation has been found to

have a range of impacts on microbial communities. The abundance, richness, and diversity of soil microbial communities have been found to increase, decrease, or remain un-change when comparing crop rotation treatments (Venter et al. 2016). As an example, Zhu et al. (2014) observed that the bacterial diversity of soils from crop rotation treatments was greater compared to the soils from continuous soybean treatments. In Vietnam at a long-term experiment, bacterial diversity and richness were greater in the rice–corn–rice, rice–mung bean– corn, and rice–mung bean–rice rotations relative to continuous rice cultivation (Xuan et al. 2012). Chamberlain et al. (2020) found significant differences in the soil bacterial communities between continuous corn, continuous soybean, and corn-soybean rotation in Wisconsin.

Many studies also found no differences in response to crop rotation. For an instance, Li et al. (2010) reported that soil microbial diversity was not significantly different between continuous soybean and soybean – corn rotations in northeast China. Yuan et al. (2021) found no significant differences in the soil bacterial diversity between continuous soybean and soybean in rotation with corn. In a study conducted in Illinois, the alpha diversity (number of microbial taxa and their relative abundance) of microbial populations was not significantly different between continuous corn and corn-soybean rotations (Neupane et al. 2021). The differences in soil types, soil moisture, temperature, duration of the crop rotations, and the type of crops in the rotations may be some of the reasons for inconsistencies in microbial community responses to crop rotation treatments.

2.6 Soil Organic Carbon

Soil organic C (SOC) is considered to be an important soil health indicator as it is sensitive to agricultural management practices such as crop rotation, return or removal of crop residues, and tillage (Reeves 1997; Carter 2002; Poeplau and Don 2013; Van Eerd et al. 2014; Turmel et al. 2015; Lal 2016). Maintaining SOC at adequate levels is important for better soil structure and aggregation, nutrient and water retention, nutrient and water use efficiency, improved cation exchange capacity, increased soil microbial activities, and other rhizospheric processes (Reeves 1997; Liu et al. 2010; Lal 2016). Organic C levels in the soil can be increased by adding organic matter inputs such as crop residues, green manure, cover crops, and perennials, and by reducing organic matter losses (Paustian et al. 1997).

Soil organic C can be fractionated into several physical (size, particle density, aggregation), chemical (solubility), and biological (microbial biomass) fractions of C (Chan et al. 2001; Awale et al. 2013; Poeplau and Don 2013; Stockmann et al. 2013; Li et al. 2018). The labile SOC pool which includes particulate organic matter C, active C (permanganate oxidizable C), microbial biomass C, mineralizable C, and dissolved organic C have recently drawn attention of the scientific community (Weil et al. 2003; Liebig et al. 2004; Dou et al. 2008; Chen et al. 2009; Awale et al. 2013). These fractions are early indicators of changes in SOC pools and respond rapidly to the short and mid-term changes in the soil, which are induced by crop rotation, tillage, and fertilizer management (Liebig et al. 2004; Chen et al. 2009; Awale et al. 2013; Aziz et al. 2013).

Labile organic carbon is the active fraction of SOC (Weil et al. 2003). The inputs of labile soil C include carbohydrates, amino acids, amino sugars, peptides, lipids and followed by cellulose, hemi-cellulose, lignin, resins, fats, and wax which are less readily metabolized materials (Tirol-Padre and Ladha 2004). Labile fraction of soil C is an important component in the soil food web as their decomposition rate is relatively rapid compared to the stable C fraction (Skjemstad et al. 2006). It is readily available for soil microflora as carbohydrate substrates, thus, considered as the primary source of energy for soil microorganisms (Chantigny et al. 2000; Bongiorno et al. 2019). Because of that, labile C is considered positively correlated with soil organic matter content, microbial biomass, microbial activity such as respiration, and soil aggregate stability (Van Es et al. 2017).

Potassium permanganate ($KMnO_4$) is used to fractionate SOC via oxidation, and Weil et al. (2003) developed a method using 0.02 mol L^{-1} $KMnO_4$ to measure the active C fraction of total SOC. This labile fraction of organic C is called as permanganate oxidizable carbon (POXC) or active C. The advantages of POXC method are that it is a rapid and inexpensive method that can be modified to use in the field and in the laboratory. Active C is a sensitive soil health indictor as it responds rapidly to the changes of crop and soil management than total organic C (Tirol-Padre and Ladha 2004; Van Es et al. 2017; Jagadamma et al. 2019).

2.6.1 Effect of Crop Rotations on SOC and Active C

The effects of crop rotation on SOC have been evaluated in numerous studies (Carter et al. 1994; Yang and Kay 2001; Carter 2002; Van Eerd et al. 2014). According to a long-term study conducted in Ontario, the highest SOC level was observed in soybean-winter wheat rotation as 21.5 Mg ha^{-1} at 0-5 cm and 79.0 Mg ha^{-1} at 0-20 cm depths compared to either continuous corn or continuous soybean, where averages were 12.8 Mg ha^{-1} at 0-5 cm and 48.1 Mg ha^{-1} at 0-20 cm soil depths under continuous cropping (Van Eerd et al. 2014). The lowest SOC at 0-5 cm depth was produced at continuous soybean under conventional tillage. It may be due to lower amounts of crop residues produced by soybean compared to either corn or wheat, with enhanced decomposition and C mineralization by tillage practice.

After 20 years of a rotation study in Ontario, a soybean-winter wheat rotation had significant SOC concentrations (17.2 g C kg^{-1}) in comparison to continuous corn (16.4 g C kg^{-1}) or two years of corn following two years of alfa-alfa (16.5 g C kg^{-1}) (Yang and Kay 2001). Moreover, Meyeraurich et al. (2006) observed greater SOC levels at 0-20 cm under the rotations which included winter wheat (soybean-winter wheat-corn-corn), compared to continuous corn or soybean-soybean-corn-corn rotations in Ontario. There was a trend for increasing SOC levels with inclusion of winter wheat in the rotations in Ontario. It may be due to greater lignin content in the wheat residue, which are slower to decompose (Yang and Kay 2001; Van Eerd et al. 2014).

Crop rotations can affect the labile fraction of SOC as it is highly sensitive to management practices. Bulbul (2019) reported that active C was greater in a corn-corn-soybean rotation compared to continuous corn in a 14 – year rotation experiment at Illinois. In another long-term rotation study in Tennessee, cotton-soybean and soybean-corn rotations had the highest active C levels compared to continuous soybean for the 0–15 cm depth (Jagadamma et al. 2019). They further observed that active C only changed among crop rotations at 0–7.5 cm soil depth while total organic C showed differences among treatments at deeper soil layers of 15–22.5 cm and 30–45 cm. These findings suggest that changes in active C in response to soil management practices are more likely to be observed at the surface soil layers when treatments have a greater C input. The greater level of total organic C at deeper soil depths in the cotton-soybean rotation

compared to corn-soybean rotation may be associated with the tap roots and greater root density of cotton in relative to fibrous and shallow root system of corn (Grimes et al. 1975).

2.7 Summary

This review summarized some significant results from recent literature that evaluated the implications of crop rotations involving soybean on crop yield and biological soil health indicators of soil enzymes activities, microbial community dynamics, and soil organic C. These studies suggest that crop rotation practices can positively affect the soybean seed yield and the yields of following crops in the rotation. However, the magnitude of agronomic and soil health benefits of crop rotations depends upon the selection of combinations of crops for rotations. Soil enzymes activities and organic C levels increased by including a high residue crop such as corn in the rotation with the low residue crop of soybean. The literature highlights that the differences in microbial community diversity are not consistent among crop rotations.

Most of the studies that have been reported in this review were conducted in the soybean growing regions of the mid-west United States, China, and eastern Canada. Limited or no studies have been conducted in Manitoba to evaluate the effects of growing soybean continuously vs in rotation with canola, corn, and wheat on soybean seed yield and biological soil health. The goal of this study was to fill the gaps in knowledge on agronomic and soil health impacts of frequency of soybean in Manitoban crop rotations.

3.0 SOYBEAN RESPONSE TO CROP ROTATION SEQUENCES IN MANITOBA

3.1 Abstract

Adding soybean [*Glycine max* (L.) Merr.] to crop rotations in the eastern Canadian prairies has diversified the dominant wheat-canola crop rotation. However, how much could be too much soybean in rotation? A four-year crop sequence study was established at three locations in Manitoba in 2014 to evaluate the effect of the frequency of soybean in crop rotation on a common soybean test crop in the fourth year of the study. The four crop sequence treatments assessed were continuous soybean (S-S-S-S), canola-soybean-canola-soybean (Ca-S-Ca-S), corn-soybean-corn-soybean (C-S-C-S), and wheat-canola-corn-soybean (W-Ca-C-S). After four years, the preceding crop sequence had no effect on soybean test crop yield at all locations. Significant differences among crop sequences were found for many parameters including soybean seed quality, dry matter yield, above ground N uptake, and potential for biological nitrogen fixation. However, the continuous soybean sequence was not consistently different form sequences where soybean was grown in rotation with canola, corn, and wheat. In this study, penalties for continuous cropping when first introducing soybean into rotations were minimal. Further study is needed to evaluate how these trends may change over time.

3.2 Introduction

Soybean, a major legume crop grown globally, is a high source of plant-based protein (25.5 – 58.9%) and oil (12.0 – 23.0%) (Gao et al. 2009). Soybean has gained popularity among producers in the eastern Canadian prairies due to the availability of short season cultivars, herbicide resistance traits, high commodity prices, and tolerance to wet growing conditions relative to other commonly grown crops in the region. For example, the acreage under soybean production has recently increased from 20,200 hectares to 532,900 hectares from 2000–2021 in Manitoba (Soy Canada 2021). In general, growing soybean in crop rotations is known to reduce the incidence of pests and diseases while improving soil fertility through biological nitrogen fixation (BNF) and subsequently increasing crop yields (Gentry et al. 2001; Gentry et al. 2013; Farmaha et al. 2016; Mourtzinis et al. 2017a, 2017b).

Several field trials have shown that soybean produces greater yields when they are in rotations with other crops compared to when grown continuously (Sindelar et al. 2015; Farmaha et al.

2016; Mourtzinis et al. 2017a, 2017b). Mourtzinis et al. (2017a) observed a soybean yield increase of 24 – 31% in rotations of corn-soybean, corn-wheat-soybean, and corn-soybean-wheat in comparison to continuous soybean in Wisconsin. Within the United States Corn Belt region, soybean yield was 5% greater in a corn-corn-soybean rotation than in a soybean-corn-soybean rotation, while corn yield was 2 – 5% greater in a soybean-corn rotation compared to continuous corn (Farmaha et al. 2016). The yield advantage of corn grown after soybean has been described as an increase in soil N levels due to the BNF of the previous soybean crop as well as less N immobilization due to the lower C:N ratio of soybean residue compared to corn residue (Gentry et al. 2001; Sindelar et al. 2015).

Soybean relies on symbiotic relationships with bradyrhizobia for acquiring the plant's N demand. However, previous crops in the rotation may influence soil N levels and populations of soil microbes that could affect the development of this symbiotic association with soybean (Liebig et al. 2002; Zhu et al. 2014; Hall et al. 2019; Kim et al. 2021).

The nitrogen requirement of soybean is met by BNF and soil nitrate N uptake (Ciampitti and Salvagiotti 2018). Soybean seeds are inoculated with *Bradyrhizobium japonicum* strains before planting to facilitate BNF (Hungria et al. 2013). Salvagiotti et al. (2008) reported that on average, 50 – 60% of the N demand of soybean is fulfilled by BNF. Rapid increase in N fixation occurs between R1 – R4 developmental stages (45%) and approximately an equal portion (43%) is fixed during R5 – R7 stages (Zapata et al. 1987). Soybean use the ureides allantoin and allantoic acid as the nitrogen transporters from nodules to shoots and the amount of ureides can be estimated by relative ureide nitrogen (RUN) method (Goos et al. 2015). The previous crop in the rotation may influence the *B. japonicum* population due to changes in soil nutrient status. As an example, Sanders (2017) reported greater BNF in soybean when following soybean in a two-year crop sequence than when compared to soybean following canola or wheat.

Previous studies from soybean production regions of Ontario and the mid-western United States have mainly focused on corn-soybean rotations and have well documented the yield stability and economic profitability of the rotation practice. With the development of adapted short season cultivars and the northern expansion of soybean production into the eastern Canadian prairies, less is known about the impact of adding soybean to rotations with canola and spring wheat.

Limited studies have been conducted in Manitoba to assess the effects of growing soybean, a low residue crop, in rotation with other low residue crops such as canola, or with high residue crops such as wheat and corn (Sanders 2017; Mohr 2018). In a two-year sequence study, Sanders (2017) showed that seed yield of a soybean test crop was similar following corn, canola, soybean, or wheat in Manitoba.

It is important to identify the opportunities to optimize soybean production in rotation with commonly grown crops in the eastern Canadian prairies, as well as the potential challenges to growing soybean too frequently in rotation. To examine these issues, a four-year crop sequence study was established in 2014 at three locations with contrasting soil types in central Manitoba. The first objective of this study was to evaluate the impacts of growing soybeans continuously vs in rotation every two years or every four years with canola, corn, and wheat on soybean biomass, seed yield, and grain quality in Manitoba. The second objective was to evaluate crop sequence effects on the potential for BNF in the soybean test crop.

3.3 Materials and Methods

3.3.1 Site Characteristics

Field experiments were initiated in 2014, at the University of Manitoba Ian N. Morrison Research Farm near Carman, Manitoba (49.492261 N, 98.042497 W), Richardson International's Kelburn Farm near St. Adolphe, Manitoba (49.694081 N, 97.122981 W), and with the Westman Agricultural Diversification Organization (WADO) near Melita, Manitoba (49.246706 N, 101.016030 W). The soils were Gleyed Black Chernozem of the Rignold series at Carman, Orthic Dark Grey Black Chernozem of the St. Nobert Series at Kelburn, and Orthic Black Chernozem of the Newstead series at Melita according to the Canadian system of soil classification (Mills and Haluschak 1993). Selected soil properties and previous crop history before the initiation of crop rotations at each location are given in the Table 3.1.

Table 3.1. Selected soil properties and previous crop history for each location before the start of study in 2014.

	Carman	Kelburn	Melita
Soil pH	5.2	7.3	7.7
Soil texture	Sandy clay loam	Clay	Loam
Soil organic matter (g kg^{-1})	30	69	32
Previous crop before 2014	Oat	Spring wheat	Canola
Soybean in last 3–4 years	Yes	Yes	Never

Four crop sequence treatments were tested at each site: 1) continuous soybean (S-S-S-S), 2) canola-soybean-canola-soybean (Ca-S-Ca-S), 3) corn-soybean-corn-soybean (C-S-C-S), and 4) wheat-canola-corn-soybean (W-Ca-C-S) (Table 3.2). The experiment had a randomized complete block design (RCBD) with four replicates. Plot size was 8 m × 10 m at Carman and Kelburn, while plot size at Melita was 8 m × 6 m. The plots were conventionally tilled at all three locations. At the Kelburn location, flax was seeded instead of canola in 2015 due to farm being under quarantine after the discovery of *Verticillium longisporum* (the fungal pathogen causes verticillium stripe in canola stems) on the farm in 2014. In 2017, all four crop sequence treatments were synchronized to have a common soybean test crop in the fourth year of the experiment. Mean monthly temperatures and precipitation in 2017 were compared to the long-term average for each experiment location and are provided in Figure 3.1.

Table 3.2. Crops in each year of the four-year crop sequence treatments grown at Carman, Kelburn, and Melita, MB from 2014 to 2017.

