EXAMINING THE IMPACT OF PULSE CROPS ON QUALITY OF SOIL IN WHEAT-BASED, RAIN-FED CROPPING SYSTEM ON THE BROWN SOILS

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By

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ABSTRACT

Improving soil quality with the inclusion of pulse crops in wheat-based cropping systems may help producers to develop appropriate sequences for crop rotations with improved resource using efficiency. The objective of this study is to examine selected physical, chemical and biological soil quality attributes of pulse crops with shallow and deep root systems grown in wheat-based, semi-arid, rain-fed conditions. The study was conducted at Brooks, AB using field pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medikus) grown alternately with wheat.

A fourth rotation treatment included lentil and chickpea alternated with wheat (lentil-wheat-chickpea-wheat). All rotations with the pulse crops were compared to continuous wheat. Soils were sampled from three depths (0-15, 15-30 and 30-60 cm) in the spring of 2017 and 2018, after six and seven years of the rotation were complete. Continuous wheat enhanced the formation of macro-aggregates (>6.35 mm) and pulse crop rotations enhanced the formation of micro and meso-aggregates (1.00-0.50 mm and 0.50-0.15 mm). All of the rotations had similar fall soil moisture, soil microbial biomass, microbial community composition, total soil carbon, nitrogen and soil organic carbon at all soil depths. On a mass basis, only about 0.5 to 1.5 % of the soil organic matter was in the light fraction organic matter (LFOM). Chickpea alternated with wheat had the highest amount of LFOM and potential mineralizable nitrogen (PMN) in both sampling years. Pulse crop rotations collectively had higher LFOM and PMN values than continuous wheat in both years. Wheat alternated with field pea had the highest 1000-kernel weight, without affecting seed yield. The inclusion of grain legumes with different rooting depths into wheat-based cropping systems did not influence overall soil quality in the short time frame of this study. However, this study provides a baseline for the evaluation of the effect of inclusion of pulse crops into wheat-based cropping systems soil quality while emphasizing the importance of the subsequent wheat crop productivity.

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DEDICATION

This dissertation is dedicated to my beloved parents Badrani Devasurendra and Upali Mahinda who have been a constant source of support and encouragement during the challenges of graduate school and throughout my life. I am truly thankful for having you in my life. This work is also dedicated all my respected teachers, without whom none of my success would be possible.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BD	Bulk density
BNF	Biological nitrogen fixation
C	Chickpea
EC	Electrical conductivity
GHG	Greenhouse gases
HF	Heavy fraction
HFOM	Heavy fraction organic matter
HI	Harvest index
HSD	Honesty significant difference
IPCC	Intergovernmental Panel on Climate Change
L	Lentil
LF	Light fraction
LFOM	Light fraction organic matter
NH_4^+	Ammonium
NO_3^-	Nitrate
P	Field pea
PLFA	Phospholipid fatty acid
PMN	Potential mineralizable nitrogen
PO_4^{3-}	Phosphates
SIC	Soil inorganic carbon
SOC	Soil organic carbon
SOM	Soil organic matter
W	Wheat

1.0. INTRODUCTION

Re-designing and shaping agricultural practices is vital to building economically and environmentally sustainable agricultural systems (Martens et al., 2015; Hamel and Saindon, 2017). Wheat, one of Canada's dominant field crops is grown on an average of over 10 million hectares (Friesen, 2018) and produced around 29 million tonnes in 2017-2018 (Agriculture and Agri-Food Canada, 2019). Canadian prairie wheat producers traditionally relied on continuous cereal-cereal or cereal-summer-fallow cropping using mechanical tillage because it was more profitable than switching crops each year (Campbell et al., 1986 as cited in Zentner et al., 2002). Despite the benefits, this practice may have counter-productive effects such as over-reliance on chemicals (Peterson, 1999; Ali, 2004), soil nutrient loss (Dusenbury et al., 2008; Guo et al., 2010), soil degradation (Massah and Azadegan, 2016) and emission of greenhouse gases (Granli and Bøckman, 1994). Low market prices for cereal grains and increasing production costs in continuous wheat systems (Hume et al., 1991; Fernandez et al., 1998) has led to diversification of cereal-based cropping systems with alternative crops by Canadian prairie grain farmers.

Incorporation of pulse crops into cropping systems have many on-farm agronomic benefits in the semi-arid prairies, including: less nitrogen (N) fertilizer dependency (Krupinsky et al., 2002); decreased disease (Krupinsky et al., 2002) and weed populations in the following crops (Seymour et al., 2012); improved nutrient and water use efficiency (Miller et al., 2003) reduced use of non-renewable energy (Hardarson and Atkins, 2003); increased yield and quality of subsequent crops (Miller et al., 2003) and decreased carbon (C) footprints (Lemke et al., 2007). Pulse crop production in Canada has been increasing and making Canada a world leader in pulse crop trade (Pulse Canada, 2018). In 2017-2018, lentil, field pea and chickpea production was 4,112, 322, and 102 thousands of tonnes, respectively (Agriculture and Agri-Food Canada, 2019).

Soil is an essential part of the entire terrestrial ecosystem, necessary for maintaining most life processes due to its unique ecological composition (Weil and Brady, 2017a). In addition, soil is also a critical natural source for agricultural production in which there are diverse organisms involved in nutrient cycling, regulation of soil organic matter (SOM), soil structure modification,

and enhancement of plant health (Doran, 2002). Thus, soil conservation is key to developing sustainable agriculture systems (Forge, 1998). In the Canadian prairies, agricultural soil quality degradation has been an ongoing phenomenon resulting in increase in soil salinity, acidity and compaction, reduction of water infiltration and the loss of organic matter. This ultimately leads to less productivity that has to be compensated by using more synthetic fertilizers (Forge, 1998). Annual grain legumes with cereals improve soil quality, including soil physical, chemical (Campbell et al., 2000) and biological attributes (Biederbeck et al., 2005). Selection of appropriate crops and growing them in a proper sequence are vital to enhance sustainability, profitability, and resilience within cropping systems. Plant root architecture, soil microbial communities and quality of plant residues, and composition of root exudates vary among different plant species. These characteristics play a key role in nutrient cycling and development of soil structure (Drinkwater and Snapp, 2007). Therefore, incorporation of appropriate pulse crops into cereal-based cropping system under proper conditions can provide pronounced environmental and economic benefits (Van Kessel and Hartley, 2000; Lemke et al., 2007; Nemecek et al., 2008).

Pulse crops add nutrients and enhance water uptake and yield of a subsequent crop. However, physical, chemical and biological soil quality analysis of different pulse crops grown in wheat-based, semi-arid and rain-fed conditions are rare. Similarly, the scientific literature information regarding pulse crops with different rooting depths (shallow- and deep-rooted) are limited especially as related to soil quality in wheat-based cropping systems. Changing the soil quality with the inclusion of pulse crops in wheat-based cropping systems may help producers to develop appropriate sequences for crop rotations with improved resource use efficiency since the inclusion of pulse crops with variations in the rooting depths of different pulse crops and wheat may improve water and nutrient use. Alternative crops, including pulse crops with different morphological traits have been developed and are widely grown in different regions of the Canadian prairies. Thus, their root morphology, including rooting patterns would be one of vital determinants for the sustainable productivity of production systems. Therefore, developing more efficient pulse crop rotations in wheat-based cropping systems may lead to agricultural sustainability by minimizing the application of agro-chemicals (fertilizers, pesticides), reducing production costs, increasing crop productivity and simultaneously being beneficial for the entire environment.

This thesis organized in traditional (standard) format. The hypotheses of this study were:

(1) Alternating pulse crops with wheat in a production system would improve soil quality attributes compared to continuous wheat production system;

(2) The impact of pulse crops on soil quality attributes in wheat-based rain-fed crop production system could vary with pulse crop species and their rooting depth and this impact affect the crop productivity of the subsequent wheat crop in the production system.

By addressing the above hypotheses, the following objectives were pursued:

(1) Examine the impact of different pulse crop species (field pea, lentil and chickpea) alternating with wheat on selected soil physical, chemical and biological properties under rain-fed conditions on semi-arid Canadian prairies;

(2) Examine the impact of three pulse crop species with varying rooting depth (shallow- and deep-rooted) on selected soil physical, chemical and biological properties under rain-fed conditions on semi-arid Canadian prairies;

(3) Examine the three pulse crop species on the productivity of the subsequent wheat crop under rain-fed conditions on the semi-arid Canadian prairies and

(4) Examine the impact of three pulse crop species with varying rooting depth (shallow- and deep-rooted) on the productivity of the subsequent wheat crop under rain-fed conditions on semi-arid Canadian prairies.

2.0. LITERATURE REVIEW

2.1. Wheat-based Cropping Systems on the Semi-arid Canadian Prairies

The Canadian prairies are located in the interior of North America and expand west from Hudson Bay to the crest of the Rocky Mountains (Natural Resources Canada, 2018). These are the most important agricultural regions in Canada as they account for 80% of arable land (Shrestha et al., 2013). Over 40% of the cultivated land is located in the semi-arid Brown and Dark Brown soil zones (Gan et al., 2002). Wheat, one of Canada's most important crops since early settlement (Campbell, 2013), is grown on an average of over 10 million hectares (Friesen, 2018). The production and export of wheat from the late 1880s to the 1950s provided the foundation for infrastructure development to support an expanding Canadian prairie economy (Lafond and Harker, 2012). In 2018, Canada produced around 29 million tonnes of wheat (Agriculture and Agri-Food Canada, 2019). The majority of national wheat production comes from Manitoba, Saskatchewan and Alberta, with a relatively small area in British Columbia and eastern Canada (McCallum and DePauw, 2008).

2.1.1. Management of cropping systems

Wheat producers in the Canadian prairies have traditionally relied on a continuous cropping system involving cereal-cereal cropping or cereal-summer-fallow that used mechanical tillage (Zentner et al., 2002). Continuous cropping is where the same crop species is sown repeatedly in the same field (Cook and Weller, 2004). In industrial crop production, most producers use this cropping system as it is more profitable than switching crops in each year. Continuous cropping encourages increased mechanization for planting, harvesting and distribution of pesticides and fertilizers across large pieces of land using specialized farm equipment. These practices reduce the labor required for production and increase efficiency. Therefore, continuous cropping reduces the cost of production by eliminating labor costs.

Despite the benefits, this practice has counter-productive effects. Continuous cropping provides favorable habitat for the weeds, diseases and pests, specific to the respective crop. If all plants in

a field are equally susceptible to certain weeds, diseases and insect pests, these pests have the potential to spread expeditiously through a crop, necessitating the use of herbicides and pesticides (Thomas and Kevan, 1993). Overreliance on single chemicals has accelerated the development of resistant weed species, which has immediate and long-term costs (Peterson, 1999). For example, the development of weeds species with resistance to herbicides has increased the use of different, more toxic herbicides, such as 2, 4-D and dicamba (Peterson, 1999; Sebukyu and Mosango, 2012; Schütte et al., 2017).

Continuous cropping can also lead to soil nutrient losses due to excessive utilization of inorganic fertilizers, especially nitrates (NO_3^-) and phosphates (PO_4^{3-}) (Dusenbury et al., 2008; Guo et al., 2010; Savci, 2012). Over-fertilization results in high levels of residual NO_3^-, which could contaminate surface and groundwater bodies via surface and subsurface flow (Almasri and Kaluarachchi, 2004). This can lead to acute toxicity on aquatic organisms and human health issues, including the development of cancer and birth defects (Bruning-Fann and Kaneene, 1993; Weyer et al., 2001; Ward et al., 2005).

Application of excessive N fertilizers in continuous cropping systems plays a key role in emissions of greenhouse gases (GHG) especially nitrous oxide (N_2O) since N is the substrate for nitrification and denitrification processes in soil (Granli and Bøckman, 1994). Nitrogen fertilization also influences methane (CH_4) exchange between croplands and the atmosphere (Cai et al., 1997). Moreover, the effect of N fertilizers on CH_4 emission varies. A recent study on rice paddy fields concluded that the emission of CH_4 was stimulated by small amounts of N fertilizer, whereas the emission was inhibited by large amounts of N fertilizer (Banger et al., 2012; Linquist et al., 2015). However, Brock et al. (2016) revealed that continuous cropping systems contribute highly to GHG emissions. Total GHG emissions from continuous wheat cropping system was 225 kg carbon dioxide equivalents (CO_2-e) t^{-1} grain for 3 t ha^{-1} production, compared with wheat following canola, which contributed 199 kg CO_2-e t^{-1} and wheat following field pea, which contributed 172 kg CO_2-et^{-1}. The production and transport of fertilizers further contribute to higher emissions of GHG (23-28 % of total GHG emissions) compared to their use in the field (16-23 % of total GHG emissions) (Brock et al., 2016).

In addition, over-use of synthetic fertilizers causes formation and accumulation of mineral salts that can lead to the development of a soil compaction layer, which restricts both movement and

storage of soil water, air and key plant nutrients (Massah and Azadegan, 2016). Excessive use of synthetic chemicals alters soil pH and soil microbial composition. In addition, pesticide utilization can result in the development of toxic effects on other non-targeted, valuable organisms and it may bring long-term food web changes which may never completely recover (Ali, 2014; Pereira et al., 2009). These can result in the deterioration of soil fertility and further loss of soil health (Savci, 2012).

Summer-fallow is the practice of leaving the land free from production for a growing season with the anticipation of getting a higher yield in the next season. Traditionally, crop producers on the Canadian prairies used this practice as a means of risk management and to improve the growing conditions of the crop in the following year (Carlyle, 1997; Gan et al., 2002; Shrestha et al., 2012). One of the primary reasons for implementing a summer-fallow system in semi-arid regions is to minimize the consequences of highly variable precipitation. The amount of soil moisture retained in the fallow period depends on the methods used for weed control. When tillage intensity is reduced by using herbicides, more crop residues remain on the soil surface for longer periods of time during the fallow period. Increased residue retention is responsible for decreased runoff, decreased evaporation, and increased water infiltration resulting in greater precipitation storage (Freebairn and Wockner, 1986; Baumhardt and Lascano, 1996).

Nitrogen is the most limiting plant nutrient in the North American Great Plains (Grant and Flaten, 2019). Nitrogen can be applied as a synthetic fertilizer and over-fertilization can lead to environmental problems. Fallow enhances NO_3^- accumulated through mineralization of organic matter in the presence of high soil moisture and aeration. Amounts of accumulated NO_3^- during the fallow year varied with the amount of organic matter and available soil moisture (Bauder et al. 1993; Campbell et al., 1995).

In addition, fallow can be used as a weed control strategy. This strategy reduces the weed seedbank by allowing weed seeds to germinate and then killing them either using tillage or herbicides. This method is suitable for both annual and perennial weeds and is especially effective on weed seeds with short dormancy, such as goat's beard (*Aruncus dioicus*) and hare's ear mustard [*Conringia orientalis* (L.) Dumort] (Frick and Johnson, 2002).

However, summer-fallow has increasingly come under attack for contributing to environmental degradation. Therefore, the area under summer-fallow has decreased and the annual crop area increased (Agriculture and Agri-Food Canada, 2016). Cropping systems that employ a fallow period significantly increase organic matter loss due to the reduced production of crop residue and tillage practices for weed control (Follett and Schimel, 1989; Havlin et al., 1990; Bowman et al., 1999; Ortega et al., 2002). Intensive tillage encourages soil erosion which generates dust that affects soil, air and water quality, and causes changes in aggregate stability, soil bulk density, porosity and water retention (He et al., 2009; Sharratt et al., 2010; Maraseni and Cockfield, 2011; Laudicina et al., 2015). Tillage and herbicides used for weed control during summer-fallow use fossil fuels that emit greenhouse gases, thereby contributing to climate change (Dyer and Desjardins, 2009; Shrestha, 2013). Soil organic matter loss also contributes to the production of CO_2 (Cheng and Johnson, 1998). As a result, frequent summer-fallowing increases the C footprint of agriculture.

2.2. Agricultural Sustainability and Soil Quality

Conversion of natural areas to cropland and utilization of highly unsustainable agricultural practices have drastically altered the structural and functional integrity of the prairie ecosystems (Martens et al., 2013). Sustainable agriculture has been defined as a long-term methodological structure that incorporates economic profitability, environmental stewardship and social responsibility. In any agricultural system, sustainability relies on the interaction of climate, soil quality, plant nutrition, management, weed and disease incidence and economics (Hulugalle and Scott, 2008). Proper soil management is critically important for crop productivity, local, regional and global environmental sustainability, and human health. The forecasted increase in world population and the consequent need for more food, energy and clean water all link to proper management of soils (White et al., 2012; Valin et al., 2014).

Soil is a vital natural resource that shapes economic and socio-economic potential. It supports the production of food and raw materials, recycles waste, filters and retains water. It also maintains diversity of plant and animal species (Weil and Brady, 2017a). Soil quality is a useful concept when assessing the sustainability of agricultural activities and has been referred to as the "capacity of a living soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, to maintain or enhance water and air quality, and to support plant and animal

health" (Doran, 2002). Soil properties, climate, topography and land management all impact soil function (Alberta Agriculture and Forestry, 2018). Generally, the quality of soil contains a combination of inherent and dynamic soil properties. The inherent soil properties, such as soil texture, depth to bedrock and cation exchange capacity are influenced by topography and parental material. The dynamic soil properties are strongly influenced by land management, including nutrient status, organic matter and soil structure (Carter, 2002; Cotching, 2006).

Indicators of soil quality also can be categorized as physical, chemical and biological properties, which are sensitive to changes in the environment and land management (Martinez et al., 2010). Physical indicators provide information about soil hydrologic characteristics, such as water entry and retention, which influences availability of water to plants. Some indicators are related to nutrient availability by their influence on rooting volume and aeration status while others are related to erosional status. These indicators include measures of soil texture, structure, bulk density, available moisture content, porosity and aggregation (Moebius-Clune et al., 2016). Biological indicators provide details about the organisms that form the soil food web that are responsible for decomposition of organic matter and nutrient cycling. Information about the numbers of organisms, both individuals and species can indicate a soil's ability to function or bounce back after disturbance. Biological indicators include measures of organic matter content, microbial community structure, soil protein, soil respiration and soil enzymes (Doran and Parkin, 1996). Soil chemical properties, such as pH, cation exchange capacity, electrical conductivity and chemical composition, determine a soil’s ability to supply available plant nutrients and affect its physical properties as well as the health of its microbial population (Ramahlo, 2013).

2.3. Crop Diversification

The widespread implementation of minimum tillage and no-tillage in the 1990s facilitated the replacement of the cereal-fallow cropping systems with more diverse and intensive cropping systems. Crop residues retained on the soil surface in no-till management reduce soil erosion and improve soil moisture conservation (Zentner et al., 2002). Moreover, lower prices for cereal grains, changes in government policies and programs, development of new market opportunities, improvements in machinery design and soil management practices, and growing concerns about soil and environmental degradation have stimulated significant changes in crop management practices (Hume et al., 1991; Fernandez et al., 1998; Smith and Young, 2000).

Crop diversification involves the allocation of different crop species into a cropping system with the aim of increasing overall productivity and market stability by reducing the farm economy's reliance on income from a single crop. Crop diversification is beneficial for nutrient cycling, pest and disease control, soil conservation and ecological diversification (Jolliff and Snapp, 1988; Smith et al., 2015). However, different crops within a crop rotation system may need specific knowledge about their growth and management, as well as the knowledge about specific pests and diseases (Saskatchewan Pulse Growers, 2017).

The inclusion of pulse crops into cereal-based cropping systems has been widely recognized for the rotational benefits. Policies and strategies that promote pulse crop usage are encouraged because pulses in crop rotations provide economic and environmental benefits to agricultural production in Canada (Gan et al., 2002; Johnston et al., 2007).

Research has also revealed that oilseed crops are well adapted to the cool climatic conditions of the Canadian prairies and the inclusion of oilseed crops could elevate net returns while reducing risk through improved production stability (Lafond et al., 1993; Dhuyvetter et al., 1996; Zentner et al., 2002).

2.4. Impact of pulse crops in cropping systems

Pulse crops belong to the Fabaceae (Leguminosae) family and produce high protein edible seed known as 'pulses', which are used for both human and animal consumption (Balasubramanian, 2015). Field pea (*Pisum sativum* L.), lentil (*Lens culinaris* Medikus), dry bean (*Phaseolus vulgaris* L.) and chickpea (*Cicer arietinum* L.) are the predominant pulse crops produced in Canada, and faba bean (*Vicia faba* L.), lupin (*Lupinus angustifolius* L.***)*** and mung bean [*Vigna radiata* (L.) R. Wilczek)] are grown on a smaller scale (Balasubramanian, 2015). The genetic improvement in soybean varieties with a short growing season and high cold tolerance expanded the boundaries from eastern Canada to western Canada (Soy Canada, 2019).

Pulse crops are the 5th largest crop group grown in Canada and in 2017, making Canada a world leader in pulse crop trade (Pulse Canada, 2018). In 2017-2018, lentil, field pea and chickpea production was 2,559, 4,112 and 102 thousand tonnes, respectively. The major pulses (field pea, lentils, chickpea and dry beans) in Canada accounts for $2.5 billion income in 2017-2018 (Agriculture and Agri-Food Canada, 2019). The main export destinations of Canadian pulses are

Turkey, India, China, and the United States (Statistics Canada, 2018). Even though, the Canadian pulse crop industry is a multi-billion-dollar industry, the market experiences considerable volatility of prices. India is the largest pulse customer of Canada, purchasing 49% of all Canadian pea and 37% of all Canadian lentil in the 2016/2017 crop year. In 2017, The Government of India imposed an import tariff on pea, lentil and chickpea, posing a challenge for crop producers with low prices and export volumes (Saskatchewan Pulse Growers, 2019; The Western Producer, 2019).

Pulse crops are considered to be a great contributor to and diversifier of crop rotations. An important attribute of pulse crops is their ability to fix atmospheric N through a symbiotic relationship with rhizobia bacteria (e.g. *Rhizobium* spp., *Bradyrhizobium* spp., *Sinorhizobium* spp.). Symbiotic N fixation can reduce dependency of the rotation on N fertilizer (Krupinsky et al., 2002). In addition, the inclusion of pulse crops in rotations as a break crop, can decrease disease incidences (Krupinsky et al., 2002) and diminish weed populations in the following crops (Seymour et al., 2012). Other economic and environmental benefits associated with pulse crops in rotation include: enhancement of nutrient uptake by a subsequent crop resulting in increased yields (Miller et al., 2003; Miller et al., 2006); reduction in the use of non-renewable energy (Hardarson and Atkins, 2003); decreased C footprints (Lemke et al., 2007; Dusenbury et al., 2008); and enhanced soil fertility (Gan et al., 2002; Johnston et al., 2007). Furthermore, some physical, chemical and biological properties of soils can be markedly changed following the cultivation of pulse crops, as compared to those of cultivated non-pulse crops and uncultivated fallow (Ganeshmurthy, 2009).