Crop Sequence	2014	2015	2016	2017
S-S-S-S	Soybean	Soybean	Soybean	Soybean
Ca-S-Ca-S	Canola	Soybean	Canola	Soybean
C-S-C-S	Corn	Soybean	Corn	Soybean
W-Ca-C-S	Wheat	Canola	Corn	Soybean

C – Corn, Ca – Canola, S – Soybean, W – Wheat

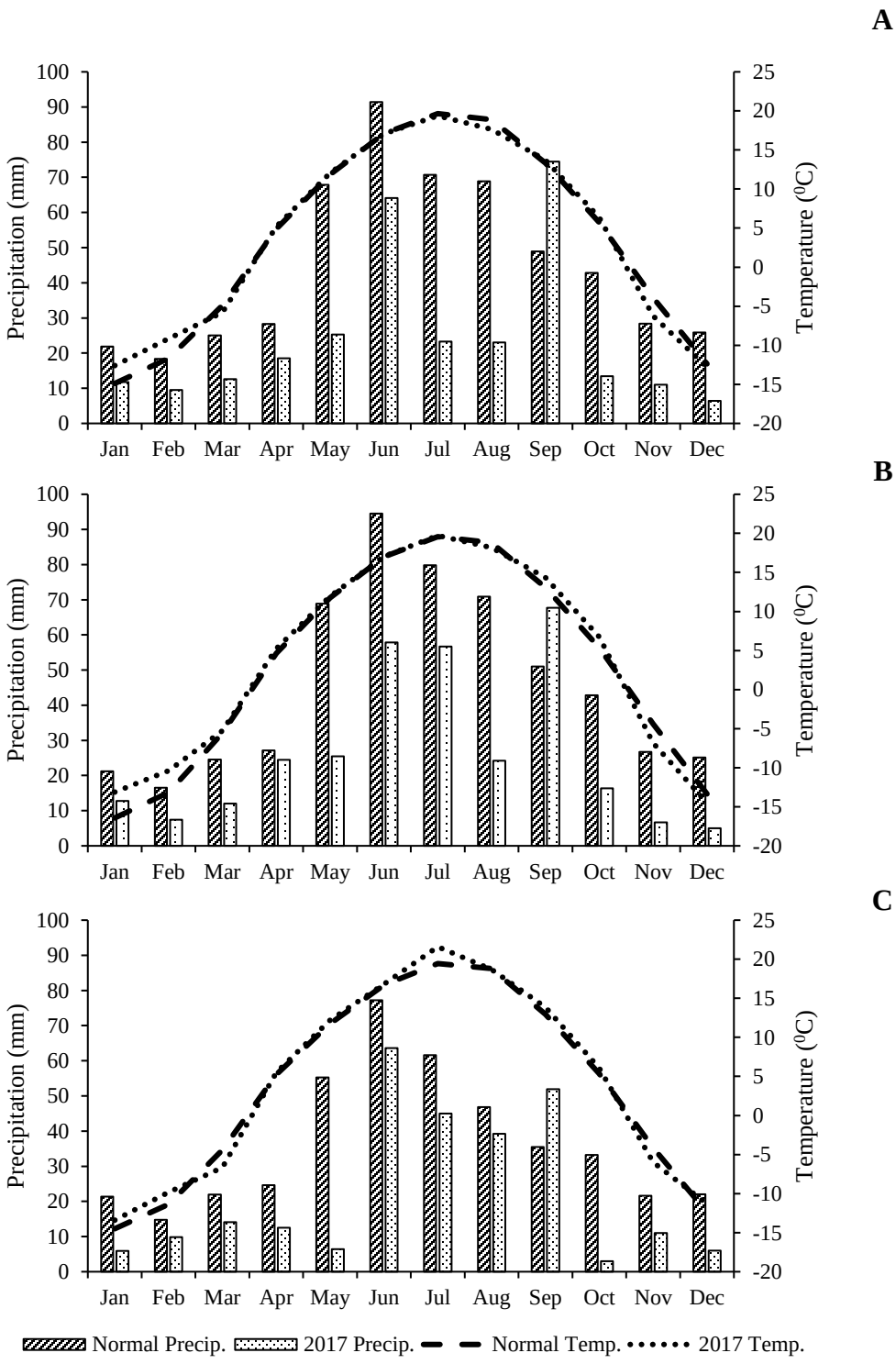

Figure 3.1. Mean monthly precipitation and temperatures in 2017 compared to the long-term normal precipitation and temperature at (A) Carman, (B) Kelburn, and (C) Melita, MB.

3.3.2 Soybean Test Crop Management

Soybean (cv. 24-10 RY), canola (cv. 74-44 BL in 2014 and cv. 73-75 RR in 2016), corn (cv. DKC 26-28 RIB), and wheat (cv. Carberry) were sown to achieve a target population of 47, 110, 7, and 311 plants m^{-2} with a row spacing of 38, 19, 76, and 19 cm, respectively. Fertilizer was broadcast and incorporated at Kelburn and Carman locations. Fertilizer rates for each crop type in the sequence were based on spring soil test recommendations. Nitrogen (N), Phosphorous (P), and Sulphur (S) were applied as urea (46-0-0-0), mono-ammonium phosphate (11-52-0-0), and ammonium sulphate (20-0-0-24) respectively. Fertilizer was banded at the Melita location. Nitrogen as liquid urea-ammonium nitrate (28-0-0), P as mono-ammonium phosphate (12-61-0), potassium (K) as potash (0-0-60), and S as ammonium sulphate (20-0-0-24) were applied at Melita. Fertilizers were not applied to soybean crops at Carman and Kelburn, but P, K, and S fertilizers were applied to soybean crops grown at Melita. Soybean seeds were inoculated with *B. japonicum* (Cell-Tech liquid, Bayer Crop Science, Canada) each year before planting. Herbicides at recommended rates were used to control weeds throughout the study. The most frequently used herbicide was glyphosate (RoundUp WeatherMax®, Bayer Crop Science, Canada) as soybean, canola, and corn varieties selected for the study were glyphosate resistant.

3.3.3 Soybean Grain Yield and Quality

At physiological maturity, soybean yield was harvested from the central 38.1 m^2 area of the plot using a small plot combine (Kincaid Equipment, Haven, Kansas). The moisture content of the grain was measured using a grain moisture meter (Dicky-JohnGAC 2500-AGRI grain analysis computer, Auburn, Illinois) using one sub-sample per plot. Soybean yield was adjusted to a grain moisture content of 13%. The oil and protein concentration of the grain was determined using a Foss Infratech Grain Analyzer (Foss Industries, Hillerod, Denmark) using one sub-sample per plot. The internal calibration for soybean grain protein and oil for concentration was supplied by the manufacturer.

3.3.4 Soybean Dry Matter Yield and Nitrogen Uptake

Above ground soybean biomass at the early-pod fill stage (R5) (Fehr et al. 1971) was sampled from a 0.381 m^2 area in the front and back of each plot to determine the dry matter yield (DMY), N concentration, and total above ground N uptake. Soybean biomass was cut just above the

ground with a sickle, oven-dried at 60 ºC for two days and weighed to determine the DMY. Dried biomass samples were ground to pass through a 1 mm mesh. The N content in the soybean plant tissues was determined by the Dumas Combustion method (Buckee 1994). From the sieved sample, 0.1 g was analyzed using a LECO FP-528 Nitrogen/ Protein Determinator (St. Joseph, Michigan). Total above ground N uptake in kg ha^{-1} was calculated using Equation 3.1.

$$\text{Total above ground N uptake } (kgha^{-1}) = \frac{\text{Nitrogen Concentration (\%)} \times DMY(kgha^{-1})}{100} \quad \text{Equation 3.1}$$

3.3.5 Nodulation and Nitrogen Fixation Assessment

Five plants from the front and five plants from the back of the plot were dug at the R5 growth stage of soybean. These ten plants were soaked in water to remove the soil adhered to the roots. After washing gently with water in the field, roots were cut off from the stems using scissors, stored in plastic bags, kept in the coolers, and transported to the University of Manitoba. The roots were stored at 4 ºC in a fridge for approximately two weeks until nodules could be counted.

From the same five soybean plants, the leaves were removed. Stem and petioles of the plants were cut into smaller pieces and dried at 60 ºC in a forced air oven. Dried stem and petiole samples were ground to pass through a 1 mm mesh and combined into one composite sample for each plot. The sieved samples were analyzed for ureide N (Goos et al. 2015) and nitrate N (Cataldo et al. 1975). An extraction mixture was prepared by adding 0.2 g of sieved plant sample and 20 ml of distilled water. The mixture was heated at 90 °C for 30 minutes in a water bath, cooled to room temperature, shaken on a mechanical shaker for 30 minutes, filtered through a funnel with a filter paper (Whatsman no. 40) into test tubes, and stored in a refrigerator until analysis of N solutes.

For ureide N analysis, calibration standards were prepared by pipetting 0, 0.5, 1.0, 2.5, 5.0, 7.5, and 10 ml of 250 mg L^{-1} allantoin stock solution into 25 ml volumetric flasks to make 0, 5, 10, 25, 50, 75, and 100 mg L^{-1} allanoin-N respectively. The solutions were diluted to volume with deionized water. A 0.3 ml aliquot of each standard and sample extracts were pipetted into test tubes and added 0.3 ml of 0.5 mol L^{-1} sodium hydroxide into each tube and capped. The tubes were placed into 90 °C water bath and heated for 30 minutes and allowed to cool. After that, 7 ml of color reagent [deionized water, mixed acid (phosphoric acid and sulfuric acid), 2, 3

Butanedione monoxime solution, and Thiosemicarbazide solution] was added to each tube, capped, and heated again for 1 hour for color development. After heating the tubes were allowed to cool in a different water bath at room temperature for 5 minutes. The color was immediately read on a spectrophotometer at 525 nm. The reading from the spectrophotometer was in ppm and then converted into mM using the ratio of ppm/ molecular weight (158.12) and multiplying by the dilution factor of 100. The resulting value was the concentration of ureide N (mM) in a sample.

The calibration standards for nitrate were prepared by pipetting 0, 0.25, 0.5, 1.25, and 2.5 ml of 1000 mg L^{-1} NO_3-N stock solution into 25 ml volumetric flasks to make 0, 10, 20, 50, 100, and 200 ppm NO_3-N respectively. The solutions were diluted to volume with deionized water. A 0.1 ml aliquot of each standard and sample extracts were pipetted into test tubes and mixed with 0.4 ml salicylic acid/ sulfuric acid mixture and left on bench for 20 minutes until the solution was clear. The solution was mixed further if it was cloudy. Next, 9.5 ml of 2 mol L^{-1} of sodium hydroxide was added to each tube, left on bench for 10 minutes, and read the color on a spectrophotometer at 410 nm. The reading from the spectrophotometer was in ppm and then convert into mM using the ratio of ppm/ molecular weight (62) and multiplying by the dilution factor of 100. The resulting value was the concentration of nitrate N (mM) in a sample.

Relative ureide N (RUN) % was calculated using Equation 3.2 where *a* is the molar concentration of ureide N in soybean stem and petiole tissues and *b* is the molar concentration of plant nitrate N from the same sample (Herridge and Peoples 1990).

$$RUN = \frac{4 \times a}{(b + 4 \times a)} \times 100 \qquad \text{Equation 3.2}$$

3.3.6 Soil Nitrate Nitrogen

Soil samples were collected from each plot at 0-15 and 15-60 cm depths in the spring of 2017 before planting the soybean test crop. Soils were analyzed for nitrate N using 2.0 mol L^{-1} potassium chloride (KCl) and the Cadmium reduction method using 20 g of air-dried and 2 mm sieved soil (Gelderman and Beegle 2015). Since there were no depth interactions and thus the data was presented for the 0-60 cm profile in the analysis presented.

3.3.7 Statistical Analysis

The data was analyzed using analysis of variance (ANOVA). Normality of the data was assessed using the Proc Univariate procedure of SAS 9.4. (SAS Institute 2014). Data distributions were considered normal if the W statistic of the Shapiro-Wilk test was close to one. Residual plots were used to confirm the variance assumption for ANOVA. The treatment variances were considered equal, if the dots in the residual plot had equal spread on either side of the mean.

The data were then subjected to statistical analysis using a linear mixed model in Proc Glimmix (SAS version 9.4). Crop sequence, experiment location, and their interaction were included as fixed effects in the model. Block was nested within location and was included as a random factor in the model. Least squares means were separated using p-diff lines option to partition significant differences between means. The level of significance was set as $p \leq 0.05$. Seed yield, DMY, total above ground N uptake, nodule count, and soil nitrate N data were analyzed with the normal distribution. Although kernel oil content is a percentage, the data followed a normal distribution and normal distribution was selected for the analysis because the fit statistics (AIC, AICC, BIC) values were lower for the normal distribution when compared to the beta distribution as it had. For other percentage variables (kernel protein content, N concentration in soybean tissues, and RUN content), a beta distribution (link = logit) was used.

3.4 Results and Discussion

3.4.1 Growing Season

The mean perception during 2017 growing season was lower than the long-term normal precipitation at all three locations (Figure 3.1). Dry conditions were observed during the critical reproductive stage of soybean, especially in July and August. Between the three locations, the lowest precipitation during July – August was reported at the Carman location. The mean temperature in 2017 was close to the long-term normal temperature at three locations.

3.4.2 Soybean Yield

Soybean yield was an important performance measure of the soybean test crop year in this crop sequence study. It was hypothesized that grain yield will be lower in the continuous soybean treatment. Unexpectedly, there was no significant crop sequence effect on soybean seed yield (Table 3.3). There were differences in soybean test crop yield between experiment locations. The highest seed yield was observed at Carman (3.16 Mg ha^{-1}) followed by Kelburn (2.21 Mg ha^{-1}) and Melita (2.01 Mg ha^{-1}) locations. Thus, different soil types and environmental conditions experienced across three locations influenced the relative performance of the soybean test crop in this study, but there were no differences between crop sequence treatments.

Table 3.3. F-test probability of the ANOVA for crop sequence, location, and their interaction on soybean seed yield, kernel oil, kernel protein, dry matter yield, total above ground N uptake and N concentration in soybean tissues at R5 growth stage, root nodules, relative ureide nitrogen (RUN), and spring soil nitrate N in 2017.

Source of variation	Seed yield	Kernel oil	Kernel protein
Sequence	0.6710	0.0035	0.0001
Location	<.0001	<.0001	0.0007
Sequence × Location	0.0945	0.1054	0.0003
Source of variation	Dry matter yield at R5	Total above ground N uptake at R5	N concentration in soybean tissues at R5
Sequence	0.4841	0.7743	0.0479
Location	<.0001	0.0004	0.0567
Sequence × Location	0.0192	0.0012	0.0027
Source of variation	Root nodules	RUN	Spring soil nitrate N (0-60 cm)
Sequence	0.0073	0.0432	0.0018
Location	0.0118	<.0001	<.0001
Sequence × Location	0.0178	0.0037	<.0001

Seed yield is the economically important product of a soybean crop. Numerous studies have shown that soybean produces greater yield when grown in rotation compared to continuous soybean (Wilhelm and Wortmann 2004; Munkholm et al. 2013; Sindelar et al. 2015; Farmaha et al. 2016; Mourtzinis et al. 2017a, 2017b). Surprisingly, in this study there were no differences in soybean test crop yield even with four years of continuous soybean. Similar results were reported by Sanders (2017) in a two-year sequence study in Manitoba. The author reported that a preceding crop of canola, corn, or wheat did not have a significant impact on the seed yield of the following soybean test crop.

Across the mid-western United States, corn – soybean rotations are common, and many studies have evaluated the yield benefit of soybean after corn (Marburger et al. 2015; Sindelar et al. 2015; Farmaha et al. 2016; Mourtzinis et al. 2017a, 2017b). Recent studies in Wisconsin reported a 24 to 31% (Mourtzinis et al. 2017a), 20 to 22% increase (Mourtzinis et al. 2017b), and 15.8% increase (Marburger et al. 2015) in soybean yield when grown in a corn-soybean rotation rather than continuous soybean. The yield advantage of soybean after corn may be due to reduced pest and disease pressure by breaking their life cycles. As soybean remains a relatively new crop to Manitoba, the disease, weed, and insect problems may still be limited but could build with time. Continuing this experiment for a longer time frame is important to evaluate changes in yield for the continuous soybean treatment occurs due to new pests and diseases.

3.4.3 Kernel Oil and Protein Content

Soybean seed quality is mainly characterized by kernel oil and protein content. Crop sequences had a small but significant influence on kernel oil content in the 2017 soybean test crop year. The highest kernel oil content was reported in the W-Ca-C-S sequence (17.1%) followed by S-S-S-S (16.9%), Ca-S-Ca-S (16.7%), and C-S-C-S (16.7%) sequences. There were also significant differences in kernel oil content between Carman (17.3%), Kelburn (16.8%), and Melita (16.4%). The reason for increased oil content of soybean kernels at Carman is unclear in this experiment. However, the changes to soil moisture, temperature, and nutrient states at each location may have affected the kernel oil content (Bellaloui et al. 2010).

Soybean seeds are important as a source of plant-based protein. There was a significant crop sequence × location interaction for kernel protein content (Table 3.3). There were no differences

among crop sequences at the Carman and Kelburn locations (Table 3.4). At Melita, the W-Ca-C-S sequence was 13% lower than the average of the other three treatments which were not different from one another. The Melita location had lower spring soil N levels (Table 3.5) compared to Carman and Kelburn. In addition, the location did not have a history of soybean before this experiment. Therefore, bradyrhizobia population in Melita soil would be lower due to reduced potential for BNF (Iturralde et al. 2019; Ordonez 2020; Halwani et al. 2021). Furthermore, the liquid *B. japonicum* inoculum on the seeds may have died prior to planting in 2017 test crop year. Low soil available N, inadequate inoculation, and decreased N fixation could have contributed to the lower kernel protein in the W-Ca-C-S sequence at Melita (Fabre and Planchon 2000).

Table 3.4. The interaction effect of crop sequence × location on kernel protein content in 2017 soybean test crop year.

Location	Sequence	Kernel protein (%)
Carman	S-S-S-S	34.3 *abc*
	Ca-S-Ca-S	34.6 *abc*
	C-S-C-S	34.0 *bc*
	W-Ca-C-S	33.3 *c*
Kelburn	S-S-S-S	34.9 *ab*
	Ca-S-Ca-S	35.2 *ab*
	C-S-C-S	35.6 *a*
	W-Ca-C-S	35.4 *ab*
Melita	S-S-S-S	34.6 *abc*
	Ca-S-Ca-S	34.8 *abc*
	C-S-C-S	35.5 *ab*
	W-Ca-C-S	30.5 *d*

Means followed by the different letters are significantly different at $\alpha = 0.05$.

Kernel oil and protein content of the soybean test crop were influenced by crop sequence treatments in this study. Similarly, a four-year of rotation study conducted with soybeans in Mississippi found that total kernel protein and oil increased with C–S–C–S, C–C–S–S, and S–S–C–S rotations compared to continuous soybean (Bellaloui et al. 2010). In contrast, Missouri, Houx III et al. (2014) observed that kernel protein concentration was greater in continuous soybean compared to corn–soybean rotation whereas oil concentration was not influenced by rotation system after 20 years of continuous soybean compared to a corn–soybean rotation.

3.4.4 Dry Matter Yield and N Uptake at the R5 Stage

In addition to soybean yield and quality, soybean test crop performance between crop sequences was assessed by evaluating the above ground dry matter yield and N uptake. It was hypothesized that the S-S-S-S treatment would have the lowest DMY and N uptake due to limited crop diversity in the rotation sequence. However, this was not the case. There was a significant crop sequence × location interaction for DMY at the R5 stage of the soybean test crop (Table 3.3). At Carman, there was no difference in DMY among crop sequences treatments (Table 3.5). At Kelburn, the DMY of the Ca-S-Ca-S sequence was 24% lower than the S-S-S-S sequence, while at Melita the S-S-S-S sequence was among the treatments with the highest DMY.

Nitrogen uptake followed similar patterns to DMY yield at the Carman and Kelburn locations. There were no differences among crop sequences at Carman. At Kelburn, N uptake of the S-S-S-S sequence was equivalent to all other crop sequences while the W-Ca-C-S sequence was 49% greater than the Ca-S-Ca-S sequence. At Melita, the W-Ca-C-S sequence had the lowest above ground N uptake at R5 due to the combination of lowest N concentration and lower DMY. Both factors of lower soil N and no previous history of soybean could have contributed to this finding for the W-Ca-C-S treatment at Melita relative to the other crop sequences at the location or relative to the same treatment at the Carman and Kelburn location.

This four-year rotation study found that the DMY of S-S-S-S was not significantly lower compared to other crop sequences. In contrast, Peterson and Varvel (1989) and Sindelar et al. (2015) observed 6% and 13% lower soybean DMY with continuous soybean when compared to a soybean-corn rotation in long-term experiments in Nebraska. The reason for increased DMY in the S-S-S-S sequence of our crop rotation study is unclear.

Table 3.5. The interaction effect of crop sequence × location on dry matter yield (DMY), total above ground N uptake and N concentration in soybean tissues at R5 growth stage, relative ureide N (RUN) content, and spring soil nitrate N in 2017 soybean test crop year.

Location	Sequence	DMY	Total above ground N uptake at R5	N concentration in soybean tissues at R5	No. of root nodules per plant	RUN	Spring soil nitrate N
		(kg ha^{-1})	(kg ha^{-1})	(%)		(%)	(kg ha^{-1})
Carman	S-S-S-S	5129 *ab*	164 *a*	3.08 *ab*	58.2 *a*	78.40 *bc*	64.5 *e*
	Ca-S-Ca-S	4994 *ab*	169 *a*	3.25 *a*	42.0 *bcdef*	52.11 *d*	103.7 *cd*
	C-S-C-S	5103 *ab*	150 *abc*	2.78 *b*	52.0 *ab*	71.86 *c*	82.1 *cde*
	W-Ca-C-S	5628 *a*	163 *a*	2.95 *ab*	37.7 *cdefg*	66.48 *c*	106.8 *c*
Kelburn	S-S-S-S	4526 *bcd*	127 *bcd*	3.15 *a*	32.9 *efg*	93.04 *a*	172.9 *a*
	Ca-S-Ca-S	3429 *e*	102 *de*	2.90 *ab*	47.6 *abcd*	94.55 *a*	135.6 *b*
	C-S-C-S	3831 *de*	119 *cde*	3.20 *a*	36.4 *defg*	95.61 *a*	77.1 *de*
	W-Ca-C-S	4758 *bc*	152 *abc*	3.20 *a*	33.4 *defg*	95.34 *a*	89.7 *cde*
Melita	S-S-S-S	4591 *bcd*	144 *abc*	3.13 *ab*	51.4 *abc*	91.81 *a*	19.3 *f*
	Ca-S-Ca-S	5056 *ab*	156 *ab*	3.08 *ab*	30.0 *gf*	88.66 *ab*	17.1 *f*
	C-S-C-S	4781 *abc*	146 *abc*	3.00 *ab*	45.1 *abcde*	94.08 *a*	13.7 *f*
	W-Ca-C-S	4098 *cde*	93.3 *e*	2.40 *c*	24.0 *g*	67.69 *c*	18.5 *f*

Means followed by the different letters are significantly different at $\alpha = 0.05$.

3.4.5 Root Nodulation

Root nodulation was used to estimate the impact of soybean crop sequence treatments on biological nitrogen fixation potential. There was a significant crop sequence × location interaction for root nodules (Table 3.3). At Carman and Melita locations, the W-Ca-C-S and Ca-S-Ca-S sequences had lower root nodules per plant than the S-S-S-S sequence (Table 3.5). This finding is not surprising because continuous soybean seeds were inoculated with *B. japonicum* in all four years of the rotation sequence. However, this trend was reversed at Kelburn where nodulation in the Ca-S-Ca-S sequence was 45% greater than the S-S-S-S sequence.

Root nodules are important for nitrogen nutrition of soybean plants as BNF by *B. japonicum* bacteria take place inside those nodules. The S-S-S-S sequence at both Carman and Melita had significantly greater nodulation compared to the S-S-S-S sequence at Kelburn. The greater nodulation might be due to presence of more *B. japonicum* in the soil relative to other sequences (Carciochi et al. 2019; Ordonez 2020). The lower nodulation of the S-S-S-S sequence at Kelburn might be due to the presence of greater spring soil nitrate N levels. The Kelburn location had a history of manure application before this experiment. This may have contributed for increased soil N levels. These levels were significantly greater in the S-S-S-S sequence at Kelburn compared to the S-S-S-S at Carman and Melita (Table 3.5). Greater amounts of spring soil nitrate N may have reduced nodulation at Kelburn relative to this treatment at the other two locations (Schuller et al. 1986; Arrese-lgor et al. 1997; Santachiara et al. 2019).

Nodulation in the W-Ca-C-S sequence at Melita was lower compared to the S-S-S-S and C-S-C-S at the same location. This may be because the Melita site had no previous soybean history before the 2017 soybean test crop was grown in the W-Ca-C-S sequence. Ordonez (2020) found soil populations of *B. japonicum* population was lower in the W-Ca-C-S sequence compared to other three sequences at Melita location. In addition, kernel protein content and above ground N uptake followed a similar pattern to root nodulation. Lower kernel protein content, above ground N uptake, and tissue N concentration at R5 in the W-Ca-C-S sequence was likely driven due to lower soil populations of *B. japonicum*, lower number of root nodules per plant, and reduced nitrogen fixation.

Although greater nodulation is typically expected for soybean plants when soil N levels are lower, root nodulation was unexpectedly lower in the W-Ca-C-S sequence at Melita where the soil had low soil nitrate nitrogen. Santachiara et al. (2019) reported that in order to maximize the soybean seed yield and achieve N balance in the soils with low to medium N fertility, the total amount of BNF should be greater compared to soils with adequate soil N supply. Manitoba Pulse and Soybean Grower's recommend using double inoculation (e.g. liquid + granular) in fields with no previous history of soybean. However, in this study, liquid *B. japonicum* inoculant was applied to soybean seeds at the recommended rate at three locations in the soybean test crop year. It seems likely that the amount of inoculant or the form of inoculant (liquid vs granular) was not sufficient to optimize the BNF in the W-Ca-C-S sequence at Melita location (Ordonez 2020). Double inoculation, application of greater rate of inoculant, or a different form of inoculant may have been required to improve the BNF at Melita soil considering no history of soybean and low soil available N.

3.4.6 Nitrogen Fixation

Relative ureide N was used as a measure to estimate biological N fixation by the soybean test crop. It was hypothesized that the S-S-S-S sequence would have the highest RUN content due to presence of greater *B. japonicum* population in the soil. However, there were no differences among crop sequences at Kelburn (Table 3.5). The RUN in S-S-S-S sequence was not significantly different from the C-S-C-S and W-Ca-C-S sequences at Carman and between C-S-C-S and Ca-S-Ca-S sequences at Melita. RUN was lowest in the Ca-S-Ca-S sequence (28%) at Carman and the W-Ca-C-S sequence (26%) at Melita compared to the average of other three sequences within each location. The lower RUN contents in the Ca-S-Ca-S sequence at Carman and the W-Ca-C-S sequence at Melita matched with the lower root nodules count of those sequences.

RUN is an indication of the amount of biologically fixed N by the soybean plants (Herridge 1982; Herridge and Peoples 1990). Surprisingly, greater RUN content was observed at Kelburn with a clay soil and high soil nitrate N levels that were observed in the early growing season compared to the Carman and Melita locations. In contrast, Ciampitti et al. (2021) report that BNF in soybean was negatively correlated with the soil clay content. The process of N fixation requires more oxygen compared to plant root growth (Layzell and Hunt 1990). In heavy clay

soils such as Kelburn, rhizosphere oxygenation may be reduced due to wet soil conditions, thus lowering the potential for N fixation in relative to other loamy or coarse textured soils (Schipanski et al. 2010). In this study, the reason for greater RUN content in the clay soil with greater levels of spring soil N is unclear.

At Carman, the lowest RUN content was observed in the Ca-S-Ca-S sequence. Decaying canola roots have been found to act as bio-fumigants that are toxic to pathogens such as *Sclerotinia sclerotiorum* (Potgieter et al. 2013). However, Norton et al. (1999) reported that these properties may have negative effects on beneficial microbes such as *Rhizobia* and Vesicular-Arbuscular Mycorrhizal fungi. Ordonez (2020) found populations of *B. japonicum* following canola to be lower than all other crop sequences in the spring prior to the soybean test crop in 2017. Hence, lower RUN in the Ca-S-Ca-S sequence may have been caused by suppressed nodule development as a result of fewer *Rhizobia* in the soil.

3.5 Summary

Crop sequence treatments did not affect the seed yield of soybean test crop after the four-year rotation sequences. It was surprising that even after four years of continuous soybean, the seed yield did not decline compared to other crop sequences. Crop sequence influenced seed quality, DMY, above ground N uptake, and potential for BNF. However, the continuous soybean treatment was not consistently different from sequences where soybean was grown in rotation with canola, corn, and wheat. At Melita, the W-Ca-C-S sequence reported significantly lower kernel protein content, above ground N uptake, RUN content, and root nodulation which could have caused by fewer *B. japonicum* population compared to other three sequences at the same location. Given the relatively short history of soybean production in Manitoba, the initial consequences of continuous soybean in the short-term may be limited. It is important to continue to study soybean crop rotations in Manitoba over the long-term to understand how this may change with time.

4.0 DOES FREQUENCY OF SOYBEAN IN PRAIRIE CROP ROTATIONS IMPACT BIOLOGICAL INDICATORS OF SOIL HEALTH?

4.1 Abstract

Crop rotations in Manitoba have become more diverse with the introduction of short season soybean [*Glycine max* (L.) Merr.] cultivars to the eastern Canadian prairies. This study was conducted to evaluate the effect of growing soybean in crop rotations on three biological soil health indicators, to identify which biological soil health indicators are able to detect differences between crop rotations, and to determine the best sampling times to identify differences between rotational treatments. The experiment was established in 2014 at two locations in central Manitoba. The crop sequence treatments compared were continuous soybean (S-S-S-S), canola-soybean-canola-soybean (Ca-S-Ca-S), corn-soybean-corn-soybean (C-S-C-S), and wheat-canola-corn-soybean (W-Ca-C-S). Surface soil samples (0-8 cm) were collected at multiple sampling stages in the 4th (2017) and 6th (2019) year of the rotation cycle. Soil enzymes (β-glucosidase, β-glucosaminidase, and acid phosphatase) identified differences between the crop sequence treatments but these differences were not consistent between sampling stages, crop sequences, locations, and years. However, enzyme activity was frequently greater in the C-S-C-S sequence compared to the S-S-S-S sequence across sampling stages in both years. Active C was also greater in the C-S-C-S in relative to the S-S-S-S sequence. When compared using principal coordinate analysis (PCoA), soil bacterial population were not found to be different between crop sequence treatments in both years. Growing soybean in rotation with corn enhanced biological soil health as measured by soil enzymes and active C, compared to growing soybean continuously. In this study, active C consistently identified differences between crop sequence treatments. Results from this study indicate that there is a flexibility to sample for soil health analysis at either the beginning or end of the growing season to identify rotational effects.

4.2 Introduction

Soil microbial activity is important for soil health due to the involvement of soil microbes in soil structure and aggregate formation, decomposition of organic matter, and cycling of soil nutrients (Dias et al. 2015; Usman et al. 2016; Bünemann et al. 2018; Ozlu et al. 2019). Microbes are involved in most soil processes. The composition and activity of soil and plant associated

microbes can be affected by agricultural management practices such as crop rotation, residue and tillage management, and fertilization (Guo et al. 2017; Ai et al. 2018; Li et al. 2021; Neupane et al. 2021). Soil enzymes that are associated with microbial activity, microbial community diversity, and labile organic carbon pools have been widely used as biological soil health indicators in recent research studies and have been found to be responsive to crop and soil management practices (Dodor and Tabatabai 2003a, 2003b; Dodor and Tabatabai 2005; Li et al. 2010; Jagadamma et al. 2019; Yuan et al. 2021). However, the effect of crop rotations including soybean on these biological indicators have been less studied in the Canadian prairies. We selected these three indicators to evaluate the impact of four soybean crop sequences on biological soil health of soils in Manitoba.

Enzymes that are produced by soil microorganisms hydrolyze different substrates and catalyze biochemical reactions that breakdown organic matter and release plant available nutrients (Bandick and Dick 1999; Makoi and Ndakidemi 2008; Stott et al. 2010; Acosta-Martinez et al. 2018). Soil enzymes are released as microbial secretions during microbial cell growth and division (Kandeler 2014). Changes in the concentrations of soil enzymes are rapid compared to other physical or chemical soil indicators and can be used as early indicators of changes in soil health (Dick et al. 1996). Soil enzymes can be used as indirect measures of microbial activity and are now being used as an index of soil health (Dodor and Tabatabai 2005; Alkorta et al 2003; Acosta-Martinez et al. 2018).

There are a range of soil enzymes that can be used to characterize soil health and may be important for nutrient cycling. β-glucosidase catalyzes the hydrolysis of polysaccharide cellulose and is considered an index for C cycling in the soil (Makoi and Ndakidemi 2008; Stott et al. 2010; Acosta-Martinez et al. 2018). β-glucosaminidase catalyzes the hydrolysis of chitin, which is important in C and N cycling in the soil (Ekenler and Tabatabai 2002, 2004). Phosphomonoesterase, which includes acid phosphatase and alkaline phosphatase, hydrolyze different phosphomonoesters in the soil to release plant available forms of phosphorous (Acosta-Martinez and Tabatabai 2011). Soil microbial activity and enzyme activity can be influenced by the amount of plant residue returned to soil after harvest and by the composition of soil organic matter (Bandick and Dick 1999; Ekenler and Tabatabai 2002; Balota et al. 2004).