2.4.1. Impact on soil organic matter

Soil organic matter (SOM) is a heterogeneous mixture that is comprised of biologically derived material, such as residues of plant and animal tissues in various states of decomposition, and microorganisms and their excretions within or on the soil surface (Baldock and Nelson, 1999; Alberta Agriculture and Forestry, 2013). Plant residues are the key source for SOM and dry plant matter contains mostly C (42%), oxygen (42%) and hydrogen (8%) by the weight (Weil and Brady, 2017b). The SOM fraction can be divided into different pools depending on physical properties, including size, density, location within the soil, chemical properties and rate of the decomposition. There are three main SOM pools identified based on the rate of decomposition, viz. active, slow and passive- which are indicators of the stability of C. The active pool belongs to the labile fraction

that contains microbial biomass and, plant and animal debris that decompose rapidly (from weeks to years). The slow pool is also a part of the labile fraction and is composed of refractory components of litter and weakly sorbed C with turnover times from 10 to more than 100 years (Parton et al., 1987; Trumbore, 1997). Management practices have a distinct impact on these two pools, which in turn influence soil nutrient availability. The passive pool is also called the inert or stable pool and consists of highly humified and mineral-associated organic compounds. This pool takes over centuries to complete the decomposition process (Parton et al., 1987; Trumbore, 1997). The majority of SOM in the passive pool is a well-protected portion of the humus fraction (Strosser, 2010).

Generally, SOM has a vital impact on soil physical, chemical and biological characteristics. Soil properties influenced by organic matter include: soil surface structure, porosity, water infiltration, water and nutrients holding capacity, buffering capacity, soil faunal and microbial diversity and activity, nutrient availability and surface runoff. Therefore, elevated levels of high quality SOM are vital for maintaining a sustainable agricultural system (Baldock and Nelson, 1999; Torbert et al., 2000; Janzen, 2006; Weil and Brady, 2017b).

In agricultural systems, crop residues that remain in the field after harvest have a vital effect on nutrient supply and are considered as the primary source for SOM formation (Beres and Kazinczi, 2000). Crop diversification positively influences the amount of labile SOM (Marriott and Wander, 2006; Culman et al., 2013; Tiemann et al., 2015). A meta-analysis based on 454 crop rotation observations from all over the world in McDaniel et al. (2014) revealed the inclusion of one or more crop into a continuous cropping system generally increased the total soil C and N by 3.6 % and 5.3 % respectively. Moreover, with compatible mixtures and sequences of different crops, soils tend to develop high amounts of SOM, since they have different qualities and quantities of above-ground and below-ground residual biomass (Havlin et al., 1990; Mujuru et al., 2013).

The quality of residue influences residue decomposition. Residues that have smaller lignin and cellulose content and low C:N ratio are considered high quality residues and have high decomposition rates (Chaves et al., 2004; Manzoni et al., 2008; Gentile et al., 2009). Carbon to nitrogen ratio in residues is crucial to determining microbial competition for N, the potential rate of decomposition and the availability of soil nutrients (Weil and Brady, 2017b). Soil organisms metabolize organic compounds to obtain mainly C and N and other nutrients, which are important

for their cellular metabolism. In addition, they require N as constituents of cellular components, such as amino acids, enzymes and DNA. If the C:N ratio of the residues is greater than 40:1, microbes will scavenge the soil solution to obtain available N and must find additional N from other sources in the soil (Ladd and Foster, 1988 as cited in Comeau, 2012). This will lead to N immobilization in soil. Conversely, if the residues have a low C:N ratio (20:1), it promotes N mineralization, where excess N will be released into soil for plant uptake. Generally, C:N of plant residues varies from 8:1 to 500:1 and the ratio declines as plants mature (Weil and Brady, 2017b). Cereal residues have C:N between 70:1 and 100:1 and pulse crop residues have smaller C:N ratios ranging from 25:1 to 40:1 (Stevenson and van Kessel, 1996). Therefore, pulse crops residues are considered high quality residues. Nonetheless, residues with high quality (low C:N) mineralize rapidly and fertilize the soil without significantly contributing to passive SOM accumulation (Brady and Weil, 2008). In contrast, low quality residues facilitate the development of humified and passive SOM (Brady and Weil, 2008).

2.4.2. Impact on soil aggregates

The formation of aggregates in soil is a complex process, regulated by physical-chemical and biological processes. The major physical, chemical processes are flocculation and shrink-swelling behavior of expansive clay masses. The prominent biological processes include ingestion activities of soil fauna, production of sticky exudates by soil microorganisms and binding of soil particles by plant roots and fungal hyphae (Weil and Brady, 2017b). Based on the diameter of soil particles, soil consists of three categories of aggregates namely macro-aggregates (>250 μm), meso-aggregates (53-250 μm) and micro-aggregates (<53 μm) (Chan et al., 1994; Six et al., 2000).

The resistance of soil aggregates to disruption by external forces is termed 'aggregate stability' (Angers and Carter, 1996; Papadopoulos et al., 2009). This crucial soil physical property is an indicator of soil quality, which is important for soil crusting, susceptibility of erosion, seed germination, root growth and penetration of the crops, physical protection of SOM, biological activity, soil aeration, water infiltration and nutrient cycling in soil (Lynch and Bragg, 1985; Le Bissonnais, 1996; Jastrow and Miller, 1997; Angers and Caron, 1998). Weakly aggregated soil results in formation of soil surface crusts and individual soil particles fill the pore space near the surface and can have negative impacts on soil function such as preventing infiltration of water, interfering with plant establishment, increasing the potential for water and wind erosion, and

decreasing water-holding and air-exchange capacity (USDA Natural Resources Conservation Service, 2011). Stable soil aggregates provide good soil structure for maintaining a continuity of pores in the soil matrix which ultimately influences crop growth and development (Stirzaker et al., 1996).

Soil organic matter plays a key role in aggregate formation and stabilization. Agricultural practices are able to alter the quality and quantity of organic input over time (Abiven et al., 2009; Weil and Brady, 2017b). Soil aggregation is influenced by a variety of organic constituents, including polysaccharides and humic compounds (Feller and Beare, 1997). Incorporation of greater amounts of SOM stimulates microbial activity since this fraction acts as an energy substrate and enhances the production of aggregate glues. Micro-aggregates consist of older and more stable forms of organic matter (Barral et al., 1998; Duiker et al., 2003). Agglomeration of micro-aggregates results in macro-aggregate formation. Formation and stabilization of macro-aggregates involve less stable products from the decomposition of recent inputs of OM (USDA Natural Resources Conservation Service, 2011; Weil and Brady, 2017b). In addition, due to the elevated stability occurring with the smaller size, turn-over time of micro-aggregates is longer compared to macro-aggregates (De Gryze et al., 2005). Therefore, macro-aggregates are generally considered as more sensitive to alterations of SOM, tillage and crop sequence and are less stable than meso- and micro-aggregates. Stable soil aggregates are vital for soil quality since it protects recently deposited SOM and facilitates the development of stable organo-mineral complexes. This process is ultimately important for C sequestration (Tisdall and Oades, 1982; Jastrow et al., 1996; Angers and Giroux, 2006; Assis et al., 2006; Salton et al., 2008). When the proportion of large to small aggregates increases, soil quality generally increases (USDA Natural Resources Conservation Service, 2011). Despite these observations, the positive relationship between total SOM and aggregation is not always apparent (Angers, 1992).

Pulse crops have the potential to maintain and improve soil aggregation mainly by providing plant biomass as a microbial substrate (Sainju et al., 2003; Srinivasarao et al., 2012; Lal, 2015). The growth and activities of living roots of pulse crops also lead to aggregate stabilization (Cooke and Williams; 1972; Reid and Goss, 1981). Haynes and Beare (1997) reported that soil aggregate stability was increased by 63% with lupin compared to wheat due to more rhizodeposited C and N and longer fungal hyphae. In addition, soil aggregate stability improved after growing winter pea

and hairy vetch compared to fallow or wheat (McVay, 1989). In contrast, better soil aggregation and SOC stock was obtained from rye than hairy vetch and red clover on a fine sandy loam soil in Georgia, USA (Sainju et al., 2003). In addition, it has been reported that the inclusion of legumes into rice-based rotations affects aggregate size distribution. Rice-chickpea and rice-chickpea-mung bean rotations had 70.1 and 80.7 g macro-aggregate 100 g^{-1} dry soil, respectively, compared to the rice-wheat rotation that had 65.7 g macro-aggregate 100 g^{-1} dry soil, at the 0-20 cm soil depth (Kumar et al., 2019). A similar trend was observed for the 20-40 cm soil increment (Kumar et al., 2019).

The residual quality of pulse crops plays a major role in aggregate stability. These effects can be dependent on site, soil type and plant species. However, research information specifically comparing the impact of pulse crops, such as field pea, lentil and chickpea on soil aggregation is limited.

2.4.3. Impact on nitrogen dynamics

As a key plant nutrient, N is important for plant growth and development as it is a major chemical element in proteins, nucleic acids, chlorophyll and energy transfer compounds (Sulieman, 2011; Mosaic Crop Nutrition, 2018). In agriculture, application of fertilizer N, enhances biomass yields, but excessive N can lead to many adverse effects on the quality of the economic product and the environment. For example, application of excessive N fertilizer decreases oil concentration in camelina seeds (Malhi et al., 2014; Hossain et al., 2017). Therefore, identifying strategies that achieve high crop yields and simultaneously reduce inorganic fertilizer input is a priority for developing sustainable cropping systems (Malhi et al., 2014; Wile et al., 2014).

Pulse crops have a significant beneficial value over many crop species due to their potential to fixing atmospheric N. Biological N fixation (BNF) is the second largest global N contribution next to synthetic fertilizers. Nitrogen bio-fixation accounts for 50-70 Tg of N, which is responsible for 16% of the annual global N contribution for crop production (Herridge et al., 2008; Liu et al., 2010). The median percentage for total plant N derived from the atmosphere was 88% for irrigated faba beans, 60% for lentil, and 55% for field pea and chickpea in the northern Great Plains region of North America (Walley et al., 2007). Therefore, N fertilizer requirements by pulse crops are smaller than other crops and competition for soil N is reduced in pulse crops resulting in ‘N

sparing' (Jensen, 1994; Jensen, 1996). Biological N fixation depends on several factors, including temperature, water and nutrient availability, and soil pH (Sprent et al., 1988; Brockwell et al., 1991; Triplett and Sadowsky, 1992; Boscari et al., 2002). High amounts of inorganic N in soil reduces BNF due to the inhibition of both nodule formation and nitrogenase enzyme activity (Sprent et al., 1988). Therefore, early root growth and enhanced nodulation and BNF result from maintaining a low inorganic N level in the soil (Voisin et al., 2002).

In addition to 'N sparing', the high N content in pulse crop residues provide N for subsequent crops in a rotation sequence through mineralization, thereby decreasing the N fertilizer requirement of subsequent crops (Jensen, 1996). The rate of mineralization varies with soil temperature, moisture content, pH and the amount of organic matter and residues (Kitchen et al, 2001; Ryan et al., 2003). Gan et al. (2010) reported that under low soil moisture, the N mineralization rate in soil under lentil (2.96 kg ha^{-1} day^{-1}) and dry pea (2.54 kg ha^{-1} day^{-1}) were greater than soil under wheat (2.12 kg ha^{-1} day^{-1}).

Legume-based cropping systems are susceptible to nitrate leaching (Poss and Saragoni, 1992; Fillery, 2001; Dinnes et al., 2002). Soils with high hydraulic conductivities or artificially drained soils are more prone to NO_3^- leaching when the soil is exposed to flood irrigation or heavy rainfall. Nitrate leaching in legume-based cropping systems occurs mostly during summer or winter fallow periods, after residue incorporation without inclusion of a subsequent crop (Fillery, 2001). Several studies have reported less NO_3^- leaching from legumes compared to fertilized crops. Drinkwater et al. (1998) reported that legume and manure-based systems lost 13 kg NO_3-N ha^{-1} y^{-1} while the fertilizer-based system lost 20 kg NO_3-N ha^{-1} y^{-1}. However, the rates of fertilizer N applied, legume content of a pasture (Cuttle et al., 1992) and species of legume will affect the outcome of such studies (Dear et al., 2001).

Reducing amounts of synthetic fertilizers applied to farmland will undoubtedly reduce GHG emissions (Jensen, 1994; Haughn et al., 2013). Anthropogenic GHG emissions play a key role in altering the global climate. Atmospheric concentrations of GHGs have increased over the last century (Ritchie and Roser, 2017). Agricultural production is a major generator of GHGs such as CO_2 during soil cultivation, methane (CH_4) associated with livestock manure and nitrous oxide (N_2O) from fertilizer and crop residue decomposition. The agricultural sector was responsible for 8% of GHG emissions in Canada in 2016 (Environment and Climate Change Canada, 2016).

Both crop type and crop residue type have significant impacts on N_2O emissions. Pulse crops, which require less amount of N fertilizer inputs reported to have low N_2O emission in crop rotations compared to cereals (Lemke et al., 2002; Dusenbury et al., 2008; Jeuffroy et al., 2013). However, because of the low C:N ratios in pulse crop residues, they provide more substrate for microbial nitrification and denitrification indicated by the negative correlation between residue C:N ratio and cumulative N_2O emission (Baggs et al., 2000). Lemke et al. (2002) showed that the emission of N_2O from wheat grown on the pulse crop stubble was comparatively smaller than fertilized wheat grown on wheat stubble. However, knowledge on the potential interaction between the type of the crop residue and the fertilizers is limited on the semiarid Canadian prairies (Lemke et al., 2007).

2.4.4. Impact on carbon dynamics

Soil carbon is the main energy source for heterotrophic organisms, including those involved in SOM decomposition. Soil has the largest store of biosphere C, storing an estimated 2,700 Gt of total C globally; of which 1,550 Gt is organic C and 950 Gt is inorganic C (FAO, 2015; Department of Primary Industries and Regional Development's Agriculture and Food, 2018). Therefore, soil organic carbon (SOC) is recognized as the largest terrestrial C pool. According to Bolinder et al. (2007) both above-ground and below-ground plant parts are primary contributors to SOC. In cropping systems the below-ground parts (the roots, and the materials released from the roots as they grow, including root exudates, lysates, sloughed cells, and mucilage) are more important in SOC accumulation than above-ground parts, primarily because above-ground materials are harvested and exported as grain, feed, fiber and biofuel (Keith et al., 1986; Pietola and Alakukku, 2005). There is potential to increase the amount of SOC with land cover change and implementation of a variety of management practices, including the addition of legumes into cropping systems (Janzen et al., 1998; Laganière et al., 2010; Deng et al., 2014).

The quality of plant residue is more vital than the quantity in determining the SOC content since it influences the residue decomposition rate (Gregorich et al., 2000; Johnson et al., 2006). Generally, wheat crops produces 1.2- 2 times more residues than pulse crops (Lemke et al., 2007). At maturity, field pea generally produces 4,100-5,200, lentil 3,200-4,300, chickpea 3,100-3,700, and wheat 6,100-6,700 kg ha^{-1} of above-ground biomass. Root biomass production in field pea is typically 460-540, lentil 690-920, chickpea 670-810 and wheat 1,070-1,420 kg ha^{-1} (Gan et al.,

2009b). Therefore, pulse crops produce a similar or higher content of SOC due to the lower C:N ratio and less lignin compared to wheat (Drinkwater et al., 1998; Campbell et al., 2000; Soon and Arshad, 2002; Sainju and Lenssen, 2011).

In western Canada, cereal-based cropping systems rely on non-renewable energy extensively and 70% of non-renewable energy is due to inorganic fertilizers, especially N (Zentner et al., 2004). Because pulse crops are capable of fixing N from the atmosphere, they reduce the synthetic N fertilizer requirement of both pulses and the subsequent crops. Therefore, the addition of pulse crops into a cropping system is capable of decreasing overall CO_2 emission compared to non-pulse crop rotations (Lemke et al., 2007).

2.4.5. Impact on soil microbial community

Soil microbial communities play a key role in plant-soil systems. They are essential for nutrient cycling, organic matter decomposition, formation of soil aggregates, N fixation, and promotion of shoot and root growth. This ultimately modifies the rhizosphere environment, and facilitates plant growth (Pankhurst et al., 1995; Balser and Firestone, 2005; Loranger-Merciris et al., 2006; Gupta, 2012; Schenk et al., 2012). In addition, there are beneficial symbiotic arbuscular mycorrhizal (AM) fungi and plant growth promoting bacteria which improve crop yield and suppress plant diseases by producing antagonistic compounds and increasing plant resistance to pathogens (Conrath et al., 2002; Jousset et al., 2010; Schenk et al., 2012). Conversely, pathogenic microbes cause disease, production losses, necrosis and eventually plant death (Jennings and Lysek, 1996). Therefore, soil microorganisms are of great importance for long-term sustainability of agricultural systems due to their key roles (Kennedy and Smith, 1995).

Long-term crop rotations can increase microbial diversity compared to continuous cropping systems. The plant species included in a rotation, the sequence and frequency of the crops, the length of rotation and the soil characteristics are major factors that impact the soil microbial communities (Garbeva et al., 2004; Ellouze et al., 2008; Bernard, 2011; Bennett et al., 2012). Many studies revealed pulse crop-based crop rotation systems positively impact soil microbial communities and sustainability of agricultural ecosystems (Lupwayi et al., 1998; Chen et al., 2008).

As a C and energy provider to soil microbes, plant residue is a vital driving force enhancing the soil microbial community (Garbeva et al., 2004). The complex interactions between plants and microbes are primarily mediated by chemical signals in root exudates (Bais et al., 2006). Plant species have a substantial influence on the structural and functional diversity of the microbial community (Berg and Smalla, 2009; Breulmann et al., 2012). Chen et al. (2008) revealed that pulse crops positively influence the abundance of the bacterial and fungal community compared to the grasses grown in monoculture with high soil moisture.

The quality of the residue also influences the structure and abundance of the soil microorganisms due to their substrate preference (Nicolardot et al., 2007; Breulmann et al., 2012). Even though, both fungi and bacteria are involved in residue decomposition and nutrient cycling, fungi play a key role in decomposing residues with higher C:N ratio and lignin. Therefore, residues with higher C:N ratio and lignin favor fungi and actinomycetes over bacteria (Eskelinen et al., 2009; Gul et al., 2012). Higher quality residues with low C:N ratio increase Gram negative bacterial abundance (Bastian et al., 2009). Pulse crop residues with low C:N ratio impact microbial mineralization and soil N availability and eventually the colonization of the soil microorganisms (Pascault et al., 2010).

2.4.6. Impact on soil water utilization

Available soil moisture content plays a vital role on the productivity and product quality of pulse crop production systems on the Canadian prairies due to low and variable rainfall (Cutforth et al., 1999; Angadi et al., 2008). Appropriate rotation systems with more efficient water usage can be designed by identifying the mechanism behind the utilization of soil water at various soil depths by previous crops (Gan et al., 2009a). Miller et al. (2001) reported that water using efficiency (WUE) of field pea is greater than wheat (9.1 kg ha^{-1} mm^{-1} vs. 6.4 kg ha^{-1} mm^{-1}), and chickpea (3.8 kg ha^{-1} mm^{-1}) and lentil (4.1 kg ha^{-1} mm^{-1}) had comparatively lower WUE. According to Gan et al. (2015) chickpea used more water from below the 60 cm soil depth, suggesting that the plant has deeper rooting than the other pulse crops. In addition, the data also suggested that lentil used the least amount of water from the 0-60 cm soil profile (Gan et al., 2015). Pulse crops, such as chickpea, field pea and lentil had much shallower roots than wheat, where roots were present at 80-100 cm. Shallow rooting may leave more nutrients and water in the soil for subsequent crops. Moreover, field pea and lentil use 15-35% less water than wheat (Gan et al., 2009a). Pulse crops,

therefore are more able to survive under low moisture conditions, thereby enhancing WUE (Gan et al., 2015).

2.5. The Impact on Crop Productivity and Contribution to the Canadian Economy

Crop productivity is the quantitative measurement of crop yield produced within a known area. The primary objective of crop production is to maximize the transformation of the resources into useful products (Pellitier et al., 2011). Many studies have revealed that diversifying cropping systems with pulse crops has the potential to produce greater yields (Zentner et al., 2004; Miller et al., 2006). Zentner et al. (2004) claimed that the total energy input for the pulse crops was 53% lower than the input for continuous wheat. According to Burgess et al. (2012), pulse crops produced positive rotational benefits on subsequent wheat yield compared to wheat following wheat, even though there was no difference in energy inputs and chemical usage between the two cropping systems. A significantly higher wheat yield (28%) was observed in a pea-wheat rotation (2.15 Mg ha^{-1}), as compared to continuous wheat (1.68 Mg ha^{-1}). Furthermore, in a study with three-year cropping phases involving pulse crops (field pea, lentil and chickpea), cereals (wheat, barley and durum) and summer-fallow, the total grain production and protein yield of wheat in the cereal-pulse system increased by more than 35% and nearly 60%, respectively compared to the summer-fallow system. In addition, the fertilizer-N-use efficiency for grain was increased by 33% over the conventional summer-fallow system. Moreover, a similar quantity of grain and protein yield were produced by the cereal-based monoculture as the pulse crop system while using a more N fertilizer to achieve the same grain yield (Gan et al., 2015).

To meet the growing human population demand, it is required to double global wheat production by 2050 based on the forecast (Godfray et al., 2010). Due to limited availability of uncultivated farmland on the planet and growing concerns around deforestation, pulse crop-based cropping systems have the potential to act as an alternative system to increase total grain production without the need for new additional croplands (West et al., 2010; Garnett, et al., 2013). More research is needed, however, to quantify the potential environmental benefits that are associated with pulse crops-based cropping systems.

3.0. MATERIALS AND METHOD

3.1. General Description of Study and Experimental Design

My study is a part of an on-going long-term crop rotation study at the Crop Diversification Centre South (CDCS), Alberta Agriculture and Forestry Research Centre in Brooks, AB (Lat. 50° 33′ 51″ N; Long. 111° 53′ 56″ W; Elev. 758 m). The dominant soil at the Brooks test site is an Orthic Brown Chernozem with a loam to silty loam surface.