Soil microbial community analysis is another tool used to determine biological soil health in agroecosystems (Kennedy and Stubbs 2006). Principal coordinate analysis (PCoA) is a method use in microbiome analysis to visualize similarities or dissimilarities of microbial populations. However, there have been inconsistencies between the findings of previous studies on soil bacterial population dynamics and their diversity under crop rotation systems. For example, Zhu et al. (2014) observed that the soil bacterial diversity was greater for soybean grown in rotations compared to the continuous soybean cropping. In contrast, Li et al. (2010) and Yuan et al. (2021) found no significant difference in soil bacterial alpha-diversity between continuous soybean and soybean in rotation with corn. Another study showed that the relative abundance of beneficial bacteria (*Bradyrhizobium* sp. and *Gemmatimonas* sp.) and beneficial fungi (*Mortierella* sp. and *Paecilomyces* sp.) increased over time in long term continuous soybean cropping treatments while the abundance of pathogenic fungi (*Fusarium* sp.) decreased (Liu et al. 2020). These contrasting results suggest that soil microbial diversity and abundance may vary with the type of crops in the rotation, rotation duration, soil type, and local environmental conditions such as temperature and precipitation (Ishaq et al. 2020; Yuan et al. 2021).

Labile organic carbon is an active fraction of soil organic carbon and is readily available for soil microorganisms as the primary source of energy (Chantigny et al. 2000; Weil et al. 2003; Bongiorno et al. 2019). Weil et al. (2003) developed a method using potassium permanganate ($KMnO_4$) to determine the amount of active soil carbon. Active C responds more rapidly to changes in crop and soil management compared to total organic carbon (Weil et al. 2003; Van Es et al. 2017). Changes in active C due to management practices such as crop rotations, cover crops, and tillage are greater in surface soil layers where a greater amount of soil organic matter is present, compared to the deeper soil layers (Jagadamma et al. 2019).

Crop rotation is an agronomic practice known to improve soil health (Ekenler and Tabatabai 2002; Balota et al. 2004; Karlen et al. 2013; Van Eerd et al. 2014). Adding soybean [*Glycine max* (L.) Merr.] has increased the diversity of crop rotations in Manitoba which were mainly dominated by canola and wheat (Statistics Canada 2021). In the United States, it has been found that incorporating soybean into rotations provides nitrogen benefits for the following cash crop as soybean can fix atmospheric N_2 into plant available forms of N (Ennin and Clegg 2001;

Gentry et al. 2001). Furthermore, numerous studies have been conducted in the United States and other soybean growing regions to assess the rotation effect of soybean on subsequent crop yields (Kelley et al. 2003; Wilhelm and Wortmann 2004; Munkholm et al. 2013; Sindelar et al. 2015; Farmaha et al. 2016).

Limited studies have been conducted in the Canadian prairies to evaluate the impacts of growing soybean in crop rotations on biological soil functions such as soil enzymes activities, microbial population dynamics, and changes to active carbon (Sanchez et al. 2001; Tonitto et al. 2006; McDaniel et al. 2014). Therefore, this study was conducted to evaluate the following objectives (1) to determine whether growing soybean continuously vs in rotations with canola, corn, or wheat influences biological soil health as measured by soil enzymes, bacterial population dynamics, and active C in the soils of Manitoba, (2) to identify biological soil health indicators that are able to detect differences between crop rotations in Manitoba soils, and (3) to determine the best sampling time to identify differences between rotation treatments.

4.3 Materials and Methods

A four-year rotation study was established in 2014 to study the impact of the frequency of soybeans in crop rotations at two locations with contrasting soil types in central Manitoba: i) Ian N. Morrison Research Station (49.492151, –98.043880), near Carman, MB and ii) Richardson International's Kelburn Farm (49.694081, –97.122981), near St. Adolphe, MB. The soils were Gleyed Black Chernozem of the Rignold series at Carman and Orthic Dark Grey Black Chernozem of the St. Nobert Series at Kelburn. Soil texture at Carman was sandy clay loam and at Kelburn was clay. Crop sequence treatments are listed in the Table 4.1. The experimental design was randomized complete block (RCBD) with four replicates. Plot size was 8 × 10 m and they were managed with conventional tillage. Tillage at the Carman location involved using a disk in the fall after crop harvest and a cultivator with cultipackers in the spring prior to spring planting. Tillage at Kelburn location involved using a disk in the fall. No tillage was used at Kelburn prior to planting due to the clay soil texture. Soybean seeds were inoculated with *Bradyrhizobium japonicum* (Cell-Tech liquid, Bayer Crop Science, Canada) each year before planting. Nitrogen and phosphorus fertilizers were applied to canola, corn, and wheat based on the spring soil tests recommendations and no fertilizer was applied to soybean. Soil health

analysis were conducted during the 4th (2017) and 6th (2019) years of the experiment (Table 4.1). A common soybean test crop occurred at the end of the first rotation cycle in 2017. In the second year of the second rotation cycle in 2019 three of the four sequences had a soybean test crop. In 2019, the W–Ca–C–S sequence was grown canola other than soybean.

Table 4.1. Crops grown in each year of the four crop sequence treatments grown at Carman and Kelburn, MB.

	Year	Sequence 1 (S-S-S-S)	Sequence 2 (Ca-S-Ca-S)	Sequence 3 (C-S-C-S)	Sequence 4 (W-Ca-C-S)
Rotation cycle 1	Year 1 (2014)	Soybean	Canola	Corn	Wheat
	Year 2 (2015)	Soybean	Soybean	Soybean	Canola*
	Year 3 (2016)	Soybean	Canola	Corn	Corn
	Year 4 (2017)	Soybean	Soybean	Soybean	Soybean
Rotation cycle 2	Year 5 (2018)	Soybean	Canola	Corn	Wheat
	Year 6 (2019)	Soybean	Soybean	Soybean	Canola
	Year 7 (2020)	Soybean	Canola	Corn	Corn
	Year 8 (2021)	Soybean	Soybean	Soybean	Soybean

C – Corn, Ca – Canola, S – Soybean, W – Wheat

*At the Kelburn location, flax was seeded instead of canola in 2015 due to farm being under quarantine after the discovery of *Verticillium longisporum* (the fungal pathogen causes verticillium stripe in canola stems) on the farm in 2014.

4.3.1 Soil Sampling

Bulk soil samples at the 0-8 cm depth were collected at multiple times during the growing season from each treatment before planting (BP), and at soybean growth stages of emergence (VE), beginning seed formation (R5), and full maturity (R8) in both years. Soil samples for BP were taken from within each row. For each of the other growth stages the samples were taken immediately adjacent to the soybean plants. Ten 2 × 8 cm soil samples from each plot were

collected randomly into zip lock bags and composited to make a homogenized sample. Field moist soils were stored at 4 ºC and subsamples were frozen at –80 ºC on the same day of sampling. A second subsample from BP and R8 stages were air dried and passed through a 2 mm sieve for active C analysis. Soils were stored at 4 ºC until used for enzyme assays.

4.3.2 Soil Enzyme Analysis

Laboratory analyses for soil enzymes were carried out using the substrates and buffers as listed in the Table 4.2. A flow chart summarizing the steps involved in soil enzyme assays is given in the Figure 4.1. The gravimetric moisture content of each soil sample was measured by oven drying approximately 10 g of field moist soil at 105 °C for 48 hours (Black 1965). For β-glucosidase and acid phosphatase assays, standard solutions of 2.5, 5.0, 10.0, 15.0, and 18.0 µg ml^{-1} p-nitrophenol (PNP) were prepared by pipetting 1.25, 2.5, 5.0, 7.5, and 9.0 ml of 100 µg ml^{-1} PNP solution in to 50 ml of volumetric flask and diluting with distilled water. For β-glucosaminidase assay, standard solutions of 2.0, 5.0, 8.0, 10.0, and 12.0 µg ml^{-1} p-PNP were prepared by pipetting 1.0, 2.5, 4.0, 5.0, and 6.0 ml of 100 µg ml^{-1} PNP solution in to 50 ml of volumetric flask and diluting with distilled water. For each enzyme assay, 1.0 g of field moist soil (weighed into volumetric flasks) was incubated for 1 hour at 37 °C with its appropriate substrate and buffer, at optimal pH for the reaction. After 1 hour, the reaction was stopped by adding the appropriate stop buffer and $CaCl_2$ (Table 4.2). The flasks were swirled to ensure termination of the reaction, allowed to sit for 5 minutes, and mixture was vacuum filtered using P4 filter papers. The absorbance of the final product, p-nitrophenol, was measured colorimetrically at 405 nm. Soil enzyme activity was determined using Equation 4.1.

$$\text{Enzyme activity (µg PNP } g^{-1} \text{ soil } h^{-1}) = \{[a + (b \times absorbance)]/\, Wt_{ds}\} \times V_T \quad \text{Equation 4.1}$$

Where a is the intercept (concentration) and b is the slope (concentration/ absorbance) of the standard curve, Wt_{ds} is the weight of field moist soil in grams adjusted for soil moisture, and V_T is the total volume of the reaction occurred. Calculations were made on a dry weight basis.

Table 4.2. Substrates, buffers, reaction pH, and absorbance wavelength used for assessing the activities of soil enzymes.

	Soil enzyme		
	β-glucosidase	β-glucosaminidase	Acid phosphatase
Substrate	4-nitrophenyl-beta-D-glucopyranoside	p-nitrophenyl-N-acetyl-β-D-glucosaminide	p-nitrophenyl phosphate
Start buffer	Modified universal buffer	Acetate buffer	Modified universal buffer
Reaction pH	6.0	5.5	6.5
Stop buffer	*THAM buffer	THAM buffer	Sodium hydroxide
Wavelength	405 nm	405 nm	405 nm
Reference	Deng and Popova (2011)	Deng and Popova (2011)	Acosta-Martinez and Tabatabai (2011)

*THAM – Tris (hydroxymethyl) amino-methane buffer

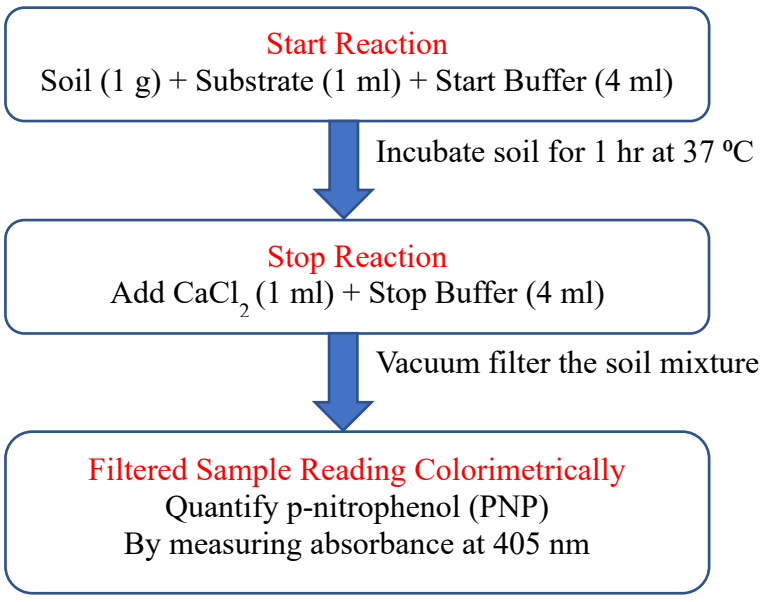

Figure 4.1. Main steps involved in analyzing soil enzyme assays.

4.3.3 Soil Active Carbon Analysis

Active C was measured following the method of Weil et al. (2003). The working stock of 0.02 mol L $KMnO_4$ was prepared by pipetting 100 ml of a 0.2 mol L $KMnO_4$ solution into a 1000 ml volumetric flask and bringing it to volume of 1000 ml using a 0.1 mol L^{-1} $CaCl_2$ solution. Air-dried and 2 mm sieved, 2.5 ± 0.01 g (0.0025 kg) of soil from each treatment were weighed into centrifuge tubes and mixed with the 0.02 mol L^{-1} $KMnO_4$ solution, which is dark purple in color. The tubes were capped tightly and shaked horizontally on a side-to-side shaker at 120 oscillations/minute for 2 min. Then, the tubes were arranged vertically in a rack, uncapped them, and the soil was allowed to settle for 5 min. While the samples were settling, 49.5 ml of distilled water was added to clean, graduated centrifuge tubes as for each sample. Then, 0.50 ml of liquid from the upper 1 cm of the soil–$KMnO_4$ suspension was slowly pipetted and transferred to the tubes of distilled water. Tubes were capped, inverted several times to mix, and a few ml of those solutions were transferred into spectrophotometer cuvettes. The absorbance of the sample was measured using a spectrophotometer set to the 550 nm wavelength. For this method, the bleaching of the purple $KMnO_4$ color is proportional to the amount of oxidizable C in soil. Standard solutions of 0.0025, 0.005, 0.01, 0.015, and 0.02 mol L^{-1} $KMnO_4$ were prepared by pipetting 0.625, 1.25, 2.5, 3.75, and 5 ml of 0.2 mol L^{-1} $KMnO_4$ solution in to 50 ml of volumetric flask and diluting with distilled water. The amount of active C in the soil was calculated using Equation 4.2 (Culman et al. 2012).

$$\text{Active C (mg kg}^{-1}) = [0.02\ mol/L - (a + b\ \times absorbance)] \times (9000\ mg\ C/mol) \times (0.02\ L\ soln./0.0025\ kg\ soil) \quad \text{Equation 4.2}$$

Where 0.02 mol L^{-1} is the initial concentration of the potassium permanganate ($KMnO_4$) solution, a is the intercept (concentration) and b is the slope (concentration/ absorbance) of the standard curve, 9000 is the amount of C in mg oxidized by 1 mol of MnO_4, 0.02 L is the volume of $KMnO_4$ solution reacted, and 0.0025 kg is the weight of soil used for the extraction.

4.3.4 Bacterial DNA Extraction

DNA extraction from frozen soil samples was carried out using the DNeasy Power Soil extraction kit (Qiagen, Hilden, Germany) using the manufacturer's protocol. Previous

optimization of the protocol had been carried out to ensure that DNA extracted was proportional to the amount of soil that was used (Ivan Oresnik, personal communication). Between 0.20 – 0.25 g of soil was used for the extraction. Soil was suspended in buffers supplied with the kit, and the sample was vortexed for 20 minutes to ensure complete lysis. The quantity of DNA was measured at 260 nm absorbance and quality was determined by 260 nm/ 280 nm absorbance ratio (Nanodrop™ Lite Spectrophotometer, Thermo Fisher Scientific Inc., Waltham, Massachusetts, United States). The ratio of absorbance ~1.8 or higher is generally accepted as "pure" for DNA. Extracted DNA was stored at –20 ºC until further analysis.

4.3.5 16s rRNA Sequencing of Bacterial DNA

Extracted DNA samples were sent to Metagenom Bio Inc. (Waterloo, Ontario, Canada) for 16S rRNA paired-end sequencing using an Illumina MiSeq. The 515FB–806RB primer was used to target the V4 region of the 16S rRNA (Caporaso et al. 2012; Apprill et al. 2015; Parada et al. 2016). The DNA was amplified using PCR as described by Ordonez (2020, pg. 25). Mothur software was used to process raw sequenced reads using the code reported by Ordonez (2020, pg. 87).

4.3.6 Data Analysis

Soil enzymes and active C data were analyzed using Analysis of Variance (ANOVA) and the Proc Glimmix procedure of SAS version 9.4 (SAS Institute 2014). The data were analyzed by year as the crops in the four-year crop sequence treatments were different in 2017 and 2019 (Table 4.1). In 2017, all the crop sequences had soybean test crop in the 4^{th} year of the experiment. In 2019, only three out of four sequences had soybean in the 6^{th} year of the experiment. Normality of the data was assessed by plotting the distribution of data using Proc Univariate. Data distributions were considered normal if the W statistic of the Shapiro-Wilks test was close to one. Homogeneity of the treatment variances were tested using scatterplot of predicted vs residuals. Treatment variances were considered equal, if the plotted residuals were consistently spread across mean on the x-axis.

Repeated measures analysis was used to conduct ANOVA for the soil enzymes and active C at different sampling stages. Sampling stage was included as the repeated factor in the model. Crop sequence and location were included as fixed effects in the model. Block was nested within

location and was included in the random statement. Least square means and the p-diff option of SAS was used for multiple means comparisons to partition the significant interactions. The level of significance was set at $p \leq 0.05$. Compound symmetry covariance structure was selected as the best covariance structure for each enzyme and active C according to the lowest values for fit statistics of AIC, AICC, and BIC.

Bacterial population data was analyzed using principal coordinate analysis (PCoA). Meta data files were uploaded into Calypso Version 8.68 for statistical analysis and visualization of the bacterial families (Zakrzewski et al. 2016). Data was filtered by removing cyanobacteria and chloroplast and normalized using cumulative sum scaling (CSS) and $\log_2$ transformed. The PCoA was conducted using the bacterial family as the taxonomic rank in the analysis. The differences of bacterial families between the crop sequence treatments and sampling stages were determined using the Bray-Curtis index in the PCoA (Bray and Curtis 1975).

4.4 Results and Discussion

4.4.1 Growing Conditions

Average monthly perception during 2017 and 2019 growing seasons were lower than the long-term normal precipitation at both locations (Figure 4.2). One exception was the average precipitation at Kelburn in July 2019 that was greater than the long term normal. Furthermore, record rainfalls occurred in September 2019 and 15 – 40 cm of snowfall occurred in early October 2019. Accumulated precipitation during the two growing seasons was between 300 – 350 mm, which was low compared to the growing season optimum of 400 – 500 mm required for soybean (Laura 2018). The mean monthly temperature in 2017 and 2019 were close to the long-term normal temperature at both locations.

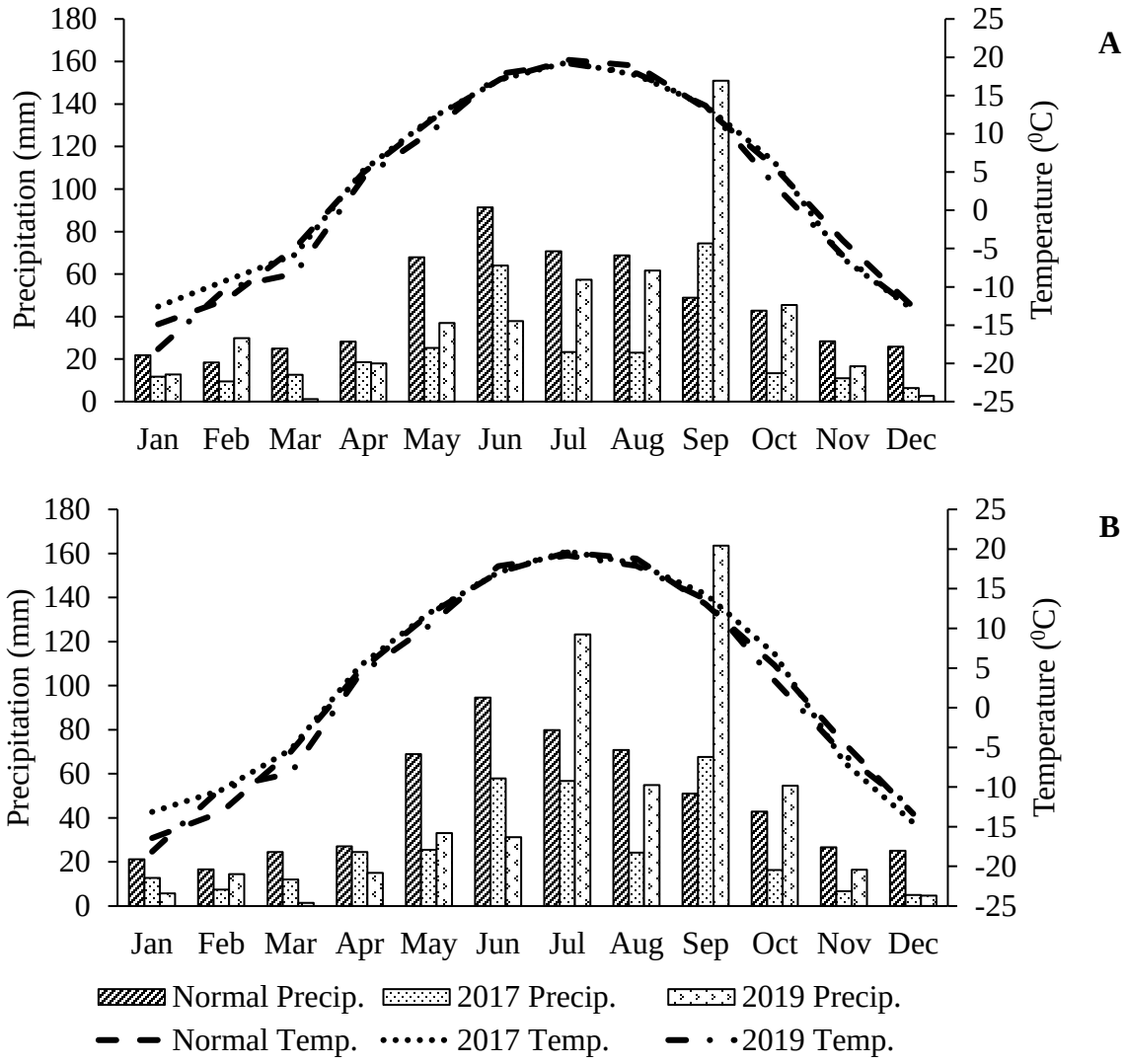

Figure 4.2. Mean monthly precipitation and temperatures in 2017 and 2019 compared to the long-term normal precipitation and temperatures at (A) Carman and (B) Kelburn, MB.

4.4.2 Rotation Effect on Soil Enzyme Activities

Soil enzymes are biological soil health indicators that have previously been shown to be sensitive to land management practices. Among those enzymes, β-glucosidase, β-glucosaminidase, and acid phosphatase were selected for this study to evaluate the effect of crop sequence treatments that include soybean in Manitoba soils. The location × crop sequence × sampling stage interaction was significant for all three soil enzymes activities in both 2017 and 2019 (Tables 4.3, 4.4, 4.5, and 4.6).

Table 4.3. F-test probability from the ANOVA of location, crop sequence, sampling stage, and their interactions on β-glucosidase, β-glucosaminidase, acid phosphatase soil enzymes as well as active C as soil health indicators in 2017 and 2019.

		β-glucosidase	β-glucosaminidase	Acid phosphatase	Active C
Study year	Source of variation	Pr >F	Pr >F	Pr >F	Pr >F
2017	Location	<.0001	<.0001	<.0001	<.0001
	Sequence	<.0001	<.0001	<.0001	<.0001
	Stage	<.0001	<.0001	<.0001	0.0199
	Sequence × Stage	<.0001	<.0001	<.0001	0.4138
	Location × Stage	<.0001	<.0001	<.0001	0.1056
	Location × Sequence	<.0001	<.0001	0.0002	<.0001
	Location × Sequence × Stage	<.0001	<.0001	<.0001	0.0009
2019	Location	<.0001	<.0001	<.0001	<.0001
	Sequence	0.0001	<.0001	<.0001	<.0001
	Stage	<.0001	<.0001	<.0001	0.9427
	Sequence × Stage	<.0001	<.0001	<.0001	0.1314
	Location × Stage	<.0001	<.0001	<.0001	0.3652
	Location × Sequence	<.0001	<.0001	<.0001	0.0176
	Location × Sequence × Stage	<.0001	<.0001	<.0001	0.3968

4.4.2.1 β-glucosidase

β-glucosidase is an important enzyme for C cycling in the soil. It was hypothesized that β-glucosidase activity will be lower in the S-S-S-S compared to C-S-C-S and W-Ca-C-S sequences as soybean produced lower biomass and add less residue to the soil than corn or wheat (Appendix B – Table 4). However, in 2017 at Carman, no differences were observed at the BP stage (Table 4.4). At the VE stage, β-glucosidase activity was greater in the C-S-C-S sequence by 36% compared to the average of other three sequences. At the R5 and R8 stages, enzyme activity was lower in the S-S-S-S by 22% and 24%, respectively, when compared to the average of C-S-C-S, Ca-S-Ca-S, and W-Ca-C-S sequences. In 2017 at Kelburn, β-glucosidase activity was significantly lower in the S-S-S-S at BP, R5, and R8 stages by 28%, 17%, and 16%, respectively, relative to the average of other sequences at each sampling stage. The W-Ca-C-S sequence had the highest β-glucosidase activity at the BP stage in 2017 Kelburn. β-glucosidase activity in 2017 at Kelburn for the VE, R5, and R8 stages was greater by 6%, 12%, and 13% in the C-S-C-S sequence than the average of other sequences at each stage.

In 2019 at Carman, the highest β-glucosidase activity at the BP and VE stages was reported in the C-S-C-S and W-Ca-C-S sequences respectively. At R5 in 2017 at Carman, the activity was greater by 26% and 18% in the C-S-C-S and W-Ca-C-S sequences respectively than in the S-S-S-S sequence. β-glucosidase activity of the S-S-S-S and Ca-S-Ca-S sequences at VE for Carman in 2017 was lower by 26% and 24%, respectively, relative to the C-S-C-S sequence. In 2019 at Kelburn, the Ca-S-Ca-S sequence had the highest enzyme activity at BP. At VE, β-glucosidase activity was greater by 28% and 26% in the C-S-C-S and Ca-S-Ca-S sequences respectively, compared to the lower activity in the W-Ca-C-S sequence. At R5, the C-S-C-S sequence had the highest activity among crop sequences at Kelburn in 2019. At R8, the lowest β-glucosidase activity was observed in the S-S-S-S sequence while the differences were not significant among other three sequences at Kelburn in 2019.

The change in β-glucosidase activity was inconsistent among sequence treatments across sampling stages in each of the two study years. However, at nine out of sixteen sampling times in both years at both locations, β-glucosidase activity was significantly greater in the C-S-C-S sequence than S-S-S-S sequence. Although the W-Ca-C-S sequence includes both corn and wheat as high residue crops in the rotation, this reported greater or similar β-glucosidase activity

compared to C-S-C-S in only three of the sixteen sampling times. Although canola was a low residue crop, in five out of sixteen sampling times, the Ca-S-Ca-S sequence had greater or similar activity in relative to C-S-C-S. On average across all sampling stages, β-glucosidase activity in 2017 and 2019 was greater in the clay soil at Kelburn (167 and 191 µg PNP g^{-1} soil h^{-1}) than in loamy soil at Carman (69 and 68 µg PNP g^{-1} soil h^{-1}).

Table 4.4. The interaction effect of location × sampling stage × crop sequence on β-glucosidase activity at 0 – 8 cm depth in 2017 and 2019 at Carman and Kelburn, MB.

			β-glucosidase (μg PNP g^{-1} soil h^{-1})	
Location	Sampling stage†	Crop sequence§	2017	2019*
Carman	BP	S-S-S-S	73.2 *ijk*	57.2 *no*
		Ca-S-Ca-S	83.1 *ih*	58.1 *no*
		C-S-C-S	83.8 *ih*	78.8 *jkl*
		W-Ca-C-S	76.7 *ij*	58.8 *no*
	VE	S-S-S-S	63.3 *klm*	62.1 *n*
		Ca-S-Ca-S	67.9 *jkl*	64.5 *mn*
		C-S-C-S	90.6 *h*	84.9 *j*
		W-Ca-C-S	68.5 *jkl*	71.8 *lm*
	R5	S-S-S-S	53.7 *nm*	50.3 *op*
		Ca-S-Ca-S	65.8 *kl*	59.7 *n*
		C-S-C-S	71.5 *jk*	62.6 *n*
		W-Ca-C-S	68.4 *jkl*	45.6 *p*
	R8	S-S-S-S	46.3 *n*	82.4 *jk*
		Ca-S-Ca-S	64.3 *klm*	74.9 *kl*
		C-S-C-S	64.5 *kl*	84.9 *j*
		W-Ca-C-S	57.7 *lm*	94.8 *i*
Kelburn	BP	S-S-S-S	126.7 *g*	218.1 *b*
		Ca-S-Ca-S	164.0 *cd*	230.5 *a*
		C-S-C-S	169.2 *c*	202.7 *c*
		W-Ca-C-S	191.8 *b*	205.3 *c*
	VE	S-S-S-S	151.3 *ef*	186.6 *d*
		Ca-S-Ca-S	149.1 *f*	206.8 *c*
		C-S-C-S	163.1 *cd*	209.3 *bc*
		W-Ca-C-S	160.6 *cde*	163.7 *ef*
	R5	S-S-S-S	132.7 *g*	153.5 *gh*
		Ca-S-Ca-S	162.6 *cd*	159.9 *fg*
		C-S-C-S	167.2 *c*	168.9 *e*
		W-Ca-C-S	154.4 *def*	149.5 *gh*
	R8	S-S-S-S	170.3 *c*	179.1 *d*
		Ca-S-Ca-S	205.2 *a*	208.4 *c*
		C-S-C-S	214.6 *a*	209.7 *bc*
		W-Ca-C-S	194.2 *b*	206.0 *c*

Means followed by the different letters are significantly different at $\alpha = 0.05$ within each year.
†BP (Before planting), VE (Emergence), R5 (Beginning seeds), R8 (Full maturity)
§S-S-S-S (continuous soybean), Ca-S-Ca-S (canola-soybean-canola-soybean), C-S-C-S (corn-soybean-corn-soybean), W-Ca-C-S (wheat-canola-corn-soybean).
*Note that in 2019 the W-Ca-C-S sequence was in the canola phase and all other sequences were in the soybean phase.

4.4.2.2 β-glucosaminidase

β-glucosaminidase is an important soil enzyme for C and N mineralization in the soil. Similar to β-glucosidase, it was hypothesized that β-glucosaminidase activity would be greater in the C-S-C-S and W-Ca-C-S sequences than in the S-S-S-S sequence due to high biomass production by corn and wheat. As hypothesized, in 2017 at Carman, the C-S-C-S sequence had the highest β-glucosaminidase activity at the BP, VE, and R5 sampling stages (Table 4.5). There were no differences between sequence treatments at the R8 stage at Carman in 2017. The S-S-S-S sequence reported lower β-glucosaminidase activity at BP (35%), VE (31%), and R5 (41%) compared to the C-S-C-S sequence at each sampling stage. In 2017 at Kelburn, enzyme activity in the C-S-C-S and W-Ca-C-S sequences was greater by 34% and 28% at the BP stage, and by 20% and 23% at the R5 stage compared to the S-S-S-S sequence, respectively. At the VE stage in 2017 at Kelburn, the C-S-C-S and S-S-S-S sequences had the highest and lowest β-glucosaminidase activities, respectively. The highest activity at R8 was observed in the C-S-C-S sequence.

β-glucosaminidase activity at the BP and R5 sampling stage in 2019 at Carman was highest in the C-S-C-S sequence. Activity in the C-S-C-S and W-Ca-C-S sequences were greater by 38% and 25% at VE and by 18% and 23% respectively at R8 than the S-S-S-S sequence in 2019 at Carman. At Kelburn in 2019, the S-S-S-S sequence reported the lowest β-glucosaminidase activity at the BP and R8 sampling stages and the differences were not significant among the other three sequences at each stage. At VE and R5 sampling stages, the highest activity was observed in the W-Ca-C-S sequence.

Although β-glucosaminidase activity was not consistent among sampling stages and crop sequences, this enzyme agreed with our hypothesis of greater β-glucosaminidase activity when soybean was followed by a high residue crop in the rotation. Among sixteen sampling times, the C-S-C-S sequence had significantly greater β-glucosaminidase activity in eleven times compared to S-S-S-S. In five out of sixteen sampling points, the W-Ca-C-S sequence had greater or similar enzyme activity in relative to C-S-C-S.

Table 4.5. The interaction effect of location × sampling stage × crop sequence on β-glucosaminidase activity at 0 – 8 cm depth in 2017 and 2019 at Carman and Kelburn, MB.

			β-glucosaminidase (μg PNP g^{-1} soil h^{-1})	
Location	Sampling stage†	Crop sequence§	2017	2019*
Carman	BP	S-S-S-S	24.3 *n*	35.4 *f*
		Ca-S-Ca-S	31.2 *ij*	31.9 *h*
		C-S-C-S	37.2 *bcdef*	44.2 *cd*
		W-Ca-C-S	25.2 *mn*	30.4 *gh*
	VE	S-S-S-S	24.0 *n*	24.5 *i*
		Ca-S-Ca-S	25.4 *mn*	33.1 *fg*
		C-S-C-S	34.5 *fgh*	30.6 *gh*
		W-Ca-C-S	28.0 *klm*	25.3 *i*
	R5	S-S-S-S	29.1 *jk*	20.8 *jkl*
		Ca-S-Ca-S	32.6 *hi*	19.9 *l*
		C-S-C-S	41.6 *a*	24.9 *i*
		W-Ca-C-S	35.8 *defg*	23.6 *ijk*
	R8	S-S-S-S	23.2 *n*	40.7 *e*
		Ca-S-Ca-S	24.7 *n*	40.3 *e*
		C-S-C-S	25.7 *lmn*	47.0 *bc*
		W-Ca-C-S	24.0 *n*	48.7 *b*
Kelburn	BP	S-S-S-S	29.5 *jk*	29.6 *h*
		Ca-S-Ca-S	34.1 *gh*	41.6 *de*
		C-S-C-S	38.5 *bcd*	42.7 *de*
		W-Ca-C-S	37.6 *bcde*	41.8 *de*
	VE	S-S-S-S	24.1 *n*	24.2 *ij*
		Ca-S-Ca-S	28.2 *kl*	25.1 *i*
		C-S-C-S	38.8 *abc*	23.0 *ijkl*
		W-Ca-C-S	35.0 *efgh*	28.6 *h*
	R5	S-S-S-S	30.3 *ijk*	20.7 *kl*
		Ca-S-Ca-S	34.4 *gh*	23.5 *ijk*
		C-S-C-S	36.1 *cdefg*	23.9 *ijk*
		W-Ca-C-S	37.4 *bcde*	30.2 *gh*
	R8	S-S-S-S	35.5 *efg*	44.3 *cd*
		Ca-S-Ca-S	36.8 *cdefg*	59.0 *a*
		C-S-C-S	39.9 *ab*	59.5 *a*
		W-Ca-C-S	35.9 *defg*	58.9 *a*

Means followed by the different letters are significantly different at α = 0.05 within each year.
†BP (Before planting), VE (Emergence), R5 (Beginning seeds), R8 (Full maturity)
§S-S-S-S (continuous soybean), Ca-S-Ca-S (canola-soybean-canola-soybean), C-S-C-S (corn-soybean-corn-soybean), W-Ca-C-S (wheat-canola-corn-soybean).
*Note that in 2019 the W-Ca-C-S sequence was in the canola phase and all other sequences were in the soybean phase.