The long-term study was established in 2011 and the data reported in this thesis are confined to the 2016/17 and 2017/18 cropping years, which are in the second cycle of the four-year, rain-fed crop rotation. My study included four crop rotation treatments and a continuous wheat treatment (Table 1.1). The pulse crops included in the crop rotation treatments were Kabuli type chickpea (*Cicer arietinum* L.) cultivar CDC Frontier, yellow pea (*Pisum sativum* L.) cultivar CDC Meadow, and red lentil (*Lens culinaris* Medikus) Clearfield type cultivar CDC Maxim. These three pulse crop species were grown alternately with hard red spring wheat (*Triticum aestivum* L.) cultivar Lillian and in one rotation lentil and chickpea were included alternately with wheat (Table 1.1).

These treatments were arranged in a randomized complete block design (RCBD) with four replicates. Each experimental unit (plot) was 12-m long and 3-m wide, and was seeded to the different crops with 12 rows spaced 25 cm apart.

3.2. Soil Sampling and Processing

Soil samples were collected on May 1, 2017 and April 27, 2018, just prior to seeding of the 7th and 8th year crops (Table 1.1). Thus, the soils were representative of the 6th (wheat phase) and 7th year (pulse crop phase) crops. Soil sampling was conducted using a soil core (4.5-cm dia.) and a metal frame (17.5 cm × 17.5 cm × 5.0 cm) for aggregate sampling.

Table 3.1. Different crop species allocated in the two, 4-year crop rotation cycles from 2010 to 2018.

Treatment		Cycle 1				Cycle 2			
	Year 0 (2010)	Year 1 (2011)	Year 2 (2012)	Year 3 (2013)	Year 4 (2014)	Year 5 (2015)	Year 6 (2016)†	Year 7 (2017)†	Year 8 (2018)
1. Continuous wheat (W)	W	W	W	W	W	W	**W**	**W**	W
2. Field pea (P) alternate with W	W	P	W	P	W	P	**W**	**P**	W
3. Lentil (L) alternate with W	W	L	W	L	W	L	**W**	**L**	W
4. Chickpea (C) alternate with W	W	C	W	C	W	C	**W**	**C**	W
5. Lentil and chickpea alternate with W	W	L	W	C	W	L	**W**	**C**	W

† Years with the bold letters correspond to the cropping years considered in this study.

3.2.1. Sampling for soil nutrients and moisture

Eight, 60 cm deep soil cores were collected from each plot from a diagonal transect across the plot. Each core was divided into three segments based on depth (0-15 cm, 15-30 cm and 30-60 cm). Soil samples at the same depth within a plot were combined to make composite samples, resulting in three composite samples per plot.

A sub-sample of soil, weighing approximately 50 g, was taken from each 0-15 cm composite sample and was immediately frozen at -80 °C to use for phospholipid fatty acid (PLFA) analysis to determine microbial community composition. A second sub-sample, weighing about 1.0 kg was stored at +4 °C for potential mineralizable N analysis.

The remaining soil from each plot was air dried for 4-5 days and sieved through a 2-mm mesh sieve. After drying, another sub-sample, weighing approximately 500 g, from each 0-15 cm composite sample was stored at room temperature for organic matter analysis. The remaining soil from all the depths was ground separately using a coffee grinder and re-ground using a ball grinder (8000D Mixer/Mill, SPEX SamplePrep® LLC., Metuchen, NJ, USA) into a fine powder after

removing visible plant materials and used for analysis of soil pH, electrical conductivity (EC), total C, total N and organic carbon (OC).

3.2.2. Sampling of soil for analysis of aggregate size distribution

Soil sampling for aggregate size distribution analysis was carried out by pressing a metal frame (17.5 cm × 17.5 cm × 5.0 cm) into the soil to a 5.0-cm depth in two randomly selected areas within each plot. The block of soil was carefully excavated using a square-point shovel and the undisturbed soil samples were placed in individual hard plastic boxes with lids to protect the intact structure during transportation and storage. These soil samples were air dried separately for 4-5 days without disturbing the structure of the soil sample.

3.3. Agronomic Practices

Cultural practices, including seeding, pest and disease control, harvest and crop data collection were carried out by the staff at CDCS in Brooks. The plots were seeded to different crops on April 29, 2016 and May 4, 2017 using a no-till plot seeder (Hege 3-point hitch seeder with ACRA-Plant Cropmaker openers spaced at 25 cm apart with 10 cm rubber metal buffer packer wheels). The seeding rates were adjusted based on seed germination percentage and field emergence rate to target an optimal plant density for each crop (Table A.1). In the 2016 cropping year, all the plots were seeded to wheat at a seeding density of 250 seeds m^{-2}. In 2017, plots were seeded to wheat, chickpea, field pea and lentil at seeding densities of 250, 50, 90 and 140 seeds m^{-2}, respectively. The granular form of inoculants containing crop-specific N-fixing bacteria *Mesorhizobium ciceri* (Nodulator®, Becker Underwood Inc., Saskatoon, SK) for chickpea and *Rhizobium leguminosarum* (Nodulator®, Becker Underwood Inc., Saskatoon, SK) for fieldpea and lentil were applied with seed according to manufacturer's recommendation (5.6 kg ha^{-1}). (Table A.1).

Mono-ammonium phosphate ($NH_4H_2PO_4$), which contains 11% N and 51% P_2O_5 to provide 39 kg ha^{-1} of phosphorus (P) was applied with the seeds of all crop species. In addition, wheat was fertilized with urea (46:0:0) at a rate of 109 kg urea ha^{-1} at seeding as a side band. No additional N-fertilizer was applied to the pulse crops. Wheat and pulse seeds were treated with Vitaflo 280 and Apron Maxx using the label rates (Table A.1). Glyphosate was applied for pre-emergence weed 'burn-off' (Table A.2). A summary of dates and treatment rates of herbicides and fungicides

at the test site is given in Table A.2-A.4. Weather data were obtained from a weather station located on the research farm, about 500 m from the plot site (Table A.5).

3.4. Analysis of Soil Samples

3.4.1. Physical properties

Soil moisture content in spring and fall

Gravimetric water content of soil in early springs of the both sampling years was determined at three depths (0-15 cm, 15-30 cm and 30-60 cm). Twenty-five g fresh soil per plot was oven dried at 105 °C- 110 °C until the dry weight of the sample became constant. Soils were reweighed and percentage moisture content was calculated as described by Topp et al. (2006). Soil moisture contents in fall of the same cropping years were measured at the same depths after crop harvest.

Soil bulk density

Soil bulk density (BD) is defined as the ratio of the mass of dry solids per bulk volume of soil. For the determination of soil BD, the total amount of collected soil in the known volume required to be air dry. However, the present study had different soil parameter analysis for each soil depth and they required different soil storage conditions (fresh soil kept at +4 °C and -80 °C or dry soil). Therefore, all the soil from a plot was unable to dry. In order to complete all the analysis, a portion with known weight of soil sample was dried and did the calculations to find the bulk density of whole sample (0-15 cm soil depth samples only) as following.

The fresh weight of each composite soil sample from the 0-15-cm depth was measured. Bulk density was calculated using the soil moisture content as follows (Soil survey staff, 2014):

$$BD_{composite}\ 'a'\ (Mg\ m^{-3}) = \frac{Dry\ soil\ weight\ (Mg)}{Soil\ volume\ (m^3)} \quad [3.1]$$

$$MC_{composite}\ 'a'\ (Mg) = \frac{MC_{25\ g}}{25\ g} \times\ W_{fresh}\ 'a'\ (Mg) \quad [3.2]$$

$$Dry\ soil\ weight\ (Mg) = W_{fresh}\ 'a'\ (Mg) - MC_{composite}\ 'a'\ (Mg) \quad [3.3]$$

$$Soil\ volume = Core\ volume = \pi r^2 h \quad [3.4]$$

Where, $BD_{composite}$ 'a' = soil bulk density of composite sample 'a', $MC_{composite}$ 'a' = moisture content of composite sample 'a', W_{fresh} = fresh weight, r = core radius (0.0225 m) and h = core height (0.15 m).

Soil aggregate size distribution

The dry aggregate size distribution of soil from the 0-5-cm depth was determined by the standard dry-sieving method (Nweke and Nnabude, 2013). The air-dried, undisturbed soil from each of sample was separated into approximately 500 g sub-samples and mechanically sieved using a rotary tap sieve shaker (RX-29, Wstyler, USA) through a nest of sieves having 6.35 mm, 2.00 mm, 1.00 mm, 0.50 mm, 0.15 mm, 0.12 mm and 0.05 mm square openings. Soils were sieved for 4.5 min. Consequently, eight aggregate size classes (ASCs) were obtained (>6.35, 6.35-2.00, 2.00-1.00, 1.00-0.50, 0.50-0.15, 0.15-0.12, 0.12-0.05 and <0.05 mm). The weights of individual soil samples with different aggregate sizes were determined.

3.4.2. Biological properties

Separation and quantification of organic matter fractions

Density fractionation divides SOM into two distinct fractions, namely light fraction (LF) and heavy fraction (HF). The SOM from the 0-15 cm depth was divided into HF and LF using sodium iodide (NaI) (density of 1.7 g mL^{-1}) as described by Gregorich and Beare (2006a). A 50 g soil sample was mixed with 100 mL NaI in clean, disposable plastic vials. The vials were capped, placed upright and shaken on a rotary shaker for 1 h at 160 rpm and then maintained at room temperature (ca. 20 °C) for 48 h. The LF floating on the surface of the NaI was decanted under vacuum through a 0.4 μm nitrocellulose membrane filter. The HF remained in the beaker. The LF and HF were washed with approximately 75 mL of 0.01 M calcium chloride ($CaCl_2$) followed by further washing with 75 mL of deionized water. The washed LF and HF were separately re-filtered and the materials dried at 60 °C and weighed. This material was retained for quantification of total C and total N.

Phospholipid fatty acid (PLFA) analysis

Phospholipids are an essential structural component of all microbial cellular membranes, which is useful in identifying the overall microbial community structure. Phospholipid content in a soil

sample is assumed to be from the living microbiota as the phospholipids rapidly degrade after microbial death. Phospholipid fatty acid analysis is a widely used technique to provide information about the overall structure of terrestrial microbial communities (Quideau et al., 2016). Phospholipid fatty acid extraction was performed according to the modified protocol of White (1979), which was adapted from the original method of Bligh and Dyer (1959) as described in Helgason et al. (2009).

Soils for PLFA analysis were previously freeze-dried and stored at -80 °C. Before analysis, the freeze-dried soil samples were aseptically ground using a mortar and pestle. For the extraction, all glassware was soaked in 4 % (v/v) Extran 300 soap bath for 2 h, then scrubbed and rinsed thoroughly with distilled water (dH_2O). The cleaned glassware were then soaked in 10 % HCl (4 h for glassware, 2 h for Teflon lined vial caps) to remove remaining lipid debris. They were tripled rinsed with dH_2O and air-dried glassware was baked at 400 °C for 4 h in a muffle furnace (Thermo Fisher Scientific Inc., Waltham, MA). All chemicals used in this extraction were HPLC grade and ultra-high pure N_2 was used for sample evaporation (Praxair Canada Inc., Mississauga, ON).

The standard protocol included lipid extraction from soil samples using a single-phase chloroform mixture, isolation of phospholipids with lipid fractionation using solid phase extraction (SPE) columns, methanolysis of phospholipids to produce fatty acid methyl esters (FAMEs), and FAME analysis using capillary gas chromatography (Quideau et al., 2016).

A freeze-dried, ground 4.0 g soil sample was extracted in a 50-mL glass vial with 19.0 mL of Bligh and Dyer extractant (5.0 mL chloroform ($CHCl_3$), 10.0 mL methanol and 4.0 mL phosphate buffer (dipotassium phosphate-2.18 g, $CHCl_3$-0.75 mL, ultra-water-250 mL) in dark conditions and then centrifuged for 15 min at 1500 rpm. The supernatant was transferred into a new 50-mL glass vial and 5.0 mL phosphate buffer and 5.0 mL $CHCl_3$ were added. The mixture was vortexed for 30 s. After sitting overnight at room temperature in the dark, the lower organic phase was transferred into a 15.0-mL vial and evaporated with N_2 at 25 °C. The dried samples were stored at -20 °C.

The SPE columns (0.50 g Si; Varian Inc. Mississauga, ON) with spigots were conditioned with 5.0 mL of acetone followed by two additions of 5.0 mL of $CHCl_3$. Samples were re-dissolved with 1.0 mL of $CHCl_3$ and transferred into labelled SPE columns for lipid separation. With the addition of 5.0 mL of $CHCl_3$ and 5.0 mL of acetone, neutral and glycolipids were sequentially eluted from

the samples. The phospholipids were eluted with the addition of 5.0 mL of methanol and the eluant collected into cleaned 15 mL glass vials. The obtained samples were dried with N_2 and stored at -20 °C.

In the lipid methylation step, the samples were allowed to return to room temperature and 0.5 mL of $CHCl_3$ and 0.5 mL of methanol were added into each sample. After the addition of 1.0 mL of 0.2 M methanolic potassium hydroxide (0.36 g potassium hydroxide and 30 mL methanol), the sealed samples were placed in a 37 °C water bath for 30 min. After the samples returned to RT, 2.0 mL of hexane, 0.2 mL of 5.75 % (v/v) acetic acid and 2.0 mL of millipore water were added. The samples were vortexed and centrifuged at 1500 rpm for 2 min. Ten µL of 0.1 µg μL^{-1} methylated internal standard (methyl nonadecanoate; 19:0) was added to labelled 4-mL amber vials and the top phase from the centrifuged samples transferred into the vials. After adding 2.0 mL of hexane to the lower phase of the sample, it was again centrifuged at 1500 rpm for 2 min. The obtained top phase were again transferred into the amber vials containing the standard and the initial supernatant. The samples were evaporated under N_2 and stored at -20 °C.

The fatty acid methyl ester (FAME) extracts were identified using gas chromatography (Hewlett Packard 5890 Series II, Hewlett Packard Scientific Instruments, Palo Alto, CA). The peaks were identified using fatty acid standard and MIDI software (MIDI Inc., Newark, DE). Total microbial biomass was quantified by summation of all identified PLFA peaks and specific biomarkers were used to determine the relative abundance of specific microbial functional groups (Table 3.2).

Table 3.2. Biomarkers used to determine the abundance of specific microbial functional groups.

Functional groups	Biomarkers	Reference
Bacteria	i14:0, i15:0, a15:0, i16:0, 16:1ω7c, 10Me16:0, i17:0, a17:0, cy17:0, 10Me17:0, 18:1ω7, 10Me18:0, cy19:0	(Helgason et al., 2010a) (Bååth and Anderson, 2003)
Gram positive	i14:0, i15:0, a15:0, i16:0, i17:0, a17:0	(Helgason et al., 2010b) (Hedrick et al., 2005)
Gram negative	16:1ω7t, 16:1ω9c, 16:1ω7c, 18:1ω7c, 18:1ω9c, cy17:0, cy19:0	(Helgason et al., 2010b) (Macdonald et al., 2004)
Fungi	18:2ω6,9	(Bååth and Anderson, 2003)
Arbuscular mychorrizal fungi	16:1ω5c	(Olsson,1999)

3.4.3 Chemical properties

Soil pH and EC

Twenty-five g of dried, ground soil from each composite soil sample at 0-15 cm depth was used to prepare a soil suspension for each treatment with 1:2 ratio of soil:deionized water (Hendershot, 2006; Miller and Curtin, 2006). The pH and electrical conductivity of these soil suspensions were measured using a calibrated pH and conductivity meter (PC700, Oakton, Canada).

Potentially mineralizable nitrogen

Following the protocol of Gregorich and Beare (2006b), 5 g of fresh soil, which had been stored at 4 °C, was weighed into a 50 mL disposable centrifuge vial and was mixed well with 50 mL of 2 M potassium chloride (KCl). The suspension was centrifuged at 1900 g and the supernatant filtered through prewashed filter papers (Whatman No. 42). The extracts were stored at -20 °C until analyzed. This extraction was considered as pre-incubated ammonium-nitrogen (NH_4^+-N) for N mineralization.

For the post-incubation NH_4^+-N, another 5.0 g of soil sample was mixed with 10 mL of dH_2O and the tube sealed. The sample was incubated at a constant temperature (40 °C) for seven days. After the 7-day-anaerobic incubation, the tubes were removed from the incubator. Each post-incubated vial was filled with 40 mL (since the final volume should be 50 mL and post-incubated vials had

10 mL of dH_2O at the beginning) of 2.5 M KCl (in order to maintain the original concentration, 2.0 M KCl of the suspensions 40 mL of 2.5 M KCl was added into the post-incubated vial) and the extraction was repeated as above. The extracts were stored at -20 °C until they were analyzed. Before the analysis the frozen samples were thawed by keeping them at the room temperature for 24 hours. Thawed samples were then analyzed colorimetrically using an autoanalyzer (Technicon Autoanalyzer, Technicon Industrial Systems, Tarrytown, NY, USA). The difference between the pre-incubation and post-incubation measurements was considered as an indicator of potentially mineralizable N (Curtin and Campbell, 2006).

Total soil carbon and nitrogen

A 1.0-g sample of air-dried and ground soil samples from the 0-15, 15-30 and 30-60 cm depths were analyzed for total C and total N content using a LECO TruMac CNS analyzer (630-300-400, LECO Corporation, Saint Joseph, Michigan, USA). Similarly, 0.75-0.80 g ball-ground sub-samples of the LF and HF were analyzed for total C and N using a Costech ECS4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA).

Soil organic carbon

Samples (1.0 g) of air-dried, ground soils from the 0-15 and 15-30 cm soil depths were weighed into nickel-lined ceramic combustion boats. The samples were moistened with distilled water and acidified with 6% (w/v) sulfurous acid (H_2SO_3) to remove inorganic carbonates while keeping the samples at 70 °C. In the presence of inorganic carbonates, acid reacts with the carbonates to CO_2. Sulfurous acid was added repeatedly until no more CO_2 produced. The soil samples were oven-dried and analyzed for organic C using a Leco C632 carbon combustion analyzer (LECO® Corporation, St. Joseph, MI, USA).

3.5. Analysis of Crop Productivity

The crop productivity of the subsequent wheat crops in the different treatments were determined by harvesting individual plots in 2018. Plant density was estimated by counting the number of plants within a 1-m length of the two middle rows of the plot, 1 to 2 weeks after emergence.

Three to four days prior to crop harvest, 10 randomly selected plants were sampled. These plant samples were placed in a drier at 39-42 °C for about 1 wk until a constant dry weight was reached.

Total dry biomass was recorded. Seed was separated from the straw and seed biomass recorded. This information was used to calculate the harvest index (HI) as follows.

$$Harvest\ index = \frac{total\ seed\ weight}{Total\ above-ground\ biomass\ weight} \times 100\% \quad [3.5]$$

At maturity, six-plant rows in the center of each plot were harvested with a plot combine for the determination of kernel yield (kg ha^{-1}). Thousand kernel weight determined by weighing a subsample of 250 kernels and multiplying the weight by 4.

The number of wheat heads per m^2 area was determined by counting the head number in 1 m^2 quadrates in each plot. This information was used to calculate the number of kernels per head as follows.

$$Kernals\ per\ head = \left[\frac{Grain\ yield\ (kg\ ha^{-1}\ )}{1000\ kernal\ weight\ (kg)}\right] x\ number\ of\ wheat\ heads\ \ (ha^{-1}\ ) \quad [3.6]$$

3.6. Data Analysis

Data analysis was performed using SAS 9.4 (SAS Institute, 2017). Prior to analysis, all data were tested for normality using the Shapiro-Wilk test ($P \geq 0.05$) and homogeneity of variance using Levene's test ($P \geq 0.05$). All data, except bulk density was normally distributed and variances were homogenous. Transformations (log and square root transformations) applied to bulk density did not improve normal distribution or homogeneity of variances. Thus, the original data were statistically analyzed.

Soils sampled from the different treatments in the wheat phase (sampled in 2017) and pulse crop phase (sampled in 2018) were considered as 'repeated measures'. Thus, the data were analyzed using repeated measure mixed model in a randomized complete block design (RCBD). In this analysis, the treatment, soil depth and sampling year were considered as fixed factors, whereas block was considered as a random factor. Sampling year was included as a fixed factor to enable the evaluation of the year by depth interaction, year. Data on crop productivity and seed yield components of the 2018 wheat were analyzed in a RCBD mixed model. Overall treatment means were declared significant at $P \leq 0.05$. Mean comparisons were performed using the Tukey's Honest Significant Difference (HSD) test.

In addition, the following, pre-planned mean comparisons were carried out for each soil depth in each sampling year using an F test:

1. Continuous wheat (Treatment 1) vs. wheat grown with pulses in rotation (Treatments 2+ 3+4+5)/4 = Treatment 1 vs. Treatments (2+3+4+5)/4.
2. Shallow-rooted (lentil) and deep-rooted (chickpea) pulses grown alternately with wheat in same rotation (Treatment 5) vs. shallow-rooted (field pea and lentil) and deep-rooted (chickpea) pulses grown alternately with wheat in separate rotations (Treatment 2+ treatment 3+treament 4)/3 = Treatment 5 vs. Treatments (2+3+4)/3.
3. Deep-rooted (chickpea) pulses grown alternately with wheat (Treatment 4) vs. shallow-rooted pulses (field pea and lentil) grown alternately with wheat (Treatment 2+treatment 3)/2 = Treatment 5 vs. Treatment (2+3)/2.
4. Shallow-rooted field pea (Treatment 2) grown alternately with wheat vs. shallow-rooted lentil (Treatment 3) grown alternately with wheat = Treatment 2 vs. Treatment 3.

(**Note**: 1= W/W/W/W, 2= P/W/P/W, 3= L/W/L/W, 4= C/W/C/W, 5= L/W/C/W)

The ordination of PLFA biomarkers was performed by non-metric multidimensional scaling (NMDS) analysis using PC-Ord software version 6 (Gleneden Beach, OR 97388, USA). The Sorensen index was used to measure distance between the samples using the Autopilot slow option. Monte Carlo test was used to select the best solution for each axis by comparing the final stress value; the statistical significance of the final solution was determined. The final stress value implies the consistency of the final ordination in relation to the dissimilarities within the dataset. Lower (5 to 10) final stress values indicate better ordination of the data. If the stress value is greater than 20, misinterpretation of data is possible. The multi-response permutation procedure (MRPP) was conducted to identify group differences among the treatments and sampling years. Chance-corrected within-group agreement (A) becomes one when all the samples within the group are identical. The closer the value is to zero the more heterogeneous the samples.