4.4.2.3 Acid phosphatase

Acid phosphatase is important for P cycling as it catalyzes the hydrolysis of organic P in the soil. Similar to the other two enzymes the hypothesis was that the acid phosphatase activity will be greater in the C-S-C-S and W-Ca-C-S sequences which produce greater amount of crop residue than continuous soybean. As expected, in 2017 at Carman, the highest acid phosphatase activity at BP sampling stage was observed in the C-S-C-S sequence, while the W-Ca-C-S sequence had the highest acid phosphatase activity at both VE and R8 sampling stages (Table 4.6). At the R5 stage, enzyme activity in the C-S-C-S and W-Ca-C-S sequences were greater by 46% and 50% than the S-S-S-S sequence. In 2017 at Kelburn, acid phosphatase activity was greater by 46% and 42% in the Ca-S-Ca-S and W-Ca-C-S sequences respectively, relative to the S-S-S-S sequence. The C-S-C-S and W-Ca-C-S sequences had the highest acid phosphatase activity at the VE and R5 sampling stages, respectively. At R8 in 2017 at Kelburn, activity was greater by 37% and 35% in the Ca-S-Ca-S and C-S-C-S sequences respectively, compared to the S-S-S-S sequence. Acid phosphatase activity was lowest in the S-S-S-S sequence at BP, VE, and R5 stages in 2017 at Carman and compared to all the sampling stages in 2017 at Kelburn.

In 2019 at Carman, acid phosphatase activity in the C-S-C-S and Ca-S-Ca-S sequences was greater by 31% and 28% at the BP stage, by 23% and 19% at the VE stage, and by 12% and 11% at the R8 stage respectively, compared to the S-S-S-S sequence. At the R5 stage, activity was greater by 10% and 9% in the W-Ca-C-S and Ca-S-Ca-S sequences, respectively, than the S-S-S-S sequence. At Kelburn in 2019, acid phosphatase activity was mostly similar among crop sequences at the BP stage. At the VE stage, enzyme activity in the S-S-S-S was 6% lower compared to the average of other three sequences. At the R5 stage, the W-Ca-C-S had greater acid phosphatase activity (18%) than the average of the other three sequences. At the R8 stage, the Ca-S-Ca-S and S-S-S-S sequences had the highest and lowest acid phosphatase activity, respectively.

There were inconsistent trends in the activity of acid phosphatase activity among sampling stages and crop sequence treatments in both years. However, seven out of the sixteen sampling times, enzyme activity was significantly greater in the C-S-C-S sequence compared to S-S-S-S sequence. Greater or similar acid phosphatase activity in the W-Ca-C-S sequence in relative to

C-S-C-S was observed in seven of the sixteen sampling times. Unexpectedly, the Ca-S-Ca-S sequence had greater or similar activity in seven times of the sixteen sampling times when compared to the C-S-C-S sequence.

Table 4.6. The interaction effect of location × sampling stage × crop sequence on acid phosphatase activity at 0 – 8 cm depth in 2017 and 2019 at Carman and Kelburn, MB.

			Acid phosphatase (µg PNP g^{-1} soil h^{-1})	
Location	Sampling stage†	Crop sequence§	2017	2019*
Carman	BP	S-S-S-S	222.4 *kl*	231.6 *op*
		Ca-S-Ca-S	312.1 *ef*	296.8 *gh*
		C-S-C-S	339.1 *d*	303.0 *g*
		W-Ca-C-S	269.8 *hi*	266.8 *kl*
	VE	S-S-S-S	239.8 *jk*	204.3 *qr*
		Ca-S-Ca-S	271.7 *hi*	242.2 *no*
		C-S-C-S	271.1 *hi*	251.0 *mn*
		W-Ca-C-S	284.5 *gh*	205.0 *qr*
	R5	S-S-S-S	215.6 *l*	207.7 *q*
		Ca-S-Ca-S	282.8 *h*	226.9 *p*
		C-S-C-S	314.2 *ef*	194.7 *r*
		W-Ca-C-S	322.8 *def*	228.0 *p*
	R8	S-S-S-S	209.6 *l*	241.6 *no*
		Ca-S-Ca-S	227.0 *kl*	268.1 *kl*
		C-S-C-S	210.8 *l*	270.4 *jk*
		W-Ca-C-S	305.3 *fg*	258.0 *lm*
Kelburn	BP	S-S-S-S	221.3 *kl*	340.2 *de*
		Ca-S-Ca-S	321.8 *def*	333.4 *ef*
		C-S-C-S	282.2 *hi*	347.1 *d*
		W-Ca-C-S	312.6 *ef*	340.4 *de*
	VE	S-S-S-S	288.3 *gh*	276.6 *jk*
		Ca-S-Ca-S	324.5 *def*	300.9 *gh*
		C-S-C-S	371.1 *c*	289.5 *hi*
		W-Ca-C-S	331.4 *de*	295.5 *gh*
	R5	S-S-S-S	261.0 *ij*	265.2 *kl*
		Ca-S-Ca-S	323.1 *def*	280.3 *ij*
		C-S-C-S	331.7 *de*	291.9 *gh*
		W-Ca-C-S	382.8 *c*	328.6 *f*
	R8	S-S-S-S	382.2 *c*	344.7 *de*
		Ca-S-Ca-S	521.7 *a*	477.4 *a*
		C-S-C-S	514.8 *a*	363.1 *c*
		W-Ca-C-S	476.2 *b*	417.7 *b*

Means followed by the different letters are significantly different at $\alpha = 0.05$ within each year.
†BP (Before planting), VE (Emergence), R5 (Beginning seeds), R8 (Full maturity)
§S-S-S-S (continuous soybean), Ca-S-Ca-S (canola-soybean-canola-soybean), C-S-C-S (corn-soybean-corn-soybean), W-Ca-C-S (wheat-canola-corn-soybean).
*Note that in 2019 the W-Ca-C-S sequence was in the canola phase and all other sequences were in the soybean phase.

4.4.2.4 Differences Between Crop Sequences

Previous studies have found soil enzymes to be sensitive soil health indicators as their activity responded to changes in the agronomic management practices (Dick et al., 1996; Bandick and Dick, 1999; Balota et al. 2004). This study was the first to look at using these enzymes to distinguish between crop rotation treatments in Manitoba. We observed that there were inconsistencies in the three enzyme activities among sampling stages and crop sequence treatments. However, β-glucosidase, β-glucosaminidase, and acid phosphatase activities were frequently greater in the C-S-C-S sequence across all sampling stages in both years compared to the S-S-S-S sequence. Similarly, Ekenler and Tabatabai (2002), Dodor and Tabatabai (2003a), and Dodor and Tabatabai (2005) found that β-glucosaminidase, acid phosphatase, and β-glucosidase activities were greater by 70%, 12%, and 31% in the 0 N kg ha^{-1} fertilizer applied C-S-C-S rotations compared to 0 N kg ha^{-1} fertilizer applied continuous soybean in a 17-year rotation study conducted in Iowa. The range of β-glucosidase and acid phosphatase activity between C-S-C-S and S-S-S-S in this long-term study was similar to our experiment. The reported range of β-glucosaminidase activity in this long-term study was greater compared to the activity observed in our study (approximately 30%).

The W-Ca-C-S sequence had greater β-glucosaminidase and acid phosphatase activities on average than the S-S-S-S sequence. In the W-Ca-C-S sequence, corn was grown as the previous crop before the soybean test crop in 2017 and wheat as the previous crop before canola in 2019. Corn and wheat add a greater amount of crop residue to the soil compared to soybean or canola (Appendix B – Table 4). Hence, including corn or wheat in rotations increases the supply of substrate available for microbial growth. β-glucosidase activity increases in soils with more residue as substrates because this enzyme plays a major role in organic matter decomposition, as it catalyzes the hydrolysis of cellulose (Deng and Popova 2011). Ekenler and Tabatabai (2002) described that improved soil structure, stabilized microclimate, and greater root density may be the reasons for greater β-glucosaminidase activity in soils under diverse crop rotations. Furthermore, Dodor and Tabatabai (2003a) reported that the quality of the organic residues as determined by the C:N ratio, significantly influences phosphatase activity by enhancing the soil microbial activities due to increased organic C content.

Canola is a low residue crop compared to corn and wheat (Appendix B – Table 4). Unexpectedly, this study found greater or similar β-glucosidase and acid phosphatase activities when soybean was grown in rotation with canola as a previous crop when compared to corn as previous crop. Canola has greater C:N ratio (on average 49) in relative to soybean (on average 34) which could increase the amount of carbohydrates for soil microbes and facilitate enzymes secretion (Dodor and Tabatabai 2003a). Cool temperature and high soil moisture at the beginning and end of the growing season, drier conditions in the mid-season, changes to soil nutrient statues due to crop uptake, quantity and quality of crop residue remaining in the soil, and timing of sampling may have contributed to the inconsistent behavior of soil enzymes across sampling stages and crop sequence treatments in this study (Klose and Tabatabai 2000; Dodor and Tabatabai 2003b; Balota et al. 2004; Gałązka et al. 2017).

The Kelburn location had greater β-glucosidase activity on average than at the Carman location across all sampling stages and in both years. This may be due to differences in the soil organic C concentrations at two locations, where the clay soil at Kelburn had greater organic C content at 0-15 cm (on average 4.8%) compared to the loamy soil at Carman (on average 2.0%). This finding agrees with previous research reported by Ekenler and Tabatabai (2002) and Dodor and Tabatabai (2005) where β-glucosaminidase and glycosidases (α- and β-glucosidases and α- and β-galactosidases) activities were significantly correlated with soil organic C content.

4.4.2.5 Differences Between Sampling Stages

Soil enzymes activities were compared among all sampling stages at both locations in 2017 and 2019 to find the best sampling stage to identify the differences between crop sequence treatments (Figure 4.3). It was hypothesized that enzyme activities will be greater at BP and R8 sampling stages compared to mid-growing season sampling. Greater amounts of crop residue are typically available at the beginning and end of the growing season which could enhance enzyme activity. As expected, β-glucosidase activity was greater by 16% and 11% in 2017 (Figure 4.3A) and by 34% and 31% in 2019 (Figure 4.3B) at the R8 and BP sampling stages compared to the R5 sampling stage. β-glucosaminidase activity in 2017 was greater by 17% at the R5 sampling stage than the VE stage (Figure 4.3C). In 2019, β-glucosaminidase activity was greater by 113% and 59% at the R8 and BP stages than at the R5 sampling stage (Figure 4.3D). Acid phosphatase

activity was greater in the R8 sampling stage by 24% compared to the VE stage in 2017 (Figure 4.3E) and by 31% compared to the R5 stage in 2019 (Figure 4.3F).

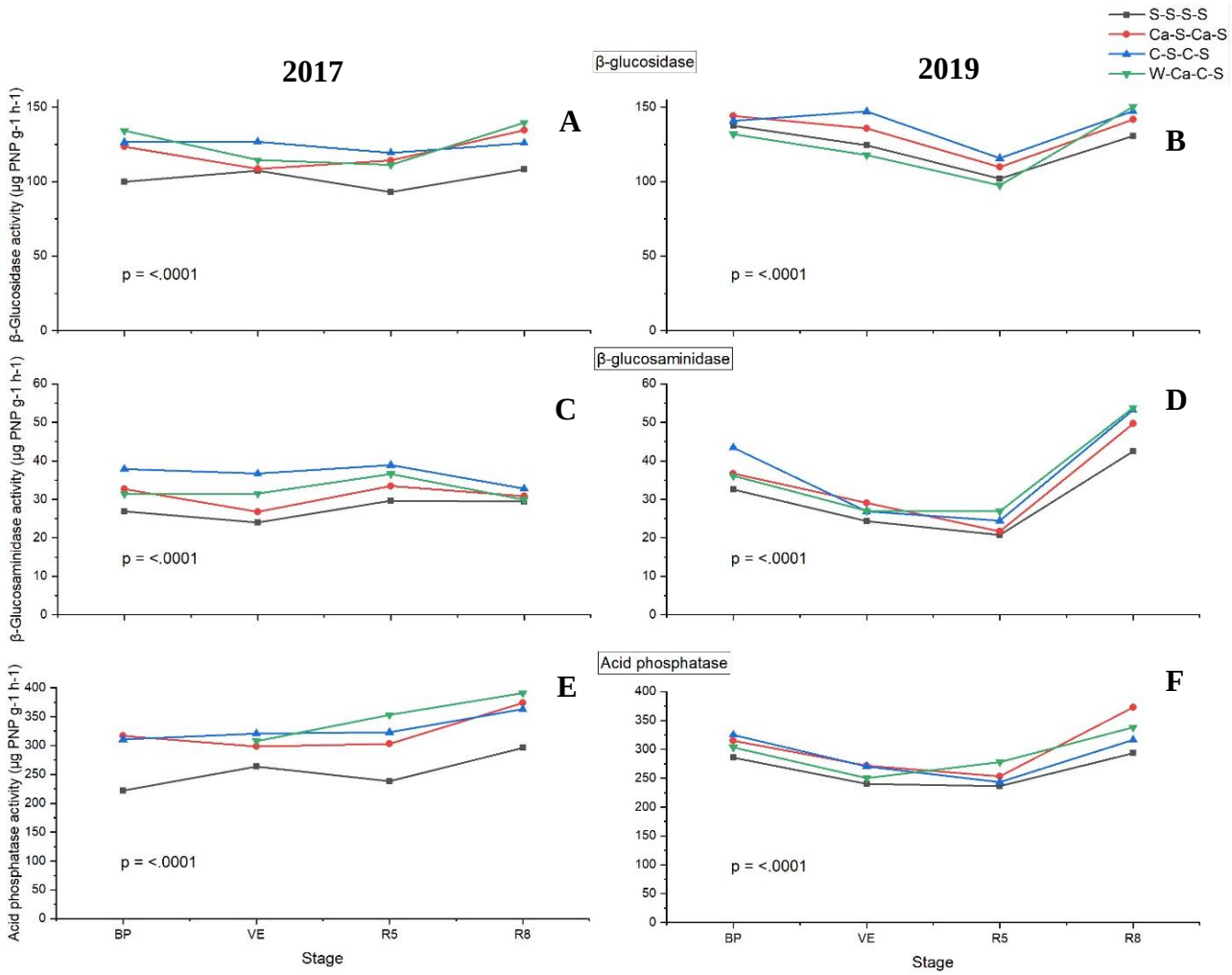

Figure 4.3. Interaction effect of crop sequence × sampling stage on β-glucosidase activity in 2017 (A) and 2019 (B), β-glucosaminidase activity in 2017 (C) and 2019 (D), and acid phosphatase activity in 2017 (E) and 2019 (F) averaged over Carman and Kelburn locations.

Biological activity in the soil is also influenced by precipitation and temperature, which can cause seasonal fluctuations in enzyme activities (Nannipieri et al. 1979; Bandick and Dick 1999). The mean monthly precipitation across both locations in April at the BP stage was closer to the long-term normal (27 mm) in 2017 (22 mm) and was lower in 2019 (17 mm) (Figure 4.2). During the mid-growing season (VE – R5 stages) from June – August precipitation was lower in both 2017 (42 mm) and 2019 (61 mm) when compared to the long-term normal (80 mm), except in July 2019 at Kelburn. At the end of the growing season (R8 stage) in September, precipitation was greater in both 2017 (71 mm) and 2019 (157 mm) than the long-term normal (50 mm). The mean monthly temperature was closer to the long-term normal and ranged from 7 – 8 °C at the beginning, 18 – 19 °C in the middle, and 13 – 14 °C at the end of the growing season across two locations in both years. We observed greater levels of soil enzyme activities on average at the beginning and end of the growing season and lower activities in the mid-growing season. Similar to this study, Tripathi et al. (2007) observed greater activities of β-glucosidase, urease, acid phosphatase, and alkaline phosphatase in the spring and a decrease in their activities during the summer under a rice cultivation system in India. Furthermore, Wolinska and Stępniewska (2012) reported that the activities of soil enzymes greatly depend on the season of the year and those greater activities are found in the spring due to increased microbial activity.

In this study, greater activity may have also been due to the presences of adequate soil moisture that are favorable to promote the decomposition of crop residue at the start and end of the growing season. After overwintering, residues from the previous crop are more available for soil microbes to feed on at the beginning of the growing season (Moulin 1994; Pelster et al. 2013). Moreover, more residues are added into the soil at the end of the growing season due to senescence of soybean leaves, which increases the amount of substrate for soil microbes. In both 2017 and 2019, drought conditions would have decreased the microbial activities and enzymes production in the mid-growing season (Sardans et al. 2008).

4.4.3 Rotation Effect on Soil Active Carbon

Active C is a labile fraction of soil organic C and has previously shown to be sensitive to agricultural management practices such as crop rotations. It was hypothesized that soil active C levels will be greater in the C-S-C-S and W-Ca-C-S sequences as they produce more residue in comparison to the S-S-S-S treatment. There was a significant location × sampling stage × crop sequence interaction for active C in 2017 (Table 4.3). In 2019, the location × crop sequence interaction was significant (Table 4.3).

As expected, the C-S-C-S sequence and S-S-S-S sequence had the highest and lowest active C level, respectively, at the BP and R8 sampling stages at both locations in 2017 (Table 4.7). In 2017 at Carman, active C levels at the BP stage in the S-S-S-S, Ca-S-Ca-S and C-S-C-S sequences were not significantly different from the same crop sequences at the R8 stage, while active C in the W-Ca-C-S sequence decreased by 4% at R8 compared to that at the BP stage. Although corn was the previous crop before the soybean test crop in 2017 for both the C-S-C-S and W-Ca-C-S sequences, active C levels were lower by 5% at Carman and by 4% at Kelburn on average across two sampling stages in relative to the C-S-C-S sequence. In 2019, the highest active C level was observed in the C-S-C-S sequence at Carman, while at Kelburn greater levels were reported in the Ca-S-Ca-S and C-S-C-S sequences compared to S-S-S-S (Table 4.8). The S-S-S-S sequence had the lowest active C at both locations. Although canola is a low residue crop (Appendix B – Table 4), active C was greater on average by 6% in 2017 and by 5% in 2019 across two locations in relative to the S-S-S-S sequence. On average, a greater active C level was observed in the clay soil at Kelburn in both 2017 (57%) and 2019 (58%) compared to the loamy soil at Carman.

Table 4.7. The interaction effect of location × sampling stage × crop sequence on soil active C at 0 – 8 cm depth in 2017 at Carman and Kelburn, MB.

Location	Sampling stage†	Crop sequence§	Active C (mg kg^{-1} dry soil)
Carman	BP	S-S-S-S	573 *i*
		Ca-S-Ca-S	644 *g*
		C-S-C-S	674 *f*
		W-Ca-C-S	637 *g*
	R8	S-S-S-S	575 *i*
		Ca-S-Ca-S	641 *g*
		C-S-C-S	662 *f*
		W-Ca-C-S	614 *h*
Kelburn	BP	S-S-S-S	948 *e*
		Ca-S-Ca-S	983 *bc*
		C-S-C-S	1027 *a*
		W-Ca-C-S	978 *cd*
	R8	S-S-S-S	946 *e*
		Ca-S-Ca-S	965 *d*
		C-S-C-S	1026 *a*
		W-Ca-C-S	993 *b*

Means followed by the different letters are significantly different at $\alpha = 0.05$.
†BP (Before planting), R8 (Full maturity)
§S-S-S-S (continuous soybean), Ca-S-Ca-S (canola-soybean-canola-soybean), C-S-C-S (corn-soybean-corn-soybean), W-Ca-C-S (wheat-canola-corn-soybean).

Table 4.8. The interaction effect of location × crop sequence on soil active C at 0 – 8 cm depth in 2019 at Carman and Kelburn, MB.

Location	Crop sequence§	Active C (mg kg^{-1} dry soil)
Carman	S-S-S-S	537 *f*
	Ca-S-Ca-S	571 *e*
	C-S-C-S	589 *d*
	W-Ca-C-S*	559 *e*
Kelburn	S-S-S-S	847 *c*
	Ca-S-Ca-S	915 *a*
	C-S-C-S	921 *a*
	W-Ca-C-S*	885 *b*

Means followed by the different letters are significantly different at $\alpha = 0.05$.
§S-S-S-S (continuous soybean), Ca-S-Ca-S (canola-soybean-canola-soybean), C-S-C-S (corn-soybean-corn-soybean), W-Ca-C-S (wheat-canola-corn-soybean).
*Note that in 2019 the W-Ca-C-S sequence was in the canola phase and all other sequences were in the soybean phase.

Active C, also known as permanganate oxidizable C is the labile fraction of soil organic C that is readily available for soil microorganisms (Culman et al. 2012). This fraction of soil C is mainly present in the topsoil layer and responds rapidly to agronomic management practices (Weil et al. 2003; Van Es et al. 2017; Jagadamma et al. 2019). In this study, soil active C levels increased in the crop sequences with corn rather than the continuous soybean at both locations in both years. This is consistent with the observations of Jagadamma et al. (2019) where S-C rotations had greater active C levels at 0 – 15 cm depth compared to S-S treatment in a long-term rotation study at Tennessee. Furthermore, in a long-term rotation study conducted in Ontario, Van Eerd et al. (2014) found that C-S rotations had greater soil organic C levels at 0 – 5 and 0 – 20 cm depths compared to S-S treatment.

Corn adds a greater amount of crop residue (on average 4870 kg ha^{-1}) to the soil compared to soybean (on average 2646 kg ha^{-1}), which can increase the organic matter content and labile C in the soil (Culman et al. 2013; Van Eerd et al. 2014). Furthermore, the C:N ratio of corn is greater (on average 64) than soybean (on average 34) which could enhance soil organic C content. Greater C:N ratio of canola residue (on average 49) would have increased the active C level in the Ca-S-Ca-S sequence in relative to the S-S-S-S sequence in this study. Similar to soil enzymes activities, we observed greater active C level in the clay soil at Kelburn location where it had greater soil organic C concentration compared to the loamy soil at Carman location.

4.4.4 Rotation Effect on Bacterial Population

The differences in bacterial community between crop sequence treatments and sampling stages were analyzed using principal coordinate analysis (PCoA). Differences and similarities between crop sequence treatments were identified using PCoA graphs by looking for separate clusters between crop sequence treatments. It was hypothesized that there would be differences in the bacterial families among crop sequence treatments. Unexpectedly, after 4 and 6 years of growing soybean continuously and in contrasting rotations, PCoA did not identify differences in the bacterial families between the crop sequence treatments as there were no separation among clusters (Figure 4.4).

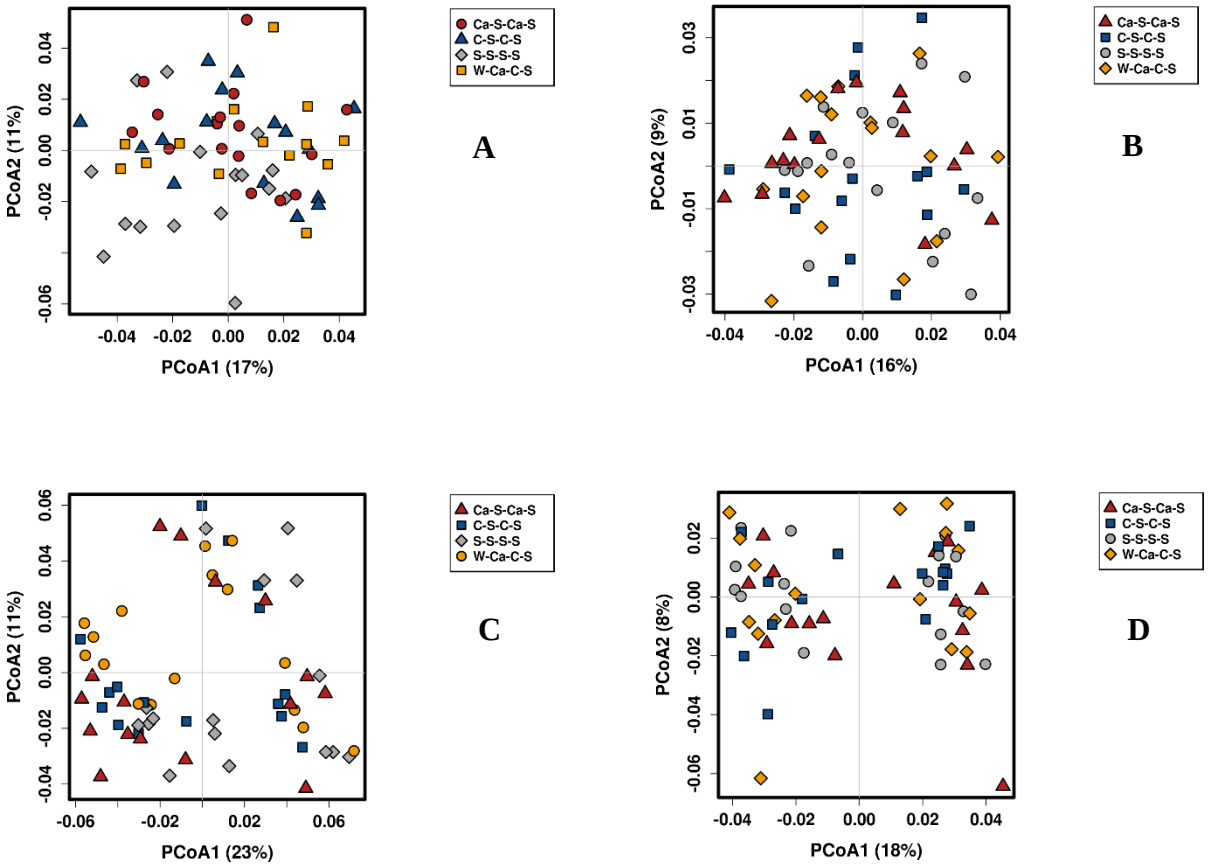

Figure 4.4. Principle coordinate analysis of bacterial families between continuous soybean (S-S-S-S), canola-soybean-canola-soybean (Ca-S-Ca-S), corn-soybean-corn-soybean (C-S-C-S), and wheat-canola-corn-soybean (W-Ca-C-S) crop sequence treatments at (A) Carman 2017, (B) Kelburn 2017, (C) Carman 2019, and (D) Kelburn 2019 in MB.

Although PCoA did not identify differences among crop sequence treatments, it did identify differences between sampling stages (Figure 4.5). At Carman in 2017, which was the common soybean test crop year, bacterial families at the BP and R8 stages formed their own clusters that were distinct from other sampling stages (Figure 4.5A). At VE the clusters were more scattered and overlapped with other sampling stages. Bacterial families at the R5 stage occurred in a cluster between the BP and R8 stages. In 2017 at the Kelburn location, the BP and R8 bacterial families formed distinct clusters from other sampling stages (Figure 4.5B). At VE, bacterial families were more tightly clustered and distinct from the rest of the sampling stages. Bacterial families at the R5 stage clustered between the BP and R8 sampling stages. In 2019, where three out of four crops were soybean and one was canola, bacterial families at the BP, R5, and R8

stages formed their own separate clusters at the Carman location (Figure 4.5C). At the Kelburn location in 2019, bacterial families at the BP and R8 stages formed separate clusters that were distinct from other sampling stages (Figure 4.5D). The R5 bacterial families overlapped with R8 but were distinct from the BP and VE stages. At both locations in 2019, the VE clusters were more scattered and distinct from the R5 and R8 stages but were overlapped with the BP stages.

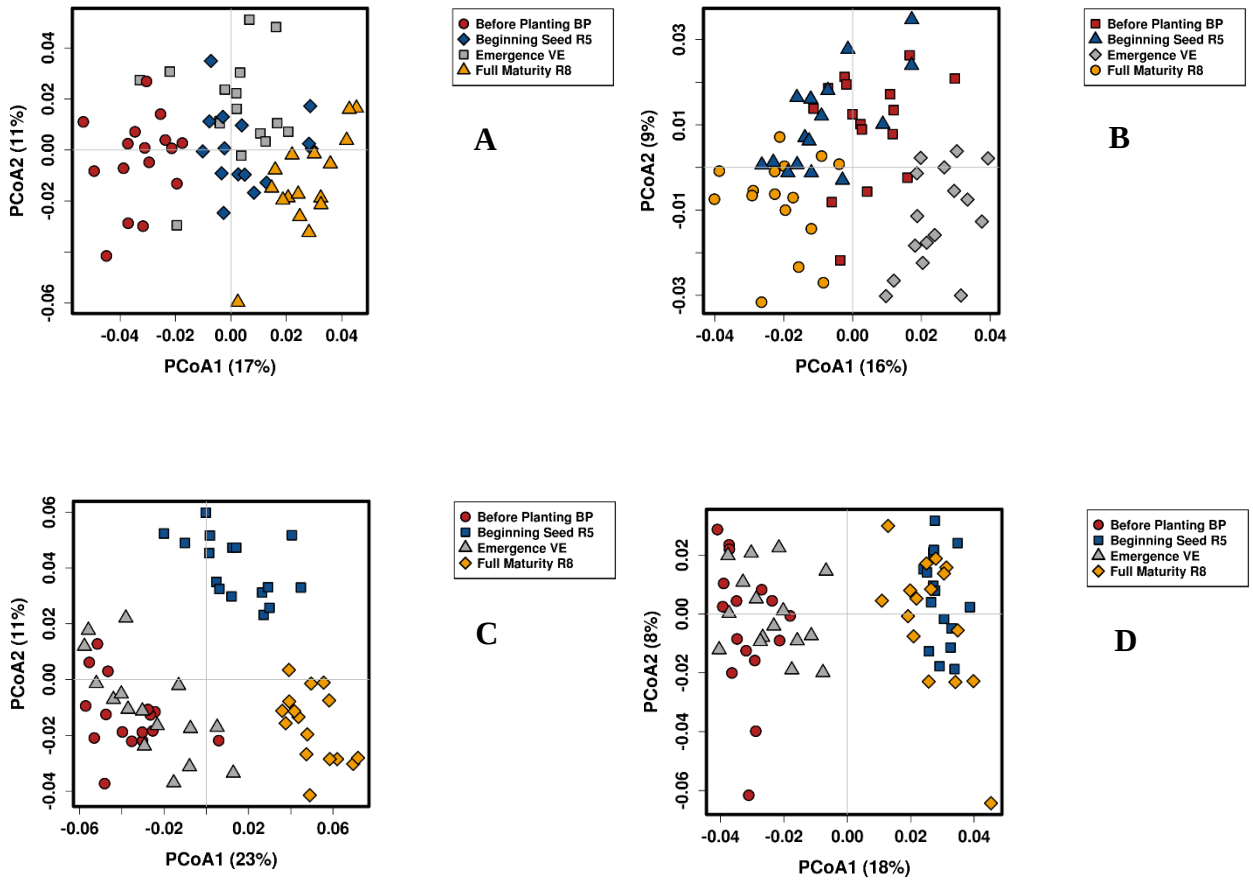

Figure 4.5. Principle coordinate analysis of bacterial families between sampling stages of before planting (BP), emergence (VE), beginning seeds (R5), and full maturity (R8) at (A) Carman 2017, (B) Kelburn 2017, (C) Carman 2019, and (D) Kelburn 2019 in MB.

Microbial community analysis is another measure of biological soil health as these communities are sensitive to the changes in crop and soil management practices (Lin et al. 2017; Liu et al. 2020; Yuan et al. 2021). Although microbial communities have been found to be responsive to land management practices, we did not find them to be responsive to our crop sequence treatments based on PCoA analysis. Similarly, Li et al. (2010) reported that soil microbial diversity was not significantly different between S-S and S-C rotations in northeast China. Furthermore, Neupane et al. (2021) found that the microbial populations were not significantly different between C-C-C, C-C-S, C-S-C, and S-C-S rotations in a long-term study (12 years) conducted in Illinois. This different response of bacterial communities to crop rotation treatments may be due to differences in the soil types, soil moisture, temperature, duration of the crop rotations, and the type of crops across the studies (Ishaq et al. 2020; Yuan et al. 2021).