4.0. RESULTS

4.1. Soil Physical Parameters

Soil moisture content in fall season

By considering soil sampling over the two years as a repeated data collection procedure, 2017 and 2018 soil moisture data of the three soil depths were analyzed in a repeated measure model. In both years, the grain legume crop treatment had no impact on fall soil moisture content, but soil moisture content was dependent on sampling year (P=0.04; Table 4.1). On average, soil moisture content in the fall of pulse crop phase was higher than the moisture contents in the fall of the wheat phase (0.087 kg kg^{-1} of soil vs. 0.080 kg kg^{-1} of soil). In addition, soil moisture content averaged over the two years varied with soil depth (P<0.0001). On average, soil moisture content in the 0-15 cm layer was lower than the two deeper layers (0.070 kg kg^{-1} of soil vs. 0.088 and 0.095 kg kg^{-1} of soil). None of the interactions involving sampling year, treatment and soil depth were significant for soil moisture content (P>0.05; Table 4.1).

A priori comparisons suggested that soil moisture content in fall of pulse crop phase at the 15-30 cm soil depth of the plots, where wheat was alternated with the chickpea (C/W/C/W), was lower than the average of the plots, where wheat was alternated with lentil (L/W/L/W) or field pea (P/W/P/W) (0.084 kg kg^{-1} of soil vs. 0.102 kg kg^{-1} of soil) (P=0.04; Table 4.1).

Total precipitation for the April-September period in 2015 (145.5 mm) and 2017 (143.3 mm) were lower than the normal 30-yr average (244.7 mm), whereas in 2016 precipitation (317.1mm) was higher than the 30-yr average (Figure. 4.1). The higher rainfall in July in 2016 (105.7 mm) was the main contributor to the higher precipitation in the 2016 growing season (Figure. 4.1).

Table 4.1. Effect of cropping sequence treatments on soil moisture content at three soil depths in falls of the 2016 and 2017 sampling years.

Treatment[ł]	Moisture content (kg kg^{-1} of soil)								
	0-15 cm		15-30 cm		30-60 cm		2017	2018	Overall
	2017[‡]	2018	2017	2018	2017	2018	mean	Mean	mean
1=W/W/W/W	0.072	0.073	0.092	0.096	0.085	0.089	**0.083**	**0.086**	**0.084**
2=P/W/P/W	0.062	0.079	0.080	0.108	0.108	0.094	**0.083**	**0.093**	**0.089**
3=L/W/L/W	0.055	0.077	0.070	0.096	0.092	0.090	**0.072**	**0.088**	**0.080**
4=C/W/C/W	0.055	0.074	0.085	0.084	0.089	0.103	**0.077**	**0.087**	**0.082**
5=L/W/C/W	0.075	0.072	0.083	0.084	0.102	0.098	**0.086**	**0.085**	**0.086**
Mean	**0.070**		**0.088**		**0.095**		**0.080**	**0.087**	-
	b[§]		**a**		**a**		**b**	**a**	
	Contrast and *P* values for *a priori* comparison[¶]								
1 vs.(2+3+4+5)/4	0.010	-0.002	0.012	0.003	-0.013	-0.007	-	-	-
	0.54^{ns}	0.57^{ns}	0.24^{ns}	0.71^{ns}	0.34^{ns}	0.33^{ns}	-	-	-
5 vs. (2+3+4)/3	0.018	-0.005	0.005	-0.012	0.006	0.002	-	-	-
	0.10^{ns}	0.12^{ns}	0.64^{ns}	0.15^{ns}	0.68^{ns}	0.72^{ns}	-	-	-
4 vs. (2+3)/2	-0.004	-0.004	0.010	-0.018	-0.011	0.011	-	-	-
	0.75^{ns}	0.21^{ns}	0.38^{ns}	0.04*	0.48^{ns}	0.14^{ns}	-	-	-
2 vs. 3	0.007	0.002	0.010	0.012	0.016	0.004	-	-	-
	0.57 ^{ns}	0.74^{ns}	0.50^{ns}	0.22^{ns}	0.36^{ns}	0.65^{ns}	-	-	-

***P* values for main and interaction effects of sampling year (Y) × treatment (T) × soil depth (D)**

	Y	T	D	Y×T	Y×D	T×D	Y×T ×D	-	-
P value	0.04**	0.44^{ns}	<0.0001 ***	0.40^{ns}	0.18^{ns}	0.68^{ns}	0.38^{ns}	-	-

ł W=Wheat, P=Field pea, L=Lentil, C=Chickpea.

‡ The soils sampled in the spring of 2017 and 2018 are representative of the 2016 wheat crop phase and 2017 pulse crop phase of rotations, respectively.

§ Values with same letter within each category (treatment and sampling year) are not significantly different at $P>0.05$, according to Tukey's HSD test.

¶ The contrast value was taken from the subtraction of the value on right from the value on the left in the comparison and the *P* value was mentioned following the contrast value.

^{ns}: non-significant ($P>0.05$), *, ** and *** denote significant at $P\leq0.05$, $P\leq0.01$ and $P\leq0.001$, respectively, according to Tukey's HSD test.

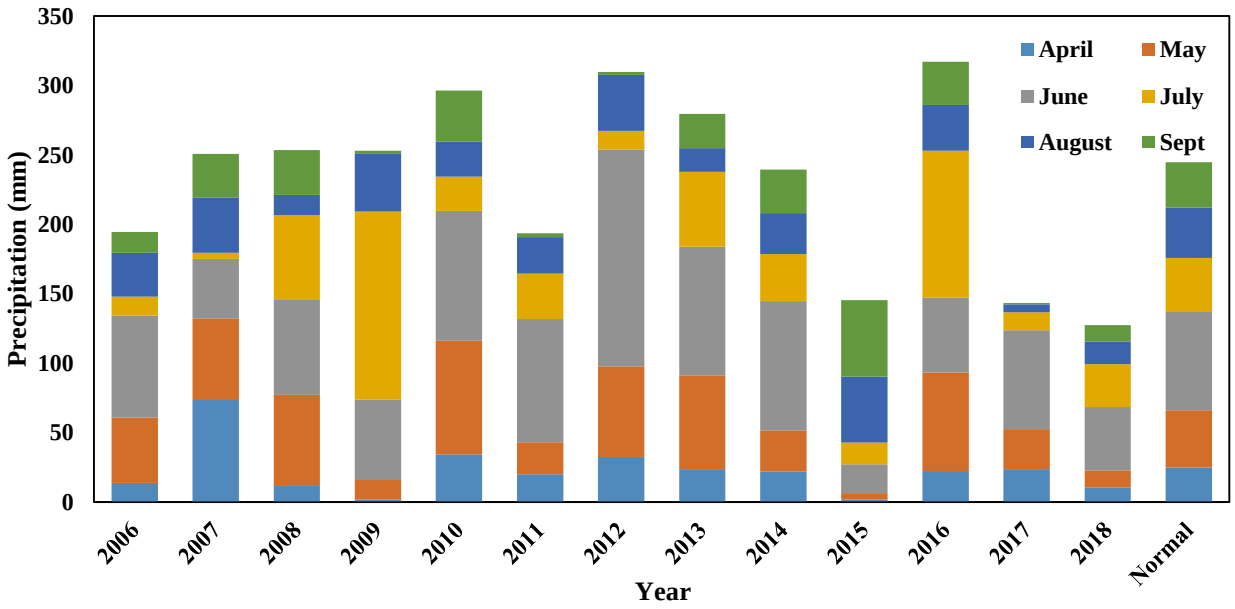

Fig. 4.1. Precipitation (mm) from April to September at the Brooks, AB field site from 2006 to 2018. Normal precipitation is the 30-year average precipitation (2006-2018).

Soil aggregate size distribution

Soil aggregates were separated into eight size ranges, namely >6.35 mm, 6.35-2.00 mm, 2.00-1.00 mm, 1.00-0.50 mm, 0.50-0.15 mm, 0.15-0.12 mm, 0.12-0.05 mm and <0.05 mm (Figure 4.2). The soils sampled in the spring of 2017 are representative of the 2016 wheat crop phase of the rotation and the soils sampled in the spring of 2018 are representative of the pulse crop phase of the rotations (Table 4.2). In general, the continuous wheat cropping system had the highest amount of largest macro-aggregates (>6.35 mm) compared to the pulse crop rotations, in both sampling years. The formation of larger aggregates was not influenced by pulse crop rotations. The wheat crop rotated with field pea, lentil and chickpea had elevated amounts of micro and meso-aggregates within the 1.00-0.50 mm and 0.50-0.15 mm ranges compared to the continuous wheat cropping system.

The soils in the continuous wheat treatment had the highest average amounts (238 g kg^{-1} of soil) of the largest aggregates (>6.35 mm) among all treatments. *A priori* comparison also suggested that the amount of the largest aggregates in the continuous wheat soil was higher in both sampling years compared to the soils from rotations that included pulse crops (Table 4.2). On average, >6.35 mm aggregates in 2018 were lower than 2017 (133 g kg^{-1} of soil vs. 152 g kg^{-1} of soil) (Table B.2).

Moreover, the year × treatment interaction for the largest aggregate size was significant due to the amount of >6.35 mm aggregates in the P/W/P/W and L/W/C/W rotations decreasing from 2017 to 2018. No decrease occurred in any of the other treatments (Table 4.3; Table B.2).

On average, the amount of the aggregates in the 6.35-2.00 mm category varied from 114 to 138 g kg^{-1} of soil (Table B.2). *A priori* comparison suggested that in both sampling years, the aggregates in the 6.35-2.00 mm category in rotations that included chickpea were different from the P/W/P/W and L/W/L/W rotations. However, the changes were not consistent; in 2017, the C/W/C/W rotation had higher amount of aggregates in the 6.35-2.00 mm category compared to the P/W/P/W and L/W/L/W rotations. The reverse was observed in 2018.

The soil from rotations with field pea, lentil and chickpea had statistically comparable amounts of 2.00-1.00 mm aggregates ($P>0.05$), but varied from 109 to 123 g kg^{-1} of soil (Figure 4.2 and Table B.2). From the 2017 to 2018, the amount of the aggregates in the 2.00-1.00 mm category increased from 97 g kg^{-1} of soil to 135 g kg^{-1} of soil (Table B.2). *A priori* comparisons suggested that the amount of this size category of soil aggregates from the W/W/W/W treatment was lower compared to the average of rotations with wheat alternated with field pea, lentil and chickpea in the wheat crop phase (79 g kg^{-1} of soil vs. 102 g kg^{-1} of soil) ($P<0.0001$; Table 4.2). There is also an indication that this size class of aggregates was higher in the rotation with chickpea (deep-rooted pulse crop) alternating with wheat, compared to the two rotations with field pea or lentil (shallow-rooted pulses) alternating with wheat in the 2017 (Table 4.2; Figure 4.2). The difference was not apparent in 2018 soils.

The W/W/W/W treatment had the smallest average amount of aggregates in the 1.00-0.50 mm category among all treatments (Figure 4.2) and the amounts in this category were statistically comparable among the wheat/pulse crop rotations (Table B.2). *A priori* comparison also revealed that continuous wheat had the lowest amount of aggregates in this size category compared to the rotations with pulse crops in both sampling years. On average, the amount of aggregates in the 1.00-0.50 mm category was higher in the pulse crop phase than the wheat crop phase (158 g of kg^{-1} of soil vs. 105 g of kg^{-1} of soil). Moreover, the year × treatment interaction for the amount of aggregates in the 1.00-0.50 mm category indicated the amount of this aggregate category in the P/W/P/W, C/W/C/W and L/W/C/W rotations increased from the 2017 to 2018 ($P<0.0001$), but no change occurred in the other treatments, including the W/W/W/W treatment ($P>0.05$; Table B.2).

A priori comparison revealed that the amount of 1.00-0.50 mm soil aggregates was lower in the treatment P/W/P/W compared to the L/W/L/W in 2017 to 2018 (95 g kg^{-1} of soil vs. 127 g kg^{-1} of soil) and a reverse trend was observed in 2018 (186 g kg^{-1} of soil for P/W/P/W and 159 g kg^{-1} of soil for L/W/L/W) (Table B.2).

On average, the soils from the W/W/W/W treatment had the lowest amount of 0.50-0.15 mm soil aggregates (226 g kg^{-1}) of all the treatments (Figure 4.2; Table B.2). Pulse species had no impact on the 0.50-0.15 mm category (P>0.05; Figure 4.2). *A priori* comparison also revealed that continuous wheat had the lowest average amount of aggregates in this size category compared to the rotations with pulse crops in both sampling years (208 g kg^{-1} of soil vs. 266 g kg^{-1} of soil in 2017, and 226 g kg^{-1} of soil vs. 289 g kg^{-1} of soil in 2018) (Figure 4.2; Table B.2). This aggregate size distribution varied with the sampling year (P<0.0001); on average, there were more aggregates in this size category in 2018 than in 2017 (Table 4.3).

The treatment L/W/C/W treatment had the highest amount (55 g kg^{-1} of soil) of aggregates in the 0.15-0.12 mm category (Table B.2). In contrast W/W/W/W and L/W/L/W had the lowest amount of this size class of soil aggregates (P<0.0001; Table 4.2; Table B.2). The amount of the aggregates in the 0.15-0.12 mm category showed no difference between the 2017 and 2018 cropping years (P>0.05).

The L/W/C/W had a small amount of aggregates in the 0.12-0.05 mm category of all of the treatments (Figure 4.2;Table B.2). This was confirmed by the *a priori* comparison (Table 4.2). On average, the amount of aggregates in the 0.12-0.05 mm category was lower in 2018 compared to 2017 (97 g kg^{-1} of soil vs. 151 g kg^{-1} of soil;Table B.2).

Soils from the treatments W/W/W/W, P/W/P/W and C/W/C/W had larger amounts of the smallest aggregate size category (<0.05 mm) than other treatments (P=0.0008) (Table 4.3;Table B.2). Furthermore, amounts of the smallest aggregates were lower in 2018 after the pulse crop year, than 2017 after the wheat crop year of the rotation (32 g kg^{-1} of soil vs. 77 g kg^{-1} of soil) (Table B.2).

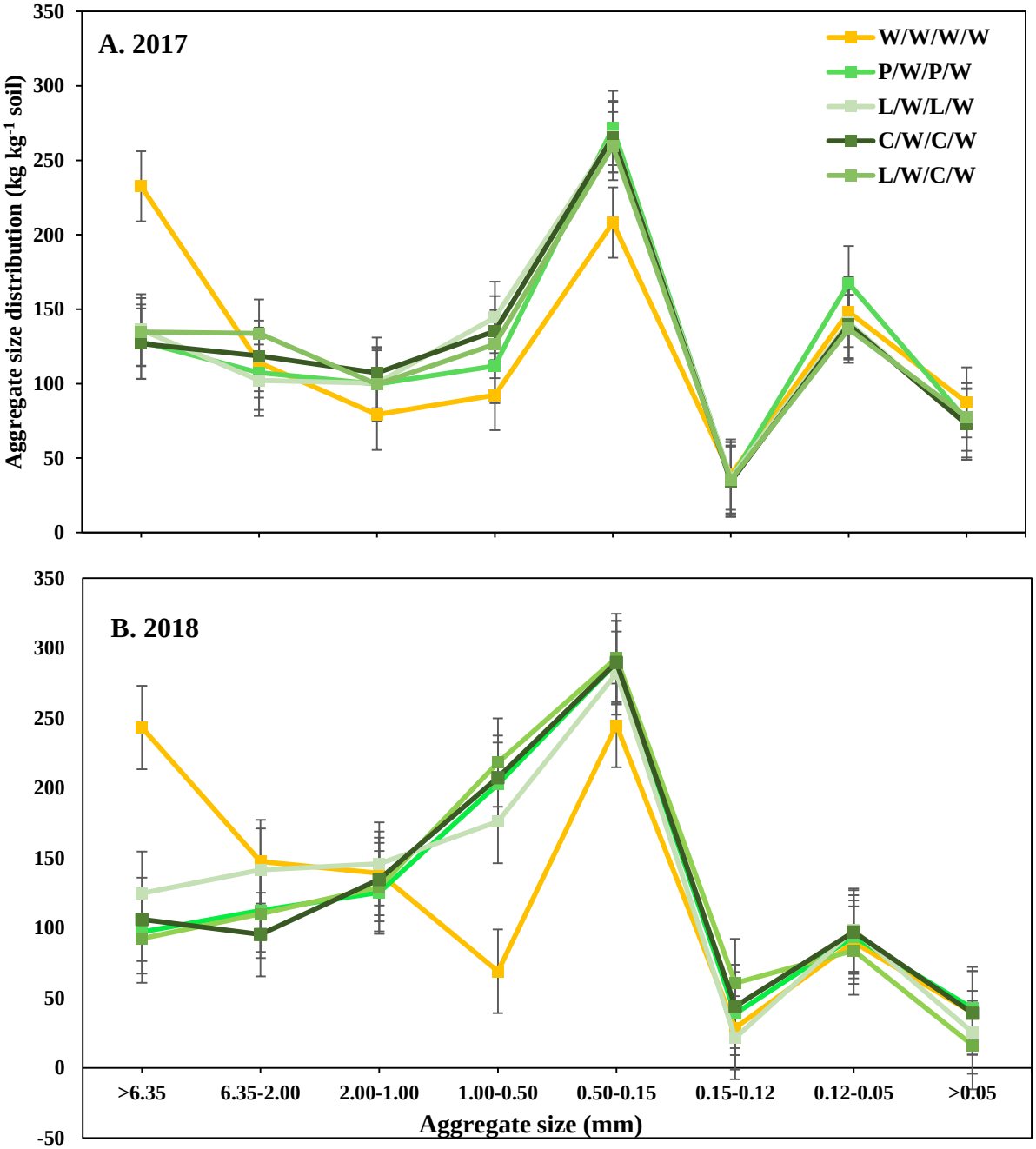

Fig. 4.2. Soil aggregate size distribution in 0-5 cm soil depth in two sampling years, the soils sampled in the spring of 2017 and 2018 are representative of the 2016 wheat crop phase and 2017 pulse crop phase of rotations (B). **Note**: Bars indicate standard error of means. The significant differences among different crop rotations based on the Tukey HSD test are in Table B.2.

Table 4.2. Values for *a priori* comparisons for aggregate size distribution in 0-5 cm soil from different crop rotation sequences.

Aggregate size category (mm)	Contrast and *P* values for *a priori* comparison†							
	2017‡				2018			
	1 vs. (2+3+4+5)/4§	5 vs. (2+3+4)/3	4 vs. (2+3)/2	2 vs. 3	1 vs. (2+3+4+5)/4	5 vs. (2+3+4)/3	4 vs. (2+3)/2	2 vs. 3
>6.35	101.1	4.27	-5.29	-7.93	138.1	-17.0	-4.84	-27.7
	<0.0001***¶	0.43ns	0.36ns	0.23ns	<0.0001***	0.03*	0.35ns	0.0005***
6.35-2.00	-1.29	24.3	13.8	5.08	32.6	-6.38	-31.7	-28.9
	0.70ns	<0.0001***	0.0024**	0.24ns	0.02*	0.62ns	0.03*	0.08ns
2.00-1.00	-22.5	-2.83	7.16	-0.60	5.32	-6.10	-0.90	-20.4
	<0.0001***	0.33ns	0.03*	0.86ns	0.34ns	0.29ns	0.88ns	0.01**
1.00-0.50	-37.2	-3.94	7.00	-32.7	-132.2	22.8	18.1	26.9
	<0.0001***	0.40ns	0.17ns	<0.0001***	<0.0001***	0.02*	0.06ns	0.02*
0.50-0.15	-57.5	-8.21	-3.12	5.78	-43.9	5.86	3.80	7.65
	<0.0001***	0.19ns	0.64ns	0.45ns	0.001***	0.61ns	0.76ns	0.58ns
0.15-0.12	3.32	-0.10	-2.26	-1.06	-12.5	25.84	13.8	17.3
	0.14ns	0.96ns	0.35ns	0.68ns	0.003**	<0.0001***	0.003**	0.002**
0.12-0.05	1.93	-12.7	-14.5	26.3	-3.47	-12.6	1.03	-4.71
	0.67ns	0.01**	0.01**	0.0003***	0.48ns	0.02*	0.85ns	0.45ns
<0.05	12.7	4.07	-1.48	2.45	8.48	-19.4	5.23	17.1
	<0.0001***	0.05*	0.147ns	0.31ns	0.14ns	0.003**	0.39ns	0.02*

† The contrast value was taken from the subtraction of the value on right from the value on the left in the comparison and the *P* value was mentioned following the contrast value; ‡ the soils sampled in the spring of 2017 and 2018 are representative of the 2016 wheat crop phase and 2017 pulse crop phase of rotations, respectively. § 1= W/W/W/W, 2= P/W/P/W, 3= L/W/L/W, 4= C/W/C/W, 5= L/W/C/W; ¶ ns: non-significant (*P*>0.05), *, **and *** indicate significance at *P*≤0.05, *P*≤0.01 and *P*≤0.001, respectively, according to Tukey's HSD test.

Table 4.3. Summary of *P* values from the repeated measure analysis of aggregate size distribution in 0-5 cm soil depth from different crop rotation treatments in different sampling years (2017 and 2018).

Aggregate size category (mm)	*P* values for main and interaction effects of sampling year (Y) × treatment (T) × soil depth (D)		
	Y	T	Y×T
>6.35	<0.0001***†	<0.0001***	<0.0001***
6.35-2.00	0.22^{ns}	0.04*	0.0003***
2.00-1.00	<0.0001***	0.01**	0.0004***
1.00-0.50	<0.0001***	<0.0001***	<0.0001***
0.50-0.15	<0.0001***	<0.0001***	0.62^{ns}
0.15-0.12	0.11^{ns}	<0.0001***	<0.0001***
0.12-0.05	<0.0001***	0.002***	0.01**
<0.05	<0.0001***	0.0008***	0.004**

† ^{ns}: non-significant (P>0.05), *, ** and *** indicate significance at P≤0.05, P≤0.01 and P≤0.001, respectively, according to Tukey's HSD test.

Soil bulk density

Including pulse crops in rotation with wheat had no impact on bulk density of the soils (Table B.3).

4.2. Soil Biological Parameters

Soil organic matter fractions

Soil organic matter was separated into light fraction (LF) and heavy fraction (HF). On a mass basis, only about 0.5 to 1.5 % of the SOM was in the LF (Table 4.4). In both sampling years, the rotation, where chickpea alternated with wheat had the highest amount of LF organic matter and consequently, the lowest amount of HF organic matter. The continuous wheat and L/W/C/W consistently had the lowest amount of LF organic matter and the highest amount of HF organic matter. However, on average, soil with continuous wheat had the lowest LF and the highest HF organic matter. Field pea or lentil alternating with wheat resulted in similar amounts of LF and HF organic matter in both sampling years. The amounts of LF and HF dependent on the sampling year and on average, 2018 had lower amount of LF compared to 2017 (P<0.0001), and HF had a reverse trend (P<0.0001). The treatment x sampling year interaction for LF and HF were significant (P<0.0001). This was mainly due to a decrease in LF mass of C/W/C/W and increase in HF mass of the C/W/C/W treatment from 2017 to 2018 (Table 4.4).