In the literature, differences in microbial communities have been found to be significant when comparing long term crop rotations vs long term continuous cropping, till vs no-till, organic vs conventional, cover crop vs no-cover crop studies or with a combination of crop and soil management practices (Balota et al. 2003; Acosta-Martinez et al. 2007; Gałązka et al. 2017; Lupatini et al. 2017; D'Acunto et al. 2018; Kim et al. 2020). In this study, the use of tillage may have masked crop sequence treatment effects on soil bacterial populations at both locations. Furthermore, the relatively short duration of the crop sequence treatments (4 and 6 years) may have not caused significant changes to soil microbial populations and diversity.

This study found that bacterial family populations were significantly different between sampling stages. Differences were observed at the beginning and at the end of the growing season, as bacterial families at the BP and R8 stages formed their own clusters that were distinct from other mid-season sampling stages. Similarly, Gałązka et al. (2017) reported that total number of bacteria and fungi in the soil were significantly different between before sowing and after harvest in a nine-year corn monoculture treatment in Poland. Seasonal variation of both soil enzyme activities and bacterial population is associated with the soil moisture, temperature, and availability of substrates. The metabolism and respiration of soil microbes during the growing season can be affected by cool soil temperature and high soil moisture at the seeding, drier and warmer soil conditions in the mid-season, and lower amount of soybean crop residue added into

the soil during the growing season (Zhao et al. 2014; Gałązka et al. 2017; Ishaq et al. 2020). The findings of this study suggest that environmental and crop factors can alter the microbial activities and their abundance throughout the cropping period.

4.5 Summary

In conclusions, soil enzymes, and active C levels were significantly affected by sequence treatments, but no differences were observed in bacterial families between crop sequence treatments according to PCoA. This study found growing soybean in rotation with corn enhanced enzyme activities and active C in loamy and clay soils in Manitoba compared to growing soybean continuously. Greater amount of crop residue added from the previous corn crop likely enhanced microbial enzyme secretions and amount of active C in the soil. If farmers are growing soybean (low residue crop) frequently in rotation, it is important to balance the rotation with high residue crops (corn) to maintain or improve soil health.

According to the findings of this study, active C can be used by farmers and agronomists in Manitoba to identify the effects of crop management practices like crop rotation. Soil enzymes also showed potential as biological soil health indicators to compare cropping practices, but more research is needed to understand their behavior under different crop rotations, soil types, and environmental conditions.

This study found greater enzyme activity on average at BP and R8 stages across all sampling stages in both years, compared to the middle of the growing season (Figure 4.3). The differences in the active C were observed at the BP and R8 stages (Table 4.7 and 4.8). According to PCoA, bacterial families formed separate clusters at BP and R8 that were distinct from other sampling stages (Figure 4.5). Hence, there is flexibility to be able to sample at both time points. More research is needed to confirm the best sampling stage identified in this study. Decisions about sampling timing might depend on the history of the field and the differences a farmer wants to compare, such as the effect of crop residues from previous years vs changes that occurring during the current growing season. As soil nutrient testing is conducted in the fall and spring, the results of this study support that it may be practical to combine soil nutrient and soil health sampling in either the fall or spring.

5.0 GENERAL DISCUSSION AND CONCLUSIONS

5.1 General Discussion

The acreage of growing soybean in Manitoba crop rotations has increased since the early 2000s. Being a relatively new crop to Manitoba, it is important to identify the effects of growing soybean in rotation with other commonly grown crops in the province such as canola, wheat, and corn. Hence, this research was conducted to evaluate the impacts of frequency of soybean in crop rotations on soybean seed yield and quality, potential for biological nitrogen fixation (BNF), and belowground responses of soil enzyme activities, bacterial population, and soil active C dynamics.

The study was initiated in 2014 at three locations (Carman, Kelburn, and Melita) in Manitoba to evaluate the soybean performance under four soybean crop sequences. The crop sequence treatments were: 1) continuous soybean (S-S-S-S), 2) canola-soybean-canola-soybean (Ca-S-Ca-S), 3) corn-soybean-corn-soybean (C-S-C-S), and 4) wheat-canola-corn-soybean (W-Ca-C-S). The third chapter of this thesis discussed the rotation effect on soybean production and impacts on BNF parameters in the soybean test crop year (2017). The experiment continued for the second rotation cycle in 2018 at two locations (Carman and Kelburn) and the emphasis was shifted to soil health. The fourth chapter focused on biological soil health and discussed the changes in soil enzymes activities, bacterial population dynamics, and active C in the 4^{th} (2017 and 6^{th} (2019) years of the experiment.

Soybean is economically important as its seeds are a major source of plant-based oil and protein. The study found that the preceding crops in rotation had no effect on soybean yield over the four-year cropping cycle in Manitoba. Significant differences among crop sequences were found for soybean seed quality, dry matter yield (DMY), above ground N uptake, and potential for biological nitrogen fixation. However, the continuous soybean sequence was not consistently different form sequences where soybean was grown in rotation with canola, corn, and wheat.

The effect on symbiotic associations of bradyrhizobia for BNF with soybean was evaluated in the soybean test crop year. Soybean nodulation and relative ureide N were affected by crop sequence treatments. The highest nodulation at Carman and Melita locations was observed in the

S-S-S-S sequence. This may be due to presence of more *Bradyrhizobium japonicum* in the soil, as S-S-S-S received rhizobium inoculant every year. The W-Ca-C-S sequence at Melita had lower kernel protein content, above ground N uptake, root nodulation, and RUN content which could be caused by a small number of *B. japonicum* compared to the other three sequences at the same location. The Melita location had no history of soybean and the soybean test crop in 2017 was the first year that W-Ca-C-S sequence received the *B. japonicum* inoculant. Both factors could have contributed to the reduced BNF in that crop sequence.

Understanding the impacts of crop rotation practices on soil health indicators provides valuable information for scientists, policy makers, farmers, and land managers for making crop management decisions. However, the magnitude of the potential soil health benefits from crop rotation depends upon the crop species diversity and length of the rotation. Furthermore, sampling timing is also important to detect the differences between cropping practices. Soils were collected from each treatment before planting (BP), and at the soybean growth stages of emergence (VE), beginning seeds (R5), and full maturity (R8) for biological soil health analysis.

Soil enzymes of β-glucosidase, β-glucosaminidase, and acid phosphatase identified differences between the crop sequence treatments, but these differences were not consistent between sampling stages, crop sequences, locations, and years. However, enzyme activity was frequently greater in the C-S-C-S sequence compared to the S-S-S-S sequence across sampling stages in both years. Corn adds more residue to the soil compared to soybean which will increase the amount of substrates available for soil microbes. Secretion of soil enzymes by microorganisms was enhanced when greater amounts of substrates were available for them to feed on. Across two years, the activities of three enzymes during the growing season were greater at the BP and R8 stages compared to the mid-season sampling. More substrates are available for soil microbes at BP from previous crop residue and, at R8 due to senescence of leaves, which would stimulate the enzymes production. Furthermore, this study found a significant rotation effect on soil active C, as rotations with corn had greater active C levels than the continuous soybean treatment. Corn can increase the organic matter content and labile C in the soil as they add greater amount of crop residue and have greater C:N ratio compared to soybean.

Bacterial population was evaluated using PCoA as another biological soil health measurement. The differences were not significant between crop sequences treatments. However, bacterial population at the BP and R8 stages formed separate clusters that were distinct other sampling stages. Soil was disturbed every growing season due to tillage, and it may have masked the crop sequence treatment effects on soil bacterial populations. Furthermore, the environmental factors such as precipitation and temperature can alter the microbial diversity. The effect on soil microbial activities and their abundance due to cool and moist conditions at seeding and drier and warmer soil conditions in the middle of both 2017 and 2019 growing seasons would have been greater in relative to the crop sequence effect.

5.2 Conclusions

Soybean test crop yield was not affected by crop sequences in this rotation experiment. It is surprising that seed yield did not decline in the continuous soybean treatment compared to other sequences even after four years of the study. Crop sequence that included canola, corn, and wheat influenced the seed quality, DMY, above ground N uptake, and BNF. However, continuous soybean treatment did not consistently different from other three crop sequences. Hence, the penalties of short-term continuous soybean may be minimal in the loamy and clay soils of Manitoba. However, future studies continued for more than four years may be required to identify the best soybean cropping sequence in Manitoba soils.

Crop sequences influenced the soil enzymes and active C. However, the differences of soil enzymes were not consistent between sampling stages, crop sequences, locations, and years. Soil enzyme activities and active C levels were frequently greater in the C-S-C-S sequence compared to the S-S-S-S sequence. Among the soil health indicators that were measured in this study, active C was better at identifying differences between rotation treatments. Furthermore, BP and R8 sampling stages were found as better sampling stages to collect soils for soil health analysis compared to mid-growing season sampling. Nevertheless, farmers have the flexibility to decide either BP or R8 time point for soil health analysis depending on the availability of resources.

Designing multiple years or long-term research studies will generate data that are helpful to evaluate the overall sustainability of crop rotation practices. Moreover, dissemination of the information gathered from the research studies that are focusing on improving crop yield while

enhancing soil physical, chemical, and biological properties is important. It will allow the farmers, agronomists, scientists, and policy makers to make agronomic decisions to maximize the profitability of the cropping systems while maintaining or even enhancing the soil health.

5.3 Future Research Works

This project has identified new ideas and questions that should be considered when planning future experiments to identify the best soybean crop rotation practice in the soils of Manitoba. If we were to repeat this experiment, I would select wheat – canola – soybean as one of the crop sequences, because it has become a common crop rotation for the framers in Manitoba. The other sequences would be corn – canola – soybean and continuous soybean. Corn and wheat are high residue crops compared to canola and soybean. The effect of previous high and low residue crops on the soybean test crop could be evaluated in relative to continuous soybean. The rotation study should continue for two or more rotation cycles before deciding the best soybean cropping sequence.

The Cornell soil health test would be worthwhile to compare the crop rotation effect on soil health. The test includes physical, chemical, and biological soil health measurements which could provide a comprehensive assessment of soil health. Furthermore, future experiments should be expanded to study other microbial populations such as soil fungal communities.

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6.2 Appendices

6.2.1 Appendix A – List of Abbreviations

ANOVA – Analysis of variance

BNF – Biological nitrogen fixation

BP – Before planting

C – Carbon

Ca-S-Ca-S – Canola-soybean-canola-soybean

C-S-C-S – Corn-soybean-corn-soybean

CSS – Cumulative sum scaling

DMY – Dry matter yield

FAME – Fatty acid methyl ester

FDA – Fluorescein diacetate

ITS – Internal transcribed spacer

K – Potassium

KCl – Potassium chloride

$KMnO_4$ – Potassium permanganate

MASC – Manitoba agricultural services cooperation

MB – Manitoba

MUB – Modified universal buffer

N – Nitrogen

NRCS – Natural Resources Conservation Service

P – Phosphorus

PCoA – Principal coordinate analysis

PLFA – Phospholipid fatty acid

PNP – p-nitrophenol

POXC – Permanganate oxidizable carbon

R1 – Beginning bloom

R4 – Full pod

R5 – Beginning seed formation

R7 – Beginning maturity

R8 – Full maturity

RCBD – Randomized complete block design

RUN – Relative ureide nitrogen

S – Sulfur

SAS – Statistical analysis system

SOC – Soil organic carbon

SOM – Soil organic matter

S-S-S-S – Continuous soybean

USDA – United States Department of Agriculture

VE – Emergence

WADO – Westman Agricultural Diversification Organization

W-Ca-C-S – Wheat-canola-corn-soybean

6.2.2 Appendix B

Table 1. F-test probability of the ANOVA for crop sequence, location, and their interaction on arbuscules, vesicles, hyphae, AMF, total above ground P uptake, spring soil P, ureide N and nitrate N in stems and petiole samples of soybean test crop in 2017.

Source of variation	Arbuscules	Vesicles	Hyphae	AMF†
Sequence	0.3265	0.0002	0.3903	0.3630
Location	<.0001	<.0001	<.0001	<.0001
Sequence × Location	0.1374	0.0094	0.8607	0.5066

Source of variation	Total above ground P uptake at R5	Spring soil P at 0-15 cm
Sequence	0.0912	0.3465
Location	0.0002	0.0033
Sequence × Location	0.0017	0.8322

Source of variation	Ureide N	Nitrate N
Sequence	0.0101	<.0001
Location	<.0001	<.0001
Sequence × Location	0.0009	<.0001

†AMF = Arbuscular mycorrhizal fungi

Table 2. The interaction effect of crop sequence × location on arbuscular mycorrhizae fungal (AMF) colonization on soybean roots of R5 stage in 2017 soybean test crop year.

Location	Sequence	Total arbuscules†	Total vesicles	Total hyphae†	Total AMF colonization†
		%	%	%	%
Carman	S-S-S-S	6.1	7.3 *a*	56.6	48.92
	Ca-S-Ca-S	6.7	3.6 *bc*	54.0	45.83
	C-S-C-S	9.0	4.3 *b*	56.0	48.42
	W-Ca-C-S	8.1	3.6 *bc*	54.1	46.33
Kelburn	S-S-S-S	8.2	3.0 *bc*	55.4	48.1
	Ca-S-Ca-S	6.4	2.0 *cd*	56.0	46.9
	C-S-C-S	7.8	0.5 *de*	56.0	44.8
	W-Ca-C-S	10.3	0.3 *e*	54.2	45.8
Melita	S-S-S-S	3.1	1.1 *de*	66.3	56.2
	Ca-S-Ca-S	2.8	1.3 *de*	69.2	57.3
	C-S-C-S	2.6	1.2 *de*	69.4	57.2
	W-Ca-C-S	1.7	1.3 *de*	65.2	55.1

Means followed by different letters are significantly different at $\alpha = 0.05$. †The interaction effect was not significant.

Table 3. The interaction effect of crop sequence × location on total above ground P uptake, spring soil P, ureide N and nitrate N of stems and petiole samples in 2017 soybean test crop year.

Location	Sequence	Total above ground P uptake at R5 (kg ha^{-1})	Spring soil P at 0-15 cm† (kg ha^{-1})	Ureide N (mM)	Nitrate N (mM)
Carman	S-S-S-S	12.56 *abcd*	14.6	10.41 *cd*	10.83 *c*
	Ca-S-Ca-S	12.77 *abc*	21.4	6.99 *d*	25.71 *a*
	C-S-C-S	12.35 *abcd*	21.0	8.02 *cd*	11.48 *c*
	W-Ca-C-S	14.37 *a*	21.8	7.99 *cd*	16.11 *b*
Kelburn	S-S-S-S	10.26 *cdef*	15.0	20.83 *a*	5.41 *d*
	Ca-S-Ca-S	7.80 *f*	15.8	22.94 *a*	5.26 *d*
	C-S-C-S	8.72 *ef*	21.0	22.70 *a*	4.13 *d*
	W-Ca-C-S	13.43 *ab*	20.6	23.36 *a*	4.54 *d*
Melita	S-S-S-S	11.38 *bcd*	10.9	18.30 *ab*	6.51 *d*
	Ca-S-Ca-S	13.79 *a*	11.3	13.33 *bc*	6.64 *d*
	C-S-C-S	11.14 *bcde*	9.0	21.80 *a*	5.40 *d*
	W-Ca-C-S	9.99 *def*	13.5	5.49 *d*	5.36 *d*

Means followed by different letters are significantly different at $\alpha = 0.05$. †The interaction effect was not significant.

Table 4. Average crop biomass produced by each crop before harvest in 2014, 2015, 2016, 2017, and 2018 across all locations.

Year	Crop Sequence	Crop	Biomass (kg ha^{-1})
2014	S-S-S-S	Soybean	2371
	Ca-S-Ca-S	Canola	3118
	C-S-C-S	Corn	7766
	W-Ca-C-S	Wheat	4730
2015	S-S-S-S	Soybean	2986
	Ca-S-Ca-S	Soybean	3483
	C-S-C-S	Soybean	3554
	W-Ca-C-S	Canola	-
2016	S-S-S-S	Soybean	3001
	Ca-S-Ca-S	Canola	3514
	C-S-C-S	Corn	5326
	W-Ca-C-S	Corn	5003
2017	S-S-S-S	Soybean	2591
	Ca-S-Ca-S	Soybean	3161
	C-S-C-S	Soybean	3341
	W-Ca-C-S	Soybean	3084
2018	S-S-S-S	Soybean	2290
	Ca-S-Ca-S	Canola	2461
	C-S-C-S	Corn	4288
	W-Ca-C-S	Wheat	4113