A priori treatment comparison further revealed that, on average, the inclusion of the pulse crops collectively increased the mass of LF (P<0.0001) and decreased the mass of HF (P<0.0001) compared to that of the W/W/W/W treatment. On average, P/W/P/W and L/W/L/W had lower LF and higher HF compared to chickpea alternated with wheat in both 2017 and 2018 (Table 4.4). The only difference was that in 2017 rotations with field pea and lentil rotations had the same amount of both fractions, but in 2018 the rotation with field pea had a higher amount of LF and lower amount of HF than the rotation with lentil (P=0.02; Table 4.4).

Total carbon (C) and nitrogen (N) abundance in different fractions in organic matter

The overall amount of total C in both LF and HF organic matter did not vary among treatments (P>0.05; Table 4.5). In contrast, the amounts of C in LF and HF were different between sampling years. On average, LF had a higher amount of C in 2017 (9.07 g kg^{-1} of soil) compared to 2018 (5.64 g kg^{-1} of soil) (P<0.0001) while C amount in HF was higher in 2018 compared to 2017 (8.48 vs. 5.29 g kg^{-1} of soil) (P<0.0001). *A priori* comparisons for C in LF in 2018 suggests that all the crop rotations with field pea, lentil and chickpea crops had lower amounts of C in LF compared to continuous wheat (P=0.02; Table 4.5).

On average, crop rotation treatments had no impact on the amount of N in LF and HF (P>0.05; Table 4.6), but the effect was significant between years (P<0.0001). Soils sampled in 2017 showed higher N content in LF and lower N content in HF than 2018. *A priori* comparisons for N in LF sampled in 2018 suggested that all of the crop rotations with pulse crops had lower LF-N contents compared to continuous wheat (P=0.05). In addition, wheat grown alternately with lentil (L/W/L/W) had a higher LF-N content than wheat grown alternately with field pea (P/W/P/W) (0.57 N g kg^{-1} of soil vs. 0.40 g kg^{-1} of soil) in 2018 (P=0.01; Table 4.6).

Table 4.4. Effect of different crop sequence treatments on the mass of soil organic matter fractions at 0-15 cm soil depth in springs of 2017 and 2018.

Treatment†	Mass of different organic matter fractions (g kg^{-1} soil)					
	Light fraction (LF)			Heavy fraction (HF)		
	2017‡	2018	Mean	2017	2018	Mean
1=W/W/W/W	4.93 ef§	4.07 f	**4.50 e¶**	996 a	995 ab	**996 a**
2=P/W/P/W	7.62 c	7.36 cd	**7.49 b**	992 d	993 cd	**992 d**
3=L/W/L/W	7.10 cd	6.10 de	**6.60 c**	993 cd	994 bc	**993 c**
4=C/W/C/W	14.92 a	9.64 b	**12.28 a**	985 f	990 e	**988 e**
5=L/W/C/W	5.52 e	5.24 ef	**5.38 d**	994 b	995 ab	**995 b**
Mean	**7.85 a**	**6.66 b**	-	**992 b**	**993 a**	
	Contrast and *P* values for *a priori* comparison#					
1 vs. (2+3+4+5)/4	-3.86	-3.02		5.00	2.00	
	<0.0001 ***¶¶	<0.0001 ***	-	<0.0001 ***	<0.0001 ***	-
5 vs. (2+3+4)/3	-4.36	-2.46		4.00	2.67	
	<0.0001 ***	<0.0001 ***	-	<0.0001 ***	<0.0001 ***	-
4 vs. (2+3)/2	7.56	2.91		-7.50	-3.50	
	<0.0001 ***	<0.0001 ***	-	<0.0001 ***	<0.0001 ***	-
2 vs. 3	0.52	1.26		-1.00	-1.00	
	0.13 ns	0.02 *	-	0.13 ns	0.02 *	-
	***P* values for main and interaction effects of sampling year (Y) × treatment (T)**					
	Y	**T**	**Y×T**	-	-	-
Weight of LF	<0.0001 ***	<0.0001 ***	<0.0001 ***	-	-	-
Weight of HF	<0.0001 ***	<0.0001 ***	<0.0001 ***	-	-	-

† W=Wheat, P=Field pea, L=Lentil, C=Chickpea.

‡ The soils sampled in the spring of 2017 and 2018 are representative of the 2016 wheat crop phase and 2017 pulse crop phase of rotations, respectively.

§ Mean values with the same letters indicates no significant difference (P>0.05) between year × treatment interactions according to Tukey's HSD test.

¶ Values with same letter within each category (treatment and sampling year) are not significantly different at *P*>0.05, according to Tukey's HSD test.

The contrast value was taken from the subtraction of the value on right from the value on the left in the comparison and the *P* value was mentioned following the contrast value.

¶¶ ns: non-significant (*P*>0.05), *, ** and *** denote significant at *P*≤0.05, *P*≤0.01 and *P*≤0.001, respectively, according to Tukey's HSD test.

Table 4.5. Carbon (C) content in light and heavy fractions at 0-15 cm soil depth from different crop rotation sequences in springs of 2017 and 2018.

Treatment†	C content in different organic matter fractions (g kg^{-1} of soil)					
	Light fraction (LF)			Heavy fraction (HF)		
	2017‡	2018	Mean	2017	2018	Mean
1=W/W/W/W	8.46 ab§	6.59 bc	**7.53**	5.67 b	8.31 a	**6.99**
2=P/W/P/W	8.66 ab	4.86 c	**6.76**	5.36 b	9.07 a	**7.22**
3=L/W/L/W	9.26 a	5.64 c	**7.45**	4.82 b	8.17 a	**6.50**
4=C/W/C/W	10.04 a	5.77 c	**7.90**	5.37 b	8.39 a	**6.88**
5=L/W/C/W	8.95 ab	5.36 c	**7.15**	5.24 b	8.42 a	**6.83**
Mean	**9.07 a**¶	**5.64 b**	-	**5.29 b**	**8.48 a**	-
	Contrast and *P* values for *a priori* comparison#					
1 vs. (2+3+4+5)/4	-0.767	1.18		0.473	-0.203	
	0.58^{ns}††	0.02*	-	0.22^{ns}	0.24^{ns}	-
5 vs. (2+3+4)/3	-0.370	-0.063		0.057	-0.123	
	0.75^{ns}	0.89^{ns}	-	0.89^{ns}	0.57^{ns}	-
4 vs. (2+3)/2	1.08	0.520		0.280	-0.230	
	0.56^{ns}	0.29^{ns}	-	0.50^{ns}	0.14^{ns}	-
2 vs. 3	-0.600	-0.780		0.540	0.900	
	0.07^{ns}	0.17^{ns}	-	0.26^{ns}	0.46^{ns}	-
	***P* values for main and interaction effects of sampling year (Y) × treatment (T)**					
	Y	**T**	**Y×T**	-	-	-
C content in LF	<0.0001***	0.25^{ns}	0.20^{ns}	-	-	-
C content in HF	<0.0001***	0.30^{ns}	0.59^{ns}	-	-	-

† W=Wheat, P=Field pea, L=Lentil, C=Chickpea.

‡ The soils sampled in the spring of 2017 and 2018 are representative of the 2016 wheat crop phase and 2017 pulse crop phase of rotations, respectively.

§ Mean values with the same letters indicates no significant difference (P>0.05) between year × treatment interactions according to Tukey's HSD test.

¶ Values with same letter within each category (treatment and sampling year) are not significantly different at *P*>0.05, according to Tukey's HSD test.

The contrast value was taken from the subtraction of the value on right from the value on the left in the comparison and the *P* value was mentioned following the contrast value.

†† ^{ns}: non-significant (*P*>0.05), *, ** and *** denote significant at *P*≤0.05, *P*≤0.01 and *P*≤0.001, respectively, according to Tukey's HSD test.

Table 4.6. Nitrogen (N) content in light and heavy fractions at 0-15 cm soil depth from different crop rotation sequences in springs of 2017 and 2018.

Treatment[ɬ]	N content in different organic matter fractions (g kg^{-1} of soil)					
	Light fraction (LF)			Heavy fraction (HF)		
	2017[‡]	2018	Mean	2017	2018	Mean
1=W/W/W/W	0.75 ab[§]	0.60 abc	**0.67**	0.63 b	0.83 a	**0.73**
2=P/W/P/W	0.78 ab	0.40 c	**0.59**	0.60 b	0.91 a	**0.75**
3=L/W/L/W	0.83 a	0.57 bc	**0.70**	0.55 b	0.84 a	**0.69**
4=C/W/C/W	0.80 ab	0.59 abc	**0.70**	0.58 b	0.85 a	**0.71**
5=L/W/C/W	0.78 ab	0.44 c	**0.61**	0.59 b	0.86 a	**0.72**
Mean	**0.79 a[¶]**	**0.52 b**	-	**0.59 b**	**0.86 a**	-
	Contrast and *P* values for *a priori* comparison[#]					
1 vs. (2+3+4+5)/4	-0.048	0.100	-	0.050	-0.035	-
	0.36^{ns}[ǂǂ]	0.05*	-	0.18^{ns}	0.49^{ns}	-
5 vs. (2+3+4)/3	-0.023	-0.080	-	0.013	-0.007	-
	0.91^{ns}	0.11^{ns}	-	0.83^{ns}	0.78^{ns}	-
4 vs. (2+3)/2	-0.005	0.105	-	0.005	-0.025	-
	0.49^{ns}	0.05*	-	0.88^{ns}	0.94^{ns}	-
2 vs. 3	-0.050	-0.170	-	0.050	0.070	-
	0.11^{ns}	0.01**	-	0.28^{ns}	0.58^{ns}	-
	***P* values for main and interaction effects of sampling year (Y) × treatment (T)**					
	Y	**T**	**Y ×T**	-	-	-
N content in LF	0.14^{ns}	<0.0001***	0.20^{ns}	-	-	-
N content in HF	0.44^{ns}	<0.0001***	0.56^{ns}	-	-	-

ɬ W=Wheat, P=Field pea, L=Lentil, C=Chickpea.

‡ The soils sampled in the spring of 2017 and 2018 are representative of the 2016 wheat crop phase and 2017 pulse crop phase of rotations, respectively.

§ Mean values with the same letters indicates no significant difference (P>0.05) between year × treatment interactions according to Tukey's HSD test.

¶ Values with same letter within each category (treatment and sampling year) are not significantly different at *P*>0.05, according to Tukey's HSD test.

The contrast value was taken from the subtraction of the value on right from the value on the left in the comparison and the *P* value was mentioned following the contrast value.

ǂǂ ^{ns}: non-significant (*P*>0.05), *, ** and *** denote significant at *P*≤0.05, *P*≤0.01 and *P*≤0.001, respectively, according to Tukey's HSD test.

Microbial community composition

Crop rotation treatments had no impact on the microbial community composition, including abundance of total PLFAs, bacterial and AMF biomarkers (P=0.20; Table 4.7). With the exception of fungal biomarkers, all of the microbial pools were affected by cropping year (Table 4.7). *A priori* comparison demonstrated that none of the treatment groups had an effect on the microbial composition, except for the Gram - bacterial composition in 2017. The crop rotation with wheat and chickpea had higher Gram- bacterial abundance compared to the rotations with lentil and field pea (Table 4.12).

Non-metric multidimensional scaling (NMDS) and MRPP analysis of PLFA profiling (mol %) indicated heterogeneity among microbial communities in both sampling years (Fig. 4.3). However, different crop rotations had no effect on microbial community structure. Microbial community profiles were mainly differentiated along axis 1, which explained 83 % of the variability in the NMDS solution. Different crop rotations in the 2017 sampling year, which had higher total PLFA, clearly separated from the crop rotations in 2018 (Fig. 4.3).

Table 4.7. Summary of *P* values from *a priori* comparisons and repeated measures analysis of soil microbial composition at 0-15 cm soil depth in springs of 2017 and 2018.

Contrasts†	Contrast and *P* values for *a priori* comparisons‡											
	Total PLFA		Bacteria		Gram positive		Gram negative		Fungi		AMF§	
	2017¶	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
1 vs. (2+3+4+5)/4	-2.52	-2.49	-2.17	-0.48	0.09	0.43	-1.87	-1.08	-0.37	-0.30	-0.18	-0.03
	0.68 ns#	0.55 ns	0.59 ns	0.84 ns	0.94 ns	0.55 ns	0.34 ns	0.44 ns	0.31 ns	0.13 ns	0.48 ns	0.81 ns
3 vs. (2+4+5)/3	14.3	7.14	9.29	2.81	2.10	0.93	5.36	1.20	0.52	0.09	0.61	0.22
	0.18 ns	0.11 ns	0.17 ns	0.28 ns	0.26 ns	0.22 ns	0.07 ns	0.40 ns	0.21 ns	0.65 ns	0.18 ns	0.10 ns
5 vs. (2+4)/2	-0.87	2.34	-0.92	1.44	0.24	0.22	-1.11	0.82	-0.50	0.13	-0.05	0.03
	0.08 ns	0.61 ns	0.08 ns	0.59 ns	0.20 ns	0.78 ns	0.04*	0.59 ns	0.32 ns	0.55 ns	0.06 ns	0.82 ns
2 vs. 4	6.67	-0.89	4.90	0.07	1.03	0.26	3.80	-0.20	0.80	-0.24	0.26	-0.02
	0.59 ns	0.86 ns	0.51 ns	0.98 ns	0.86 ns	0.77 ns	0.23 ns	0.91 ns	0.07 ns	0.34 ns	0.56 ns	0.88 ns
	***P* values for main and interaction effects of sampling year (Y) × treatment (T) analysis**											
	Y		T		Y×T							
Total PLFAs	<0.0001 ***		0.53 ns		0.30 ns		-	-	-	-	-	-
Bacteria	<0.0001 ***		0.43 ns		0.35 ns		-	-	-	-	-	-
Gram positive	<0.0001 ***		0.78 ns		0.53 ns		-	-	-	-	-	-
Gram negative	<0.0001 ***		0.14 ns		0.16 ns		-	-	-	-	-	-
Fungi	0.12 ns		0.18 ns		0.17 ns		-	-	-	-	-	-
AMF	<0.000 ***		0.38 ns		0.16 ns		-	-	-	-	-	-

† 1= W/W/W/W, 2= P/W/P/W, 3= L/W/L/W, 4= C/W/C/W, 5= L/W/C/W; ‡ The contrast value was taken from a subtraction of the value on right from the value on the left in the comparison and the *P* value was mentioned following the contrast value; § AMF: arbuscular mycorrhizal fungi; ¶ The soils sampled in the spring of 2017 and 2018 are representative of the 2016 wheat crop phase and 2017 pulse crop phase of rotations, respectively; # ns: non-significant ($P>0.05$), *, ** and *** denote significant at $P\leq0.05$, $P\leq0.01$ and $P\leq0.001$, respectively, according to Tukey's HSD test.

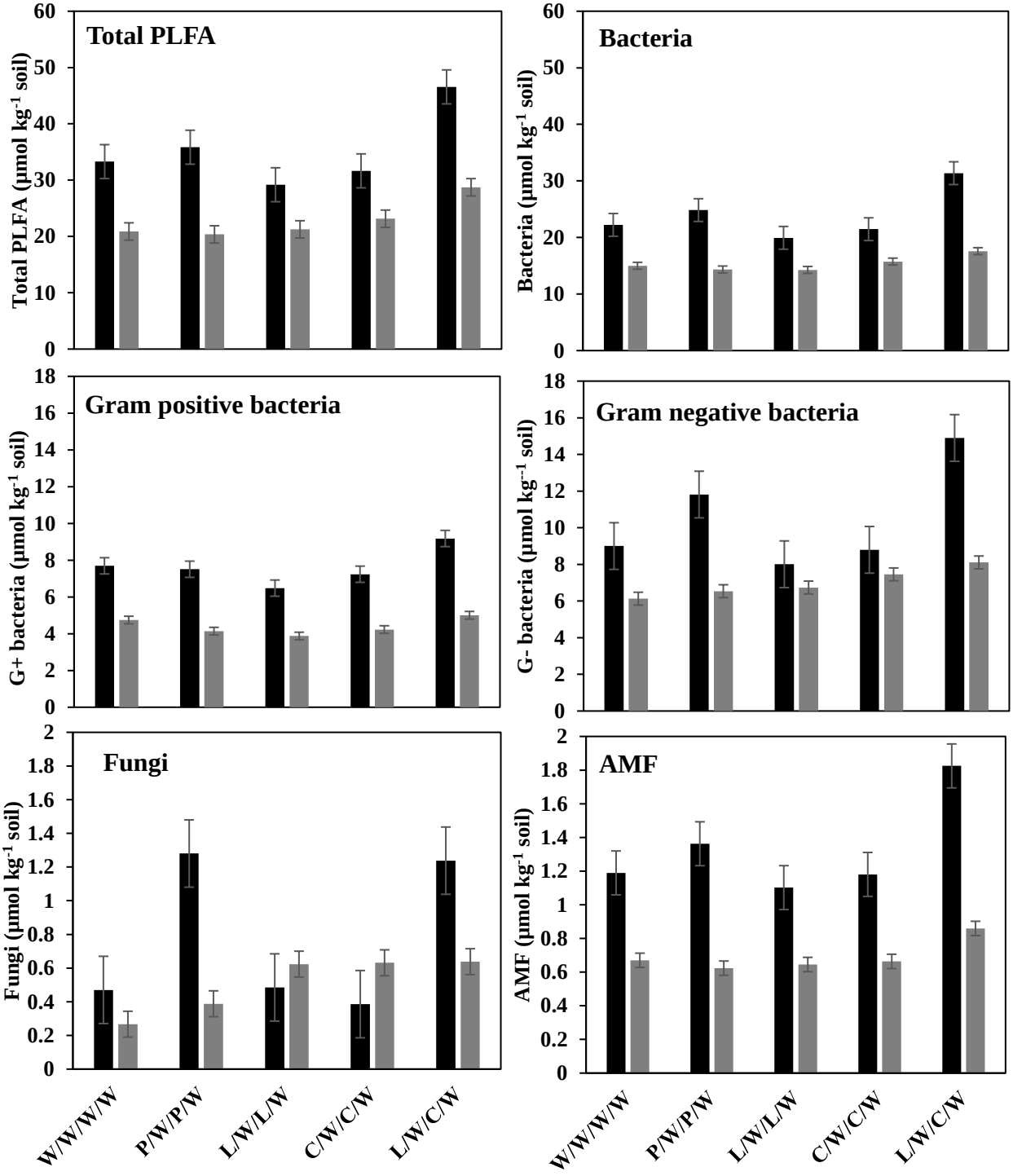

Fig. 4.3. Soil microbial community composition at 0-15 cm soil depth of different crop rotation sequences sampled in springs of 2017 and 2018. Black and grey colors represent the soil microbial abundance (µmol kg^{-1} soil) in 2017 and 2018 respectively. AMF denotes arbuscular mycorrhizal fungi. **Note:** The y axis in different graphs are in different scale.

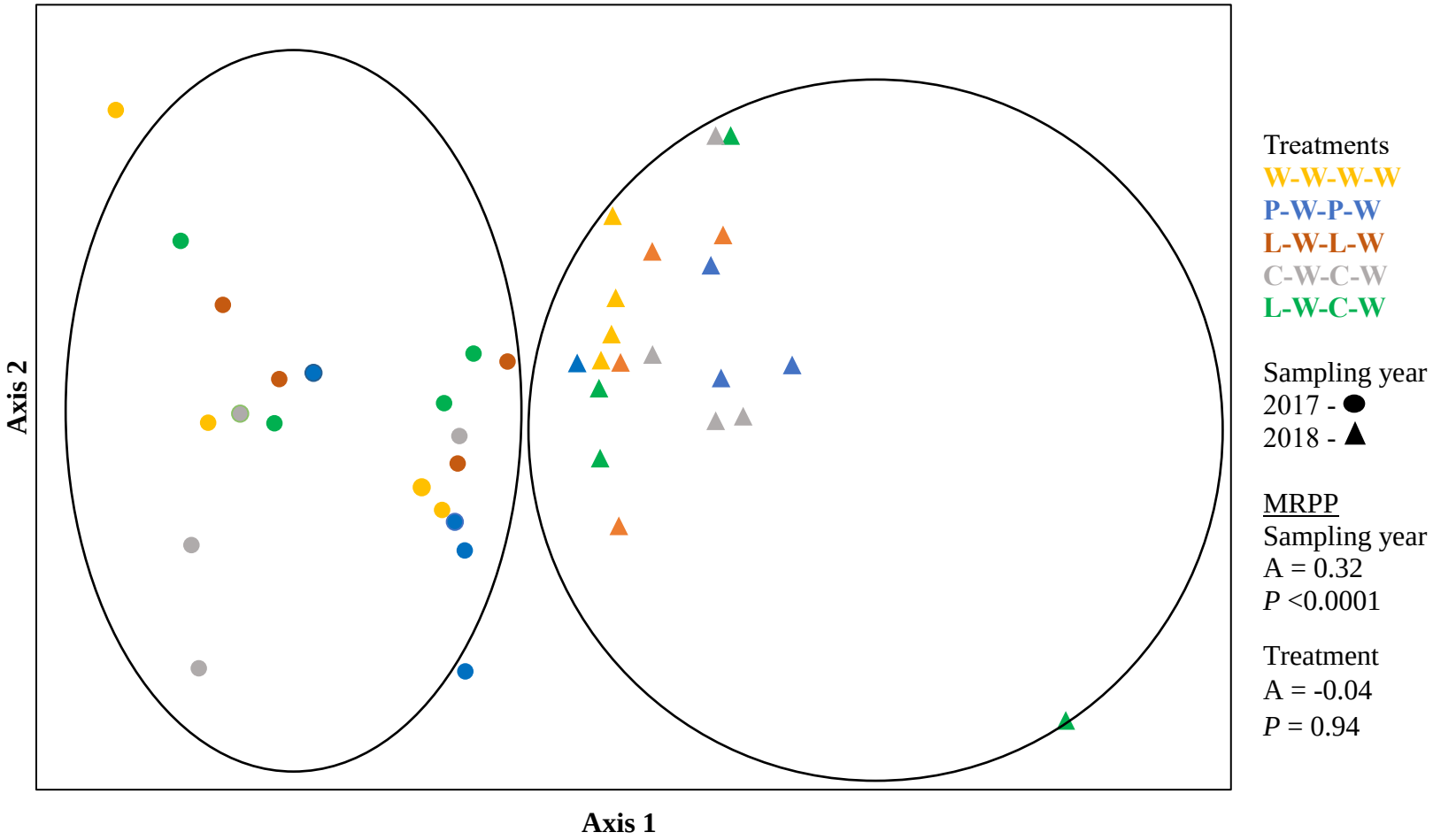

Fig. 4.4. Non-metric multidimensional scaling (NMDS) and multi-response permutation procedure (MRPP) analysis of crop rotation and year on microbial community structure (mol% PLFA). Final stress = 9.76 %. The A statistic indicates within group homogeneity; an A value 1 means the samples within a group are identical, A=0 would indicate a level of homogeneity expected by chance. **Note:** For the construction of NMDS graph, biomarkers, which have relative abundance of more than 5% were considered.

4.3. Soil Chemical Parameters

Soil pH

On average, soil pH of the W/W/W/W rotation was higher than all the other rotations in both sampling years (Table 4.8). The rotations that included pulse crops namely P/W/P/W, L/W/L/W, CW/C/W and L/W/C/W all had similar pH values. Soil pH varied with sampling year (P<0.0001) with generally higher pH values after wheat year (2017) than the pulse crop year (2018). Furthermore, the treatment × sampling year interaction for soil pH was significant (P=0.006) due to a decrease in the soil pH of the P/W/P/W treatment from 2017 to 2018 (Table 4.8).

A priori comparison indicated that the crop rotations with pulse crops had lower soil pH values compared to the continuous wheat rotation, which was persistant in both sampling years (P<0.0001; Table 4.8). Furthermore, in 2017, which was the year after wheat cultivation, all the comparisons showed variations. The pH of the C/W/C/W treatment was lower (6.59) than the average of the P/W/P/W and L/W/L/W/ treatments (7.0) in 2017 and but not consistent in 2018. (Table 4.8).

Soil EC

Soil EC was affected neither by crop rotation nor cropping year (Table 4.8). Soil EC of the W/C/W/C/W treatment was slightly higher than the average of P/W/P/W and L/W/L/W treatments (0.008 S m^{-1} vs. 0.006 S m^{-1}; Table 4.8).

Table 4.8. Soil pH and electrical conductivity (EC) at 0-15 cm depth of different crop rotation sequences in springs of 2017 and 2018.

Treatment†	Soil pH			Soil EC (S m^{-1})		
	2017‡	2018	Mean	2017	2018	Mean
1=W/W/W/W	7.47 a§	6.86 ab	**7.16 a¶**	0.007	0.006	**0.007 ab**
2=P/W/P/W	7.40 a	6.06 cd	**6.73 b**	0.006	0.006	**0.006 b**
3=L/W/L/W	6.67 bc	6.27 bcd	**6.47 bc**	0.006	0.006	**0.006 b**
4=C/W/C/W	6.59 bcd	5.90 d	**6.24 c**	0.008	0.008	**0.008 a**
5=L/W/C/W	6.60 bc	6.40 bcd	**6.50 bc**	0.006	0.008	**0.006 b**
Mean	**6.94 a**	**6.30 b**	-	**0.006 a**	**0.007 a**	-
	Contrast and *P* values for *a priori* comparison#					
1 vs. (2+3+4+5)/4	0.655	0.703	-	0.001	-0.001	-
	<0.0001***††	0.003**	-	0.27^{ns}	0.95^{ns}	-
5 vs. (2+3+4)/3	-0.287	0.323	-	-0.001	0.001	-
	0.02*	0.15^{ns}	-	0.84^{ns}	0.56^{ns}	-
4 vs. (2+3)/2	-0.445	-0.265	-	0.002	0.002	-
	0.001**	0.24^{ns}	-	0.03*	0.006**	-
2 vs. 3	0.730	-0.210	-	0.000	0.000	-
	<0.0001***	0.41^{ns}	-	0.82^{ns}	0.65^{ns}	-
	***P* values for main and interaction effects of sampling year (Y) × treatment (T)**					
	Y	**T**	**Y×T**	-	-	-
pH	<0.0001***	<0.0001***	0.006**	-	-	-
EC	0.01**	0.69^{ns}	0.91^{ns}	-	-	-

† W=Wheat, P=Field pea, L=Lentil, C=Chickpea.

‡ The soils sampled in the spring of 2017 and 2018 are representative of the 2016 wheat crop phase and 2017 pulse crop phase of rotations, respectively.

§ Mean values with the same letters indicates no significant difference (P>0.05) between year × treatment interactions according to Tukey's HSD test.

¶ Values with same letter within each category (treatment and sampling year) are not significantly different at *P*>0.05, according to Tukey's HSD test.

The contrast value was taken from the subtraction of the value on right from the value on the left in the comparison and the *P* value was mentioned following the contrast value.

†† ^{ns}: non-significant (*P*>0.05), $^{*, **}$ and *** denote significant at *P*≤0.05, *P*≤0.01 and *P*≤0.001, respectively, according to Tukey's HSD test.

Total soil carbon

Crop rotation sequence had no effect on total soil C content (P>0.05) however, the total soil C tended to decrease with soil depth (P<0.001; Table 4.9). In addition, the total soil C was dependent on the sampling year (P=0.01). On average, the total soil C content in 2017 was higher than that observed in 2018 (13.5 g kg^{-1} of soil vs. 12.8 g kg^{-1} of soil). None of the interactions involving treatment, sampling year and soil depth as factors was significant at P<0.05. *A priori* comparisons showed that, on average the continuous wheat rotation had a higher total soil C content in the 0-15 cm soil layer in 2018 compared to the rotations with pulse crops (Table 4.9).

Total soil nitrogen

Crop rotation had an impact on the total soil N and on average, varied from 1.03 g kg^{-1} soil for L/W/C/W to 1.14 g kg^{-1}soil for C/W/C/W (Table 4.10). Sampling year also had an impact on total soil N (P=0.006), with slightly higher values in 2018 (1.11 g kg^{-1} of soil) compared to 2017 (1.06 g kg^{-1} of soil). Similar to total soil C, total soil N also tended to decreased with soil depth (P<0.0001). The treatment × year and soil depth × year interactions for the total soil content were significant (Table 4.10). While the total N content of the L/W/L/W treatment increased in 2018 compared to that of 2017, the total soil N contents of the other treatments remain unchanged.

Total soil carbon:nitrogen ratio

Crop rotation sequence had no effect on the soil C:N ratio (P>0.05; Table 4.11). In contrast, C:N ratio varied with soil depth (P<0.0001), with a higher C:N ratio at the 30-60 cm depth (17.5) compared to the other soil depths 0-15 and 15-30 cm (Table 4.11). Furthermore, C:N ratio was generally higher in 2017 than in 2018 (14.5 vs. 12.3) reflecting the different phases of the rotations. Moreover, the year × depth interaction (P=0.0001) for soil C:N ratio suggests that the C:N ratio at the 30-60 cm depth was lower in 2018 compared to 2017, but no such changes occurred at other soil depths (Table 4.11).

The *a priori* treatment comparison for soil C:N ratio results showed that rotations with pulse crop had higher C:N ratio compared to that of the continuous wheat, which was not consistent in 2018. Rotations with chickpea had higher soil C:N ratio compared to rotations with lentil and field pea at 0-15 cm soil depth in both 2017 (12 g kg^{-1} of soil vs. 11 g kg^{-1} of soil) and reverse the trend in 2018 (10.7 g kg^{-1} of soil vs. 11.1 g kg^{-1} of soil; Table 4.11).

Table 4.9. Carbon (C) content at three soil depths from different crop rotation sequences in springs of 2017 and 2018.

Treatment†	Soil total C in different soil depths in different years (g kg^{-1} of soil)								
	0-15 cm		15-30 cm		30-60 cm		Mean	Mean	Overall
	2017‡	2018	2017	2018	2017	2018	2017	2018	mean
1=W/W/W/W	14.5	15.3	13.4	12.6	11.0	10.4	**13.0**	**12.8**	**12.9**
2=P/W/P/W	14.4	14.3	13.7	12.5	12.8	11.8	**13.6**	**12.8**	**13.2**
3=L/W/L/W	14.5	14.2	13.4	12.1	11.6	10.8	**13.2**	**12.4**	**12.8**
4=C/W/C/W	15.8	14.5	13.8	13.0	12.31	12.9	**13.8**	**13.5**	**13.7**
5=L/W/C/W	14.6	14.2	13.5	11.2	13.1	11.0	**13.7**	**12.4**	**13.0**
Mean	**14.6 a§**		**13.0 b**		**11.7 c**		**13.5 a**	**12.8 b**	-
	Contrast and *P* values for *a priori* comparison¶								
1 vs. (2+3+4+5)/4	-0.325	1.000	-0.200	0.400	-1.453	-1.225	-	-	-
	0.55$^{ns\#}$	0.03*	0.68^{ns}	0.63^{ns}	0.31^{ns}	0.31^{ns}	-	-	-
5 vs. (2+4+3)/3	-0.300	-0.133	-0.133	-1.33	0.863	-0.833	-	-	-
	0.54^{ns}	0.67^{ns}	0.85^{ns}	0.25^{ns}	0.56^{ns}	0.50^{ns}	-	-	-
4 vs. (2+3)/2	1.35	0.250	0.250	0.700	0.110	1.60	-	-	-
	0.02*	0.53^{ns}	0.68^{ns}	0.19^{ns}	0.93^{ns}	0.23^{ns}	-	-	-
2 vs. 3	-0.100	0.100	0.300	0.400	1.20	1.00	-	-	-
	0.92^{ns}	0.82^{ns}	0.66^{ns}	0.52^{ns}	0.52^{ns}	0.52^{ns}	-	-	-
***P* values for main and interaction effects of sampling year (Y) × treatment (T) × soil depth (D)**									
	Y	**T**	**D**	**Y×T**	**Y×D**	**T×D**	**Y×T×D**	-	-
P value	0.01**	0.26^{ns}	<0.001**	0.78^{ns}	0.43^{ns}	0.75^{ns}	0.93^{ns}	-	-

† W=Wheat, P=Field pea, L=Lentil, C=Chickpea.

‡ The soils sampled in the spring of 2017 and 2018 are representative of the 2016 wheat crop phase and 2017 pulse crop phase of rotations, respectively.

§ Values with same letter within each category (treatment and sampling year) are not significantly different at $P>0.05$, according to Tukey's HSD test.

¶ The contrast value was taken from the subtraction of the value on right from the value on the left in the comparison and the *P* value was mentioned following the contrast value.

^{ns}: non-significant ($P>0.05$), *, ** and *** denote significant at $P\leq0.05$, $P\leq0.01$ and $P\leq0.001$, respectively, according to Tukey's HSD test.

Table 4.10. Total nitrogen (N) content at three soil depths from different crop rotation sequences in springs of 2017 and 2018.

Treatment†	Soil total N in different soil depths in different years (g kg^{-1} of soil)								
	0-15 cm		15-30 cm		30-60 cm		Mean	Mean	Overall
	2017‡	2018	2017	2018	2017	2018	2017	2018	mean
1=W/W/W/W	1.39	1.41	1.18	1.14	0.67	0.73	**1.08 abc§**	**1.10 abc**	**1.09 ab**
2=P/W/P/W	1.38	1.30	1.21	1.15	0.60	0.77	**1.06 bc**	**1.08 abc**	**1.07 b**
3=L/W/L/W	1.38	1.40	1.19	1.22	0.50	0.85	**1.02 c**	**1.16 ab**	**1.09 ab**
4=C/W/C/W	1.38	1.43	1.23	1.27	0.70	0.84	**1.10 abc**	**1.18 a**	**1.14 a**
5=L/W/C/W	1.36	1.30	1.12	1.06	0.62	0.70	**1.04 c**	**1.02 c**	**1.03 b**
Mean	**1.37 a**		**1.18 b**		**0.70 c**		**1.06 b**	**1.11 a**	-
Mean	**1.38 a**	**1.37 a**	**1.19 b**	**1.17 b**	**0.62 d**	**0.78 c**	-	-	-
	Contrast and *P* values for *a priori* comparison¶								
1 vs. (2+3+4+5)/4	0.015	0.053	-0.008	-0.035	0.065	-0.060	-	-	-
	0.96^{ns}#	0.11^{ns}	0.83^{ns}	0.39^{ns}	0.25^{ns}	0.24^{ns}	-	-	-
5 vs. (2+4+3)/3	-0.020	-0.077	-0.090	-0.153	0.020	-0.120	-	-	-
	0.82^{ns}	0.06^{ns}	0.05*	0.003**	0.69^{ns}	0.02*	-	-	-
4 vs. (2+3)/2	0.000	0.080	0.030	0.085	0.150	0.030	-	-	-
	0.99^{ns}	0.08^{ns}	0.44^{ns}	0.09^{ns}	0.03*	0.51^{ns}	-	-	-
2 vs. 3	0.000	-0.100	0.020	-0.070	0.100	-0.080	-	-	-
	0.98^{ns}	0.07^{ns}	0.75^{ns}	0.19^{ns}	0.17^{ns}	0.18^{ns}	-	-	-
***P* values for main and interaction effects of sampling year (Y) × treatment (T) × soil depth (D)**									
	Y	**T**	**D**	**Y×T**	**Y×D**	**T×D**	**Y×T×D**	-	-
P values	0.006 **	0.0003 ***	<0.0001 ***	0.02 *	<0.0001 ***	0.60 ^{ns}	0.42 ^{ns}	-	-

† W=Wheat, P=Field pea, L=Lentil, C=Chickpea.

‡ The soils sampled in the spring of 2017 and 2018 are representative of the 2016 wheat crop phase and 2017 pulse crop phase of rotations, respectively.

§ Values with same letter within each category (treatment and sampling year) are not significantly different at $P>0.05$, according to Tukey's HSD test.

¶ The contrast value was taken from the subtraction of the value on right from the value on the left in the comparison and the *P* value was mentioned following the contrast value.

^{ns}: non-significant ($P>0.05$), *, ** and *** denote significant at $P\leq0.05$, $P\leq0.01$ and $P\leq0.001$, respectively, according to Tukey's HSD test.

Table 4.11. Carbon:nitrogen (C:N) ratio at three soil depths from different crop rotation sequences in springs of 2017 and 2018.

Treatment†	Soil total C:N ratio in different soil depths in different years								
	0-15 cm		15-30 cm		30-60 cm		Mean	Mean	Overall
	2017‡	2018	2017	2018	2017	2018	2017	2018	mean
1=W/W/W/W	11.0	11.3	11.7	11.4	16.2	14.3	**13.0**	**12.3**	**12.7**
2=P/W/P/W	11.0	11.5	11.8	11.1	22.4	15.2	**15.0**	**12.6**	**13.8**
3=L/W/L/W	11.0	10.7	11.6	10.2	22.9	12.7	**15.2**	**11.2**	**13.2**
4=C/W/C/W	12.0	10.7	11.6	10.6	18.4	15.7	**14.0**	**12.3**	**13.2**
5=L/W/C/W	11.2	11.4	12.4	11.4	21.6	15.8	**15.0**	**12.9**	**14.0**
Mean	**11.2 b**§		**11.4 b**		**17.5 a**		**14.5 a**	**12.3 b**	-
Mean	**11.2 c**	**11.1 c**	**11.8 c**	**11.0 c**	**20.3 a**	**14.7 b**			
	Contrast and *P* values for *a priori* comparison¶								
1 vs. (2+3+4+5)/4	-0.300	0.225	-0.150	0.575	-5.12	-0.550	-	-	-
	0.05*#	0.10ns	0.75ns	0.08ns	0.16ns	0.79ns	-	-	-
5 vs. (2+4+3)/3	-0.133	0.433	0.733	0.767	0.367	1.267	-	-	-
	0.20ns	0.003**	0.13ns	0.02**	0.91ns	0.58ns	-	-	-
4 vs. (2+3)/2	1.00	-0.400	-0.100	-0.050	-4.25	1.75	-	-	-
	<0.0001 ***	0.01*	0.84ns	0.89ns	0.27ns	0.47ns	-	-	-
2 vs. 3	0.000	0.800	0.200	0.900	-0.500	2.50	-	-	-
	0.71ns	0.0002 ***	0.77ns	0.02*	0.92ns	0.36ns	-	-	-

***P* values for main and interaction effects of sampling year (Y) × treatment (T) × soil depth (D)**

	Y	T	D	Y×T	Y×D	T×D	Y×T×D
P values	0.0001 ***	0.52ns	<0.0001 ***	0.40ns	0.0001 ***	0.72ns	0.54ns

† W=Wheat, P=Field pea, L=Lentil, C=Chickpea.

‡ The soils sampled in the spring of 2017 and 2018 are representative of the 2016 wheat crop phase and 2017 pulse crop phase of rotations, respectively.

§ Values with same letter within each category (treatment and sampling year) are not significantly different at $P>0.05$, according to Tukey's HSD test.

¶ The contrast value was taken from the subtraction of the value on right from the value on the left in the comparison and the *P* value was mentioned following the contrast value.

ns: non-significant ($P>0.05$), *, ** and *** denote significant at $P\leq0.05$, $P\leq0.01$ and $P\leq0.001$, respectively, according to Tukey's HSD test.

Soil organic carbon content

Overall, the rotation sequence had no effect on the SOC content (Table 4.12). The SOC content varied with sampling year (P<0.0001) and soil depth (P<0.001). On average, SOC content in 2018 was higher than 2017, and the average SOC content at the top soil (0-15 cm) layer was higher than the 15-30 cm soil depth. *A priori* comparison revealed that the continuous wheat rotation had a higher SOC content at the 0-15 cm depth compared to corresponding depth of the other crop rotations in 2018 (Table 4.12).

Potentially mineralizable nitrogen

Crop rotation sequence affected potentially mineralizable N (PMN) (P<0.0001; Table 4.13). Chickpea alternated with wheat had the highest values for PMN (12.0 mg kg^{-1} soil) compared to all the other treatments. Year also affected PMN (P<0.0001), with PMN higher in 2018 (8.71 mg kg^{-1} of soil) compared to 2017 (1.73 mg kg^{-1} of soil). Furthermore, the crop rotation × year interaction was also significant (P=0.03) for PMN, indicating that PMN of the different crop rotations varied with cropping years. The most significant difference between the years was the 10-fold higher PMN in the C/W/C/W in 2018 compared to that of 2017 (Table 4.13).

Table 4.12. Soil organic carbon (SOC) in two soil depths from different crop rotation sequences in springs of 2017 and 2018.

Treatment[ł]	SOC content in different soil depths in different sampling years (g kg^{-1} of soil)						
	0-15 cm		15-30 cm				
	2017[‡]	2018	2017	2018	Mean 2017	Mean 2018	Overall mean
1=W/W/W/W	12.6	14.4	9.0	9.9	**10.8**	**12.1**	**11.5**
2=P/W/P/W	12.1	13.4	8.9	9.7	**10.5**	**11.6**	**11.0**
3=L/W/L/W	12.2	13.0	9.0	9.7	**10.6**	**11.4**	**11.0**
4=C/W/C/W	12.4	13.5	9.3	10.2	**10.8**	**11.8**	**11.3**
5=L/W/C/W	12.1	13.1	8.5	9.1	**10.3**	**11.1**	**10.7**
Mean	12.3	13.5	8.9	9.7	**10.6 b**[§]	**11.6 a**	-
Mean	**12.9 a**		**9.3 b**		-	-	-
	Contrast and *P* values for *a priori* comparison[¶]						
1 vs. (2+3+4+5)/4	0.400	1.15	0.075	0.225	-	-	-
	0.45^{ns}[#]	0.04*	0.72^{ns}	0.59^{ns}	-	-	-
5 vs. (2+3+4)/3	-0.133	-0.200	-0.567	-0.767	-	-	-
	0.81^{ns}	0.70^{ns}	0.14^{ns}	0.06^{ns}	-	-	-
4 vs. (2+3)/2	0.250	0.300	0.350	0.500	-	-	-
	0.62^{ns}	0.59^{ns}	0.42^{ns}	0.28^{ns}	-	-	-
2 vs. 3	-0.100	0.400	-0.100	0.000	-	-	-
	0.92^{ns}	0.50^{ns}	0.80^{ns}	0.95^{ns}	-	-	-

***P* values for main and interaction effects of sampling year (Y) × treatment (T) × soil depth (D)**

	Y	T	D	Y×T	Y×D	T×D	Y×T×D
P value	<0.0001***	0.07^{ns}	<0.0001***	0.88^{ns}	0.22^{ns}	0.56^{ns}	0.96^{ns}

ł W=Wheat, P=Field pea, L=Lentil, C=Chickpea.

‡ The soils sampled in the spring of 2017 and 2018 are representative of the 2016 wheat crop phase and 2017 pulse crop phase of rotations, respectively.

§ Values with same letter within each category (treatment and sampling year) are not significantly different at $P>0.05$, according to Tukey's HSD test.

¶The contrast value was taken from the subtraction of the value on right from the value on the left in the comparison and the *P* value was mentioned following the contrast value.

^{ns}: non-significant ($P>0.05$), *, ** and *** denote significant at $P\leq0.05$, $P\leq0.01$ and $P\leq0.001$, respectively, according to Tukey's HSD test.

Table 4.13. Soil potential mineralizable N (PMN) at 0-15 cm soil depth from different crop rotation sequences in springs of 2017 and 2018.

Treatment[ł]	PMN in different sampling years (mg kg^{-1} of soil)		
	2017[‡]	**2018**	**Overall mean**
1=W/W/W/W	1.11 b[§]	4.53 b	**2.82 b[¶]**
2=P/W/P/W	1.75 b	6.56 b	**4.15 b**
3=L/W/L/W	1.74 b	3.32 b	**2.38 b**
4=C/W/C/W	2.05 b	22.0 a	**12.0 a**
5=L/W/C/W	2.30 b	7.12 b	**4.71 b**
Mean	**1.73 b**	**8.71 a**	-
	Contrast and *P* values for *a priori* comparison[#]		
1 vs. (2+3+4+5)/4	-0.850	-5.22	-
	0.30^{ns}[ǂǂ]	0.71^{ns}	-
5 vs. (2+3+4)/3	0.453	-3.51	-
	0.47^{ns}	0.30^{ns}	-
4 vs. (2+3)/2	0.305	17.06	-
	0.58^{ns}	0.10^{ns}	-
2 vs. 3	0.010	3.24	-
	0.74^{ns}	0.11^{ns}	-
	***P* values for main and interaction effects of sampling year (Y) × treatment (T)**		
	Y	**T**	**Y ×T**
P value	$<0.0001^{***}$	$<0.0001^{***}$	$<0.0001^{***}$

ł W=Wheat, P=Field pea, L=Lentil, C=Chickpea.

‡ The soils sampled in the spring of 2017 and 2018 are representative of the 2016 wheat crop phase and 2017 pulse crop phase of rotations, respectively.

§ Mean values with the same letters indicates no significant difference (P>0.05) between year × treatment interactions according to Tukey's HSD test.

¶ Values with same letter within each category (treatment and sampling year) are not significantly different at *P*>0.05, according to Tukey's HSD test.

The contrast value was taken from the subtraction of the value on right from the value on the left in the comparison and the *P* value was mentioned following the contrast value.

ǂǂ ^{ns}: non-significant (*P*>0.05), *, ** and *** denote significant at *P*≤0.05, *P*≤0.01 and *P*≤0.001, respectively, according to Tukey's HSD test.

4.4. Yield Components, Grain Productivity and Harvest Index

The impact of the different crop rotations on grain yield components, grain productivity and HI of the succeeding spring wheat crop of the study was assessed using in the wheat phase in the 8th year (2018) (Table 4.14).

Irrespective of the pulse species included in the crop rotation, the rotation sequence had no effect on plant population density of the succeeding spring wheat crop (Table 4.14). Among grain yield components, crop rotation only marginally affected 1000-kernel weight of the succeeding wheat crop, in that wheat grown after pea had a higher 1000 kernel weight than wheat grown after wheat (Table 4.14). *A priori* comparison revealed that wheat grown alternately with chickpea (C/W/C/W) had higher head density (385 heads m^{-2}) than the average head density of wheat grown alternately with field pea (P/W/P/W) or lentil (L/W/L/W) (334 heads m^{-2}). On average, wheat grown alternately with pulse crops (lentil, field or chickpea) produced slightly heavier kernels (31 g vs. 29 g 1000-kernel weight^{-1}) than the wheat crop grown as continuous wheat. Wheat grown alternately with lentil and chickpea (L/W/C/W) had heavier kernels (31 g vs. 30 g 1000-kernel weight^{-1}) than the average of wheat grown alternately with lentil (L/W/L/W), field pea (P/W/P/W) or chickpea (C/W/C/W). The wheat crop grown alternately with field pea (P/W/P/W) had heavier kernels (32 g vs. 30 g) than wheat grown alternately with lentils (L/W/L/W), which suggests that the field pea crop, as component crop in rotation, has solely been contributed to elevate the average value of 1000-kernal weight of wheat. Rotation treatment had no effect on kernel yield. Furthermore, the inclusion of pulse crops in rotation had no effect on HI.

Table 4.14. Yield components, kernel yield and harvest index of spring wheat grown from different crop rotation sequences in 2018.

Treatment†	Plant density plants m^{-2}	Heads m^{-2}	Kernels $head^{-1}$	1000-kernel weight (g)	Kernel yield ($kg\ ha^{-1}$)	Harvest index
1=W/W/W/W	142	389	20	29 c‡	2220	0.34
2=P/W/P/W	143	342	19	32 a	2069	0.29
3=/L/W/L/W	154	325	19	30 bc	1879	0.26
4=C/W/C/W	156	385	16	30 bc	1848	0.28
5=L/W/C/W	161	383	17	30 bc	1977	0.35
P value	0.28^{ns}§	0.09^{ns}	0.38^{ns}	0.005^{**}	0.58^{ns}	0.35^{ns}
	Contrast and *P* values for *a priori* comparison¶					
1 vs. (2+3+4+5)/4	-11.50	30.25	2.25	-1.50	276.8	0.045
	0.17^{ns}	0.16^{ns}	0.34^{ns}	0.01^{**}	0.18^{ns}	0.38^{ns}
5 vs. (2+3+4)/3	10.00	32.33	-1.00	-0.667	45.00	0.073
	0.24^{ns}	0.15^{ns}	0.70^{ns}	0.04^{*}	0.83^{ns}	0.30^{ns}
4 vs. (2+3)/2	7.50	51.50	-3.00	-1.000	-126.0	0.005
	0.41^{ns}	0.04^{*}	0.09^{ns}	0.09^{ns}	0.57^{ns}	0.18^{ns}
2 vs. 3	-11.00	17.00	0.000	2.00	190.0	0.030
	0.28^{ns}	0.54^{ns}	0.89^{ns}	0.03^{*}	0.46^{ns}	0.35^{ns}

† W=Wheat, P=Field pea, L=Lentil, C=Chickpea.

‡ Values with same letter within each category (treatment and sampling year) are not significantly different at $P>0.05$, according to Tukey's HSD test.

§ § ^{ns}: non-significant ($P>0.05$), *, ** and *** denote significant at $P\leq0.05$, $P\leq0.01$ and $P\leq0.001$, respectively, according to Tukey's HSD test.

¶The contrast value was taken from the subtraction of the value on right from the value on the left in the comparison and the *P* value was mentioned following the contrast value.

5.0. DISCUSSION

5.1. Physical Properties of Soil

Available soil moisture is one of the key factors that limits crop growth and productivity of pulse crops grown on rain-fed, semi-arid agricultural lands (Campbell et al., 1977; Angadi et al., 2008) with highly variable climatic conditions and precipitation (Cutforth et al., 2007). Thus, crop water use in semi-arid regions is of paramount concern. Understanding fall soil moisture (post-harvest residual soil moisture) content in different soil depths is vital to develop crop rotation systems, which are more efficient in water utilization (Wang et al., 2012).

The species of legume included in the rotation treatment had no effect on either spring soil moisture content measured before seeding (Table B.1) or post-harvest residual moisture content at any soil depth and this was consistent in both sampling years. This indicated that all of the crops in a given cropping season had equivalent soil moisture usage from the 0- to 60- cm soil depth.

In the current study, the post-harvest residual soil moisture levels at the three soil depths (0-15, 15-30 and 30-60 cm) were comparable among the treatments in both years (Table 4.1). A previous rotation study with field pea, lentil and chickpea in wheat-based cropping systems compared to continuous wheat also had similar post-harvest residual soil moisture contents at the top 0-60 cm soil depth (Gan et al., 2015). In contrast, other studies reported that pulse crops in wheat-based crop rotations used less water from deeper soil profiles, leaving more water for the next crop in a rotation compared to deeper rooting crops, such as wheat, (Gan et al., 2007; Wang et al., 2012). The comparable post-harvest residual soil moisture among the treatments, which was persistent in both sampling years may be due to similar water consumption in all the treatments. In contrast, Gan et al. (2009a) reported that wheat used a higher amount of soil water from the 0-120 cm soil depth than pulse crops including field pea, lentils and chickpea under rain-fed conditions. However, in our study moisture contents were evaluated in a shallower depth (0-60 cm) compared to the study (0-120 cm) performed by Gan et al. (2009a). Differences in root distributions among crops will affect the depth of soil that water is extracted from. Around 52% of chickpea total root biomass distributed within 0-20 cm depth, compared to field pea, lentil and wheat which had a

larger proportion of total root biomass (61-67%) in the 0-20 cm depth (Gan et al., 2009b). In addition, 77- 85 % of the roots of field pea, lentils and chickpea crops distributed within the 0-40 cm soil profile (Liu et al., 2009). This suggests that the distribution of a substantial portion of root biomass of each crop within the top soil layers may lead mature crops to withdraw similar amounts of water from this shallow depth.

The occurrence of comparable soil moisture contents among the treatments may be due to the low precipitation during the growing periods. Because of the extreme water deficit condition, all crops were probably water stressed to the point that differences in water uptake did not exist. Crops were taking up all available water despite differences in rooting depth. Even though 2016 had a higher precipitation than normal, the additional precipitation probably serves to only partially relieve the extreme dry conditions from 2015.

In both 2017 and 2018, continuous wheat showed no difference in post-harvest residual soil moisture content compared to all the pulse crop rotations. However, wheat had the highest root biomass in most soil depths (0-120 cm) compared to pulse crops namely field pea, lentils and chickpea (Gan et al., 2009b). Higher root biomass may lead a wheat crop to withdraw more soil water from the total soil profile, resulting in lower storage of water than pulse crops. Evapotranspiration also plays a key role in soil water storage capacity (Gan et al., 2015). Evapotranspiration rates are usually greater in annual legumes than in other grain crops, such as spring wheat under semi-arid conditions (Thomson et al., 1997). Slow canopy coverage in annual grain legumes (Merrill et al., 2002) results in lower amounts of soil water. The coupling effect of differential root distributions and evapotranspiration rates may result in the comparable post-harvest residual soil moistures observed among the different rotations.

On average, post-harvest soil moisture content increased with increasing soil depth, which may due to two major reasons. Firstly, density of roots, surface area and the number of root tips rapidly decrease with soil depth between 0-100 cm in semi-arid regions (Campbell et al., 1977; Benjamin and Nielsen, 2006). Therefore, fewer roots extracting less amounts of water from the soil. Secondly, the heat energy transported from the surface reduces across the soil profile, which typically leads to decrease soil temperature depth (Han and Zhou, 2012). Higher temperature facilitates soil moisture evaporation (Kidron and Kronenfeld, 2016). It can be speculated the evaporation may decrease with soil depth. Therefore, decreasing the amount of roots and water

evaporation both may have a cumulative impact on increased soil moisture with increasing soil depth. Post-harvest residual soil moisture in 2018 was higher compared to 2017 in 0-60 cm soil profile, which is probably due to the higher precipitation from July-September in 2018 (Table A.5).

Soil macro-aggregates are important in C sequestration since decomposition of SOM tends to occur at a slower rate within macro-aggregates compared to newly deposited SOM. Higher abundance of macro-aggregates decreases the susceptibility of soil to erosion by wind, water and tillage. Micro-aggregates are considered as the repository of the most stable C pool in soils (Tisdall and Oades, 1982; Six et al., 2000). However, studies regarding the impact of aggregate size distribution within pulse-diversified system are limited in western Canada.

Presence of large macro-aggregates (>6.35 mm) was most pronounced in the continuous wheat compared to the pulse crop rotations (Figure 4.1). In contrast, the rotations with field pea, lentil and chickpea had a higher abundance of micro-aggregates (especially <0.1 mm) compared to the continuous wheat rotation. This result was inconsistent with Chu et al. (2016), who reported that pulse crops increased the formation of macro-aggregates while continuous cereal increased the formation of micro-aggregates. Moreover, Chu et al. (2016) explained that macro-aggregate formation mainly depends on plant root formation and plants with tap root and coarse roots facilitated the development of macro-aggregates and plants with fibrous and fine roots develop micro-aggregates. The macro-aggregation in continuous wheat cannot be explained based on the rooting system since the fibrous rooting system in wheat led to an increase in micro-aggregates compared to macro-aggregates.

The formation of macro-aggregates relies on the production of large amounts of polysaccharide gel by fungi and AMF (Tisdall and Oades, 1982; Chantigny et al., 1997; Helfrich et al., 2008; Wilson et al., 2009). Phospholipid fatty acid analysis revealed that fungi and AMF populations were comparable in continuous wheat and pulse crop rotations. However, the soil samples for the microbial analysis was collected before seeding and might not represent microbial populations during crop growth. Changes in fungal and AMF biomass that occur during plant growth may stimulate macro-aggregation in continuous wheat cropping systems in the surface soil.

The soil light fraction is vital in stabilization soil structure by forming soil macro-aggregates (Miller and Justraw, 1990). However, in contrast to expectations, LF was lower in continuous

wheat compared to all pulse crop rotations. The lower LF and higher HF in continuous wheat rotation indicates that soils in which wheat is grown appear to be better able to process newly inputted organic matter (LF) into mineral-associated organic matter (HF) than the rotations with pulse crops.

Lehmann et al. (2006) emphasized the importance of microbial metabolites for the formation of stable micro-aggregates rather than plant debris. Micro-aggregates are important in that they represent initial phases of C stabilization. Since the pulse crop rotations had higher amount of micro-aggregates, pulse crop soils are better able to aggregate with these early C forms released by microorganisms.

5.2. Biological Properties of Soil

How soil microbial community composition and numbers of soil microorganisms are affected by different crop types and environmental factors is important for understanding the sustainability of an agricultural system (Bossio and Scow, 1995; Söderberg et al., 2002). Microbial biomass can provide an indirect indication of how the size of a microbial community is affected by agronomic practices.

The different pulse crops in the rotation treatments had no impact on microbial biomass, total PLFAs, PLFAs of bacteria, G^-, G^+, fungi and AMF (Fig. 4.3). In addition, microbial biomass in continuous wheat did not pronounce any difference compared to the rotations with pulse crops. The result of the present study was not consistent with findings in previous studies conducted using other legumes, such as black lentil, chickling vetch and feed pea grown in cereal-based cropping rotations (Biederbeck et al., 2005; Pankhurst et al., 2005). In general, soils with high SOC content had high microbial biomass since microbial biomass depends on nutrients from SOM (Hao et al., 2008; Jacobs et al., 2011; Kallenbach and Grandy, 2011). Nutritional stress can occur when SOC is less than 1% (10 g in soil kg^{-1}) (Kallenbach and Grandy, 2011). In this study, the SOC in each rotation was low (10.7-11.3 g kg^{-1} soil), and the microbial nutritional stress may have hindered the expression of the microbial biomass differences among the treatments.

In the present study, the treatment effect on microbial community composition among the treatments were not pronounced (Fig 4.3). In contrast, Alvey et al. (2003) demonstrated that introduction of legumes (cowpea and groundnut) into cereal crop rotations has a substantial effect

on the structure and diversity of soil microbial community compared to continuous cereal (maize, millet and sorghum) cropping systems. Soil texture, available water content, SOC amount and regional climatic factors have been found to strongly influence distribution of microbial community structure (Brockett et al., 2012; Yang et al., 2013; Tsiknia et al., 2014). In this study, soils were highly enriched with G^- bacteria compared to G^+ bacteria and this persisted over the two sampling years that were characterized by low available soil moisture. This was consistent with previous studies (Rinklebe and Langer, 2006; Ma et al., 2015). Therefore, the dry climate, the limited soil moisture and SOC contents may control the composition of microbial communities across the treatments.

Microbial biomass and community structure affected by the sampling seasons. This might be associated with the changes in soil moisture, pH and nutrients including SOC between the sampling years.

In the present study, bacteria were the dominant members of the soil microbial community. Breulmann et al. (2012) suggested that bacterial dominated systems are associated with more rapid rates of decomposition and nutrient cycling, leading to lesser accumulation of SOC. This is consistent with the low amounts of SOC measured in this study. However, it is possible that the pre-seeding sampling did not account for changes in microbial composition resulting from different root exudates produced by different crops. This may due to the differences in utilization of certain organic acids due to rhizosphere effects and increased exudation at during the plant growth (Griffiths et al., 2003). In addition, several previous studies, which examined microbial communities throughout a field season suggested that microbial community structure influences by seasonal variation and fatty acid composition of the microbial community associated with the time of sampling (Di Cello et al., 1997; Lottmann et al., 2000; Grayston et al., 2001).

The size of the light fraction organic matter (LFOM) pool is an indicator of the balance between above- and below-ground residual inputs and decomposition (Gregorich and Janzen, 1996). The rotation with chickpea alternating with wheat had the highest amount of LFOM and lowest amount of heavy fraction organic matter (HFOM). This indicates the possibility of higher residual input and slower decomposition rates of the chickpea residues by soil micro-organisms compared to the other rotations. In addition, chickpea may also produce higher dry matter content than lentil and field pea, which was consistent with Siddique et al. (2001).

In this study, pulse crop rotations collectively had higher LFOM fraction compared to continuous wheat in 0-15 cm soil depth in both sampling years. This probably due to the higher amount of wheat residual input following a pulse than in continuous wheat. Nuruzzaman et al. (2005) reported that the biomass of wheat grown after pulse crops including field pea, faba bean and white lupin was higher that continuous wheat. In addition, Analysis of LF-C and LF-N is an indication of the ability of a soil to supply C and N to a crop (Smith et al., 2015). Lower LF-C and LF-N values may attribute to observed low soil C and N in this study (Tables 4.5 and 4.6).

5.3. Soil Chemical Properties

Soil pH is crucial for healthy plant growth as it is determiner of nutrient availability for plants (Aini et al., 2014). The soil at this experimental site was susceptible to acidification because of its low buffering capacity attributable to low SOM content (Haynes, 1983). The present study demonstrated that the inclusion field pea, lentil and chickpea into wheat-based crop rotations resulted in 8-10 % lower pH values at 0-10 cm soil depth compared to the continuous wheat (Table 4.8). Previous studies also reported that pulse crops acidified soil compared to continuous wheat (Haynes, 1983; Coventry and Slattery, 1991; Tang et al., 1997). Soil acidification due to the growing of pulse crops may mainly result from the exchange of protons (H^+) inside the cell and cations in the soil system (Haynes, 1983) and excretion of large amounts of organic acid from pulse crop roots (Igamberdiev and Eprintsev, 2016). In addition, soil acidification by legumes may also cause by NO_3^- leaching into deeper soil layers since the N mineralization of pulse residues mainly occur in 0-5 cm soil depth (Murphy et al., 1998).

In the present study, the highest capacity of acidification was shown in chickpea alternated with wheat compared to the other pulse crop rotations in 2017 (Table 4.8). This was consistent with Marschner and Römheld, (1983). Chickpea had very low pH at 0-5 cm soil depth compared with other plants may due to extrusion of higher amount of organic anions or organic acids compared to field pea and lentil during uptake of NO_3^- (Marschner and Römheld, 1983). It was expected to observe the same result in 2018, however the comparisons did not show differences among the pulse crop rotations. The overall soil pH in sampling year after the pulse crop phase (2018) was lower compared to the sampling year after the wheat phase (2017) which may be due to the influence of the pulse crops on soil pH in 2017 (Table 4.8).

The soil in the experimental site had markedly low total soil C and N contents. It should be noted that the study site has dry and warm climatic conditions within the semi-arid region. Low soil moisture contents and high temperatures lead to slow decomposition contributing to low amounts of SOM (Li et al., 2015).

Soil C is composed of both organic and inorganic reservoirs in soil. In this study, SOC constituted more than 80% of total soil C at 0-15 and 15-30 cm soil depths; SOC was not evaluated in the 30-60 cm soil depth (Table 4.11). In other studies, soils in arid and semi-arid eco-regions had a high abundance of soil inorganic C (SIC), dominated by calcium carbonate ($CaCO_3$) (Batjes, 1998; Martens et al., 2005; Sanderman, 2012). The observed soil acidity may have contributed for the presence of low SIC.

In the present study, rotation treatments had no impact on total soil C and SOC at any depth (Table 4.9). Other studies reported a higher amount of total soil C in continuous non-legume cropping systems compared to diversified cropping systems with legume and non-legume crops without tillage in semi-arid region in the northern Great Plains (Halvorson et al., 2002; Sainju, 2014, Engel et al., 2017; Sainju et al., 2017). Alterations in SOC develop from the amount organic matter input to the soil, the rate of decomposition of SOM and oxidation of SOC, or a combination of above factors (Follett, 2001; Paustian et al., 2000). In the present study, continuous wheat contributed higher amount of above-ground residues than that of pulse crop rotations. Since higher N concentrations (low C:N ratio) of pulse crops compared to spring wheat facilitates the rapid decomposition of plant material (Kuo et al., 1997; Sainju, 2014; Engel et al., 2017). Therefore, lower amounts of SOC contents were expected from pulse crop rotations compared to continuous wheat. Equal quantities of total SOC among all the treatments may result due to the inadequate rotation history in this study. The accumulation of SOC in soil is a slow process and studies measuring changes is SOC must be of sufficient duration (Gregorich et al., 2000; Fornara and Tilman, 2008). Kern and Johnson (1993) assumed the duration of C sequestration takes 10-20 years. Soil samples in this study were collected after 6 and 7 years of 4-yr rotation cycle. Therefore, the time duration to identify change in SOC is probably insufficient. Despite that, Halvorson et al. (2016) reported that SOC is lesser responsive for the cropping sequence than other C fractions such as particulate organic matter C and soil microbial biomass C. Thus, all the above factors may contributes to the absence of impact of treatments on SOC in our study.

In the present study a higher amount of total soil C and SOC in all the pulse crop rotations compared to continuous wheat at 0-15 cm was noticed. This trend was similar with LFOM distribution in 0-15 cm soil depth for the both sampling years therefore LFOM closely reflected SOC amounts in the different treatments. Moreover, the overall soil total C and SOC decreased with the soil depth, which was accordance with Lawrence et al. (2015) which may due to declining of plant inputs with soil depth.

Soil N status is vital for regulation of rhizosphere processes, which provide feedback to plant growth and development (Gan et al., 2010; Huang et al., 2016; Borrell et al., 2017). Treatments had an impact on the overall total soil N however, the inclusion of pulse crops into wheat-based rotations did not provide additional soil N compared to the continuous wheat cropping system at any soil depth (Table 4.10). In contrast, many studies have shown, including pulse crops in rotations to be an effective strategy for improving total soil N through N-rich residual input and root depositions (Gylfadottir et al., 2007; Rasmussen et al., 2007; Gan et al., 2015). However, incorporation of N-rich pulse crop residues might not result high soil N. At crop harvest, a considerable fraction of N in pulse crops, which is stored in grains removed from the field. Thus, the residual contribution for the soil N has a minor impact (Peoples et al., 2009). This explains the presence of similar total soil N in pulse crop rotations compared to continuous wheat in this study.

Additional soil N benefit was expected from rotation with field pea alternated with wheat due to the higher capability of BNF (Walley et al., 2007) and early maturity (Government of Saskatchewan, 2019) compared to lentil and chickpea. However, biological N fixation depends on several factors, including temperature, water and nutrient availability, and soil pH (Sprent et al., 1988; Brockwell et al., 1991; Triplett and Sadowsky, 1992; Boscari, 2002). Therefore, the weather conditions of the experimental site may hinder the contribution of field pea to total soil N. In addition, early maturity of field pea facilitates decomposition of plant residues and release soil N which may prone to loss soil NO_3^- via leaching. Considering all these facts, the N contribution of pulse crops to a subsequent wheat is difficult to evaluate using short-term studies.

The soil total N content decreased with soil depth. Because of minimal disturbance under zero tillage and stratification of crop residues and organic matter near the soil surface, total soil N accumulates in top soil layers and gradually may decrease with soil depth. In addition, the highest root biomass occurs near the soil surface and gradually decreases with soil depth Slater (2015).

In the present study, total soil N was higher in 2018 (following the pulse crop phase) compared to 2017. O'Donovan et al. (2014) also reported that a following crop can gain the N benefits from the decomposition of the residues of previous pulse crops. The remaining pulse crop straw and roots after the harvest in 2017 decompose over the fall and winter providing soil N to the crops to be grown in year 2018.

It was thought that crop rotations that included field pea, lentil and chickpea would produce higher PMN compared to continuous wheat; however the effect of pulse crop on PMN was marginal. Gan et al. (2010) also found approximately similar N mineralization for continuous wheat and pulse crop rotations. The higher quantities of relatively recalcitrant non-pulse residues may be balanced by the lower input amounts of the more labile pulse residues. This balance between quality and quantity may also couple with the N fertilizer effect of a continuous wheat system, which resulted in comparable PMN values observed among continuous wheat and wheat-pulse rotations (Shah et al., 2010; Zhang et al., 2012). In addition, the increases in PMN in all of the other treatments compared to the W-C-W-C-W indicate that the residues in C-W-C-W had sufficient labile N pool and that most of the decomposer microbial communities existed in the active state.

Potentially mineralizable N values tended to increase over the sampling seasons. Substrate availability is a major factor regulating inorganic N production. The roots of pulse crops secrete large quantities of readily mineralizable N-compounds (Ofosu-Budu et al., 1990) that are lost from roots under insufficient soil moisture in spring resulting in the lower values of PMN in 2017. The higher spring soil moisture content in 2018 compare to 2017 would lead to higher microbial activity and higher PMN values.

Compared to total soil N, the values obtained for the PMN are low. Under N limited conditions in the soil, the soil microbial community experiences N deficiency, which may lead to N entering the soil microbial biomass pool via immobilization (McSwiney et al., 2013). Therefore, the amount of mineral N present in soil represents the net effect of the magnitude of the two concurrent opposing processes of mineralization and immobilization.

Soil C:N ratio is a reliable indicator of the degree of decomposition and quality of the organic matter held in the soil. However, ratios are prone to considerable variation resulting from errors in determining both variables. The overall mean of soil C:N ratio among treatments ranged from 12.7-14.0 (Table 4.12) and soil C:N ratio tended to increase with soil depth, reflecting a lower

degree of breakdown and more recalcitrant organic matter (humus) stored in the lower parts of the profile.

5.4. Grain Yield Components, Grain Productivity and Harvest Index

In the present study, the inclusion of different pulse crops or continuous wheat had no effect on plant density, number of heads m^{-2} and number of kernels per head or kernel yield of the succeeding wheat crop in the production systems. In contrast, Bonciarelli et al. (2016) revealed that crop rotations with high frequency of wheat had lower crop yield compared to the wheat in diversified cropping system. Stevenson and van Kessel (1996) reported that the inclusion of pulse crops in cereal-based rotation often leads to greater seed yields in a succeeding cereal crop. For dryland annual crops grown on the prairies, soil N availability at early growing stages plays a key role in the grain yield potential (Demotes-Mainard et al., 1999), therefore, N provided by legume biomass reflects in higher yields. The similar crop yield performance among the treatments reflects the absence of apparent variation in total soil N among the treatments.

Even though the plant N requirement is supplied through synthetic N fertilizers, soil available moisture is vital for the response of grain yield to N fertilization. Improved soil moisture leads to more yield to a point where some other factor becomes limiting. This study area consisted of rain-fed wheat production systems, which are reliant on stored soil water. Water stored in the soil profile is a vital resource to maximize yield as available water is used for increasing carbohydrate supply to the growing grains (Condon et al., 1993). The dry conditions in 2018 may be responsible for the lack of difference in yield performance of the wheat crops in the different treatments. Smith et al. (2015) reported that wheat yield varied with the amount of total precipitation from May-July in in Lethbridge, AB. During 6 years of that study, the lower than normal rainfall reduced wheat yields. However, the absence of differences in the spring and fall soil moisture content among the treatments in all the soil depths may hinder the effect of pulse crops on the yield of subsequent wheat crop.

The preceding crops grown in the rotations had a significant impact on the mean kernel weight (measured as 1000-kernel weight) of the succeeding wheat crop in 2018. The P/W/P/W rotation treatment produced the highest 1000-kernel weight of the succeeding wheat crop while reducing the number of kernels (data not shown). The 1,000- kernel weight is a measure of seed size, which is an important physical indicator for yield, market grade and harvest efficiency (Gadisa, 2018).

Kernel size plays a key role in kernel yield (Kumar and Seth, 2004) as well as biomass increment of a plant (Simmone et al., 2000). However, the highest 1000-kernel weight of the subsequent wheat crop produced in the P/W/P/W rotation did not translate to higher kernel yield. Stevenson and van Kessel (1996) reported a 62% increase in wheat yield after field pea compared to continuous wheat. However, there is a limitation of studies which investigated individual yield components, including the 1000-kernel weight. Despite that, some of previous studies claimed that pulse crops had an impact on 1000-kernel weight of corn (Idikut et al., 2009; Mohammadi and Ghobadi, 2010).

6.0. SUMMARY AND CONCLUSION

This study was carried out to examine physical, chemical and biological soil quality attributes of pulse crops with shallow and deep root systems grown in wheat-based, semi-arid and rain-fed conditions. The inclusion of grain legumes with different rooting depths into wheat-based cropping systems did not influence overall soil quality in the short time frame of this study. Chickpea, lentil and pea in wheat-based cropping system did not affect bulk density at 0-15 cm nor post-harvest residual soil moisture content at three soil depth. Using crop species with contrasting rooting structures (shallow-rooted and deep-rooted) did not improve the water sharing within 0-60 cm soil profile in semi-arid areas. However, the effects of pulse crops grown alternately with wheat in rotations readily apparent in the distribution of soil aggregate sizes, particularly >6.35 mm, 2-1 mm, 1-0.5 mm and 0.5-0.15 mm. The continuous wheat rotation had a clear benefit in terms of large macro-aggregate formation and the rotations with pulse crops facilitated the formation of micro-aggregates than the continuous wheat rotation in 0-5 cm soil depth without an impact on bulk density. Compared to the LFOM and HFOM abundance among the different treatments, this study underscore the possible contribution of microbial community composition on soil aggregation than the SOM distribution.

This study gives an insight on the potential role of including pulse crops into wheat-based cropping systems in increasing residue return thus enhancing the formation of labile SOM fractions. Chickpea caused a gradual increase in LFOM while decreasing amounts of HFOM which indicate that chickpea could be an effective proceeding crop in producing high amount of dry matter. Even though the inclusion of pulse crops into wheat-based cropping system had no impact on soil microbial biomass and microbial community composition before seeding, pulse crops in the rotations increased the total soil microbial abundance in the subsequent wheat crop.

All the treatments provided similar total soil C, N and SOC benefits at all the soil depths that demonstrated total soil C and N and SOC may not be responsive to preceding crops in the short-term under no-till cropping systems. Therefore, we suggest that the combination of pulse crops and wheat may need longer time to show changes in soil chemical properties. Similarities in N

dynamics among the treatments in this study indicate the coupling effects of plant residue quantity and quality. The overall N benefit of pulse crops in the rotations may hinder since smaller quantity of N-rich residues can contribute equal amounts of N to succeeding crops compared to more input-intensive cereals.

Overall, pulse crop rotations did not provide yield benefits in subsequent wheat crop compared with continuous wheat in monoculture; Despite that, kernel weight (measured as 1000-kernel weight) of the succeeding wheat crop in 2018 in field pea alternated with wheat and did not influence the kernel yield. However, the field pea-wheat cropping system enhanced market grade of wheat kernels.

Effects of pulse crop inclusion on some of the soil quality attributes assessed, which are more labile were easily discerned. However, it is concluded that the introduction of grain legumes with different rooting depths into wheat-based cropping systems do not influence the overall soil quality within a short time duration under no-tillage, semi-arid and rain-fed conditions.

Future Work

In the evaluation of the different treatments on soil quality, none of is a promising crop in terms of soil quality. Therefore, in order to track the changes in soil physical, chemical and biological attributes, soil quality needs to be further investigated using long-term assessments of rotation studies (probably 5-6 four-year studies), will be necessary to identify drawbacks and beneficial effects of pulse crops on soil properties, productivity and cost-effectiveness of the production system. In addition, it should be noted that, in the research presented in this dissertation, the site factor generally had influence on the evaluated parameters. Therefore, expansion of this study covering diverse growing environments in this region would facilitate identifying the best rotation options, based on growing environments.

In this study, fall soil moisture was determined only within 0-60 cm soil profile. However, evaluation of fall soil moisture in deeper soil profile (0-120 cm) could improve predictions of the soil water usage by different crops with different rooting depths. Data collection, including root nodulations and effectiveness of N fixation, based on crop growing stage would provide comprehensive understanding about the changes in the below-ground environments due to pulse crops in rotations.

It seems likely that grain yield itself could be a valuable predictor of nutrient removal and contribution. Future studies investigating the effect of treatments on soil N dynamics, the quantification of N in grain yield (harvest) and the plant straw in different treatments will be important for better understanding of contribution of N by different treatments. Furthermore, the evaluation of C footprint of different treatments will help identify the best treatment(s), which improve the environmental sustainability of the agricultural system.

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APPENDIX A

Table A.1. Crop variety, seeding rate, seed treatment, and fertilizers and agro-chemicals used in the cycle 2 of 4-year crop rotation at Brooks.

Crop variety	Cultivar	Seeding		Seed treatment		Fertilizer N-P-K	
		Rate (kg ha^{-1})	Density (seed m^{-2})	Trade name	Rate (kg ha^{-1})	Type of fertilizer	Rate (kg ha^{-1})
Spring wheat	AC Lillian (Hard red)	65	250	Vitaflo 280	100 kg^{-1}	11-51-0	39
						46-0-0	109
Chickpea	CDC Frontier (Kabuli)	200	50	Apron Maxx	100 kg^{-1}	11-51-0	39
Field pea	CDC Meadow (Yellow)	162	90	Apron Maxx	100 kg^{-1}	11-51-0	39
Lentil	CDC Maxim CL (Red)	56	140	Apron Maxx	100 kg^{-1}	11-51-0	39

Table A.2. Dates of application of agro-chemicals and agro- fertilizers in the cycle 2 of 4-year crop rotation at Brooks.

Operation	Crop(s)†	Fertilizer/chemical	Application rate	Year 1 2015	Year 2 2016	Year 3 2017	Year 4 2018
Pre-seeding burn	All	Factor 540	2500 mL ha^{-1}	-	-	-	-
off	All	Touchdown 500	1875 mL ha^{-1}	-	-	-	-
Pre-emergence	All	Factor 540	1000 mL ha^{-1}	May, 29	-	-	-
burn off							
Pre-seeding	All	Touchdown 500	1250 mL ha^{-1}	-	-	-	-
chemical	All except W	Bonanza 10G	9.0 kg ha^{-1}	April, 23	May, 02	May, 05	May, 04
	All except W	Edge	22.5 kg ha^{-1}	April, 13	April, 22	-	-
	C	Pursuit	30 mL ha^{-1}	-	-	October, 18	-
Nitrogen	W	46-0-0	146 kg ha^{-1}	-	April, 22	-	-
application	W	46-0-0	109 kg ha^{-1}	April, 22	-	May, 05	April, 28
Post-seeding	C	Pursuit	30 mL ha^{-1}	-	April, 29	May, 05	April, 28
chemical	P and L	Solo	0.029 kg ha^{-1}	April, 23	-	-	-
	P and L	Odyssey	0.043 kg ha^{-1} + merge 0.5%	June, 19	June, 03	-	-
	W	Foothills + Buctril M	237.5 mL ha^{-1} + 1000 mL ha^{-1}	-	-	June, 01	-
	W	Estraprop +Puma	1250 mL ha^{-1} + 750 mL ha^{-1}	June,19	-	June, 01	-
	C	Assure II	500 mL ha^{-1}	June, 19	June, 3	-	-
	C	Sencor	0.275 kg ha^{-1}	-	-	June, 01	-

† W-wheat, P-field pea, L-lentil, C-chickpea.

Table A.3. Dates of application of agro-chemicals and agro-fertilizers in the cycle 2 of 4-year crop rotation at Brooks.

Operation	Crop(s)†	Fertilizer/chemical	Application rate	Year 1 2015‡	Year 2 2016‡	Year 3 2017	Year 4 2018
Fungicide application	W, C and L	Bravo 500	3000 mL ha^{-1}	-	June, 14	-	-
	P	Headline	500 mL ha^{-1}	-	June, 14	June, 24	-
	C	Proline 480	375 mL ha^{-1}	June, 25	-	June, 16	-
	C	Bravo 500	2500 mL/ac	-	-	July, 05	-
	All	Bravo 500	3000 mL ha^{-1}	July, 08	-	-	-
	All	Headline and Lance	600 mL ha^{-1} and 4.25 kg ha^{-1}	-	-	-	June, 26
	C	Proline 480	375 mL ha^{-1}	July, 20	July, 07 and 15	June, 28 July, 17	-
	C	Headline and Lance	600 mL ha^{-1} and 6.25 kg ha^{-1}	-	-	-	-
Desiccation	P and L	Reglone	1750 mL ha^{-1}	Aug, 13- P, Aug, 22- L	Aug, 02	July, 22	-
	W	Roundup	6.25 kg ha^{-1}	-	Aug, 09	-	-
	C	Roundup	6.25 kg ha^{-1}	-	Aug, 26	-	-
Pre-harvest burn off	All	Touchdown 500	1875 mL ha^{-1}	-	-	-	-
Fall burn off	All	Factor 540	2500 mL ha^{-1}	-	-	-	-
		Factor 540	1250 mL ha^{-1}	-	-	-	-
		Touchdown 500	2500 mL ha^{-1}	-	Sep, 26	-	-

† W-wheat, P-field pea, L-lentil, C-chickpea.
‡Aug: August, Sep: September

Table A.4. Dates of different cultural operations and data collections in the cycle 2 of 4-year crop rotation at Brooks.

Operation	Crop(s)†	Year 1 2015	Year 2 2016	Year 3 2017	Year 4 2018
Spring soil sampling	All	April, 16	April, 21	April, 27	April, 25
Seeding	All	April, 22	April, 29	May, 04	April, 20
Re-seeding	All	June, 01	-	-	-
Plant density counts	All	June, 18	May, 26	May, 30	May, 15
Weed counts	All	June, 18	June, 01	May, 31	May, 31
Nodulation sampling	Pulses	July, 15	June, 30	June, 28	-
Weed biomass sampling	All	August, 11	July, 26	July, 19	July, 26
Harvest index sampling	P	August, 17	August, 08	July, 25	-
	L	August, 27	August, 08	July, 27	-
	W	August, 31	August, 08	August, 08	August, 10
	C	September, 09	September, 02	August, 14	-
Harvest date	P	August, 17	August, 08	July, 25	-
	L	August, 27	August, 08	July, 27	-
	W	August, 31	August, 18	August, 08	August, 10
	C	September, 10	September, 02	August, 14	-
Fall soil sampling	P	August, 18	August, 11	July, 25	-
	L	August, 28	August, 11	July, 28	-
	W	August, 31	August, 19	August, 09	August, 13
	C	September, 11	September, 02	August, 14	-

† W-wheat, P-field pea, L-lentil, C-chickpea.

Table A.5. Monthly temperature during the crop growing season at Brooks from 2015 to 2018.

Month	Temperature (°C)							
	2015		2016		2017		2018	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
April	15.1	-2.6	17.2	-0.1	12.9	-1.7	9.0	-5.6
May	19.4	1.2	18.9	3.7	21.8	4.9	23.9	5.0
June	25.4	8.8	25.0	8.8	23.9	8.4	25.2	8.1
July	27.3	10.4	25.0	11.4	29.6	11.5	28.4	9.8
August	26.1	10.2	24.4	10.1	27.2	8.7	26.9	8.4
September	19.4	4.7	19.7	4.1	21.6	4.0	15.8	2.2

† Max. = maximum temperature, Min.= minimum temperature

APPENDIX B

Table B.1. **Effect of cropping sequence treatments on soil moisture content at three soil depths in springs of 2017 and 2018.**

Treatment†	Moisture content ((kg kg^{-1} of soil)								
	0-15 cm		15-30 cm		30-60 cm				
	2017‡	2018	2017	2018	2017	2018	2017	2018	Overall mean
1=W/W/W/W	0.13	0.18	0.12	0.20	0.11	0.18	0.12	0.19	0.15
2=P/W/P/W	0.13	0.17	0.13	0.18	0.09	0.15	0.12	0.17	0.14
3=L/W/L/W	0.12	0.17	0.13	0.18	0.08	0.14	0.11	0.16	0.14
4=C/W/C/W	0.12	0.17	0.11	0.16	0.10	0.14	0.10	0.16	0.13
5=L/W/C/W	0.12	0.18	0.09	0.16	0.10	0.13	0.11	0.16	0.14
Mean	0.15 a§		0.14 a		0.12 b		0.11 b	0.17 a	
	Contrast and *P* values for *a priori* comparison¶								
1 vs. (2+3+4+5)/4	0.008	0.008	0.005	0.030	0.018	0.040			
	0.96$^{ns\#}$	0.18^{ns}	0.90^{ns}	0.02*	0.04*	0.12^{ns}			
5 vs. (2+3+4)/3	-0.003	0.010	-0.033	-0.013	0.01	-0.013			
	0.94^{ns}	0.26^{ns}	0.11^{ns}	0.30n^{s}	0.50^{ns}	0.66^{ns}			
4 vs. (2+3)/2	-0.005	0.000	-0.020	-0.020	0.015	-0.005			
	0.20^{ns}	0.92^{ns}	0.57^{ns}	0.25^{ns}	0.16^{ns}	0.62^{ns}			
2 vs. 3	0.010	0.000	0.000	0.000	0.010	0.010			
	0.68^{ns}	0.65^{ns}	0.98^{ns}	0.61^{ns}	0.52^{ns}	0.84^{ns}			
***P* values for main and interaction effects of sampling year (Y) × treatment (T) × soil depth (D) analysis**									
	Y	**T**	**D**	**Y×T**	**Y×D**	**T×D**	**Y×T×D**		
P value	<0.0001***	0.05*	<0.001**	0.58^{ns}	0.32^{ns}	0.43^{ns}	0.90^{ns}		

† W=Wheat, P=Field pea, L=Lentil, C=Chickpea.

‡ The soils sampled in the spring of 2017 and 2018 are representative of the 2016 wheat crop phase and 2017 pulse crop phase of rotations, respectively.

§ Values with same letter within each category (soil depth and sampling year) are not significantly different at α=0.05, according to Tukey's HSD test.

¶ ^{ns}: non-significant (P>0.05), *, ** and *** denote significant at $P\leq0.05$, $P\leq0.01$ and $P\leq0.001$, respectively, according to Tukey's HSD test.

The contrast value was taken from the subtraction of the value on right from the value on the left in the comparison and the *P* value was mentioned following the contrast value.

Table B.2. **The effect of different treatments on soil aggregate size distribution at 0-5 cm soil depth in springs of 2017 and 2018.**

Treatment†	Soil aggregate size distribution (g kg^{-1} of soil)											
	> 6.35 mm			6.35 – 2.00 mm			2.00 -1.00 mm			1.00 -0.50 mm		
	2017	2018	Mean	2017	2018	Mean	2017	2018	Mean	2017	2018	Mean
W/W/W/W	233a‡	243a	238a	121abcd	154a	138a	79e	139ab	109b	75fg	51g	64b
P/W/P/W	128bc	97d	113c	114bcd	119abcd	117ab	100d	125ab	112ab	95ef	186ab	140a
L/W/L/W	136b	125bc	130b	109cd	148ab	129ab	100ab	146a	123a	127cd	159bc	143a
C/W/C/W	127bc	106cd	116c	125abcd	102d	114b	107cd	135ab	121a	118de	190ab	154a
L/W/C/W	135b	92d	112c	140abc	117abcd	129ab	100d	129ab	114ab	109b	201a	155a
Mean	152a	133b	-	122a	128a	-	97b	135a	-	105b	158a	-

Treatment †	Soil aggregate size distribution (g kg^{-1} of soil)											
	0.50 -0.15 mm			0.15 – 0.12 mm			0.12-0.05 mm			< 0.05 mm		
	2017	2018	Mean	2017	2018	Mean	2017	2018	Mean	2017	2018	Mean
W/W/W/W	208c	245bc	226b	45bc	36cd	41bc	153ab	94c	123ab	87a	39b	63a
P/W/P/W	272ab	290a	281a	43c	46bc	44b	172a	98c	135a	75a	43b	59ab
L/W/L/W	266ab	282a	274a	43bc	28d	36c	146b	103c	124ab	74a	25bc	49bc
C/W/C/W	266ab	290a	278a	41bc	51b	46b	144b	101c	123ab	73a	39b	56abc
L/W/C/W	260ab	293a	276a	42bc	67a	55a	141b	88c	115b	78a	16c	47c
Mean	254b	280a	-	43a	45a	-	151a	97b	-	77a	32b	-

† W: wheat; P: field pea; L: lentil; C: chickpea.

‡ Same letter following mean indicates no significant difference between (P>0.05) between the sampling year*treatment interactions according to Tukey's HSD test.

¶ Values with same letter within each category (treatment and sampling year) are not significantly different (P>0.05) according to Tukey's HSD test.

Table B.3. **The effect of different treatments on soil bulk density at 0-15 cm soil depth in springs of 2017 and 2018.**

Treatment†	**Soil bulk density in different sampling years (Mg m^{-3})**		
	2017‡	**2018**	**Overall mean**
1=W/W/W/W	1.18	1.18	**1.18**
2=P/W/P/W	1.03	1.09	**1.06**
3=L/W/C/W	1.32	1.24	**1.28**
4=L/W/L/W	1.10	1.13	**1.12**
5=C/W/C/W	1.13	1.04	**1.08**
Mean	**1.15**	**1.15**	
	***P* values for *a priori* treatment comparison**		
1 vs. (2+3+4+5)/4	0.82 ^{ns}§	0.68 ^{ns}	-
5 vs. (2+3+4)/3	0.09 ^{ns}	0.28 ^{ns}	-
4 vs. (2+3)/2	0.64 ^{ns}	0.62 ^{ns}	-
2 vs. 3	0.66 ^{ns}	0.82 ^{ns}	-
	***P* values for main and interaction effects of Sampling year (S) × Treatment (T) analysis**		
	S	**T**	**S×T**
P value	0.78 ^{ns}	0.34 ^{ns}	0.94 ^{ns}

† W: wheat, P: field pea, L: lentil, C: chickpea.

‡ The soils sampled in the spring of 2017 and 2018 are representative of the 2016 wheat crop phase and 2017 pulse crop phase of rotations, respectively.

§ $^{ns, *, **}$ denote non-significance at $P<0.05$, significance at $P<0.05$ and $P<0.01$ respectively, according to Tukey's HSD test.