THE INFLUENCE OF LENTIL, CANOLA, PEA AND WHEAT ON CARBON AND NITROGEN DYNAMICS IN TWO CHERNOZEMIC SOILS

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Abstract

Even though the sustainability of crop rotation with pulses has been demonstrated by ancient civilizations, the effects of legume crops on soil organic carbon (SOC) and soil organic nitrogen (SON) dynamics has not yet been adequately quantified in the Canadian Prairies. The main goal of this thesis was to analyze the impacts of incorporating lentil and pea in rotation with wheat on SON mineralization and the fate of recently fixed C in soil. Two soil types were analyzed in this study, a Brown Chernozem (Cz) from Swift Current SK. and a Dark Brown Cz from Scott SK. To quantify the effect of pulse crops on Nitrogen (N) gross mineralization, stable ^{15}N isotope dilution was used and eight different crop rotations were selected (five on the Dark Brown Cz: canola-wheat, pea-wheat, pea-canola-wheat, continuous wheat without N fertilizer, continuous wheat with N fertilizer; and three on the Brown Cz: lentil-wheat, continuous wheat without N fertilizer, and continuous wheat without N fertilizer). These rotations were in the wheat phase at sampling. Additionally, lentil-wheat in the lentil phase was included. Gross N mineralization was evaluated at seeding (May 2009) and anthesis (July 2009). Mean gross N mineralization was not significantly different between crop rotations on any date. Timing of sampling (seeding versus anthesis) had the strongest effect on mineralization with mineralization rates significantly higher at anthesis than at seeding. No significant effect of the soil type was found and rotations with pulse crops had higher mineralization rates than continuous wheat only when compared to unfertilized continuous wheat.

Soil N mineralization and SOC dynamic are interconnected and therefore the transformation of crop residue carbon (C) into SOC was also investigated. Lentil, canola, pea and wheat were grown in intact soil cores extracted from the field plots of the N mineralization experiment (at Swift Current and Scott) and pulse-labeled with $^{13}CO_2$ in a controlled environment to trace crop residue decomposition. At the end of the labeling season (first growing season) 50% of the soil cores were destroyed to estimate root biomass with a ^{13}C technique. Before the second growing season (without ^{13}C labeling), ^{13}C-enriched shoot residues were ground and incorporated into the soil. At the end of the second growing season the remaining cores were destroyed to assess the amount of remaining derived C from the root and the shoot. For both growing seasons, the soil was fractionated into water extractable organic matter (WEOM) very light fraction (VLF), light fraction (LF) and heavy fraction (HF) and the $\delta^{13}C$ was determined for each soil fraction. For all

crops, estimated root biomass production was markedly higher than estimates in the literature based on root washing and counting methods. In addition, lentil had root biomass production (0-10 cm) significantly higher than the other crops and the lowest shoot:root ratio at 2.5. Since canola produced three times more straw residue than the other crops, its shoot:root ratio was significantly higher at 13.2. At the end of the second growing season, the amount of root - derived C remaining in the VLF, LF and HF had decreased 91%, 61% and 60% respectively. No significant difference was found among crops. At the end of the second growing season on the Swift Current soil (Brown Cz), lentil had more derived C per ha^{-1} than wheat and on the Scott soil (Dark Brown Cz), canola and pea had more than wheat. Based on these results, the deduced transformation of plant residue C into SOC was VLF first, then LF, then HF, with all fractions contributing C to the WEOM and the WEOM transferring C back to the HF.

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Dedication

I would like to dedicate this Master thesis to Professor Darwin W. Anderson for encouraging me to do my master studies in the department of Soil Science at the University of Saskatchewan.

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List of Abbreviations

AAFC- Agriculture and Agri-Food Canada

ANOVA - Analysis of variance

C – Carbon

Cz - Chernozem

FA – Fulvic acid

GHG – Greenhouse gas

HA – Humic acid

HF – Heavy fraction

HuC – Humin clay

HuS – Humin sand

IRGA - Infrared gas analyzer

LF – Light fraction

N - Nitrogen

NaI – Sodium iodide

NH_4^+ - Ammonium

NO_3^- - Nitrate

PURENET - Pulse Research Network

PVC - Polyvinyl chloride

SOC - Soil organic carbon

SE – Standard error

SOM - Soil organic matter

SON – Soil organic nitrogen

VLF- Very light fraction

WEOM – Water extractable organic matter

1. Introduction

The Neolithic Revolution began with the cultivation of wheat (*Triticum spp*) approximately 10,000 BC in northern Syria (Gupta, 2010). However, civilization did not emerge until 3,100 BC in Egypt when farmers adopted ploughing practices and crop rotations with legume species (Wengrow, 2006). The benefits of cereal-legume (Poaceae-Fabacea) mixed planting and rotation were many; cereals provide a diet high in carbohydrates and legumes supply vital amino acids. In agricultural fields Fabaceae plants form symbiosis with *Rhizobium*, which enriches the soil with N and increases yields (Campbell et al., 1993; Entz et al., 2001). In addition, cereal-legume rotations help control weeds, pests and diseases while enhancing soil biota activity (Ominski et al., 1999). Like the ancient Egyptians, each agricultural civilization developed its own cereal-legume rotations. In Europe it was wheat/barley and pea/lentil/chickpea; in America, maize and bean/groundnut; in Africa millet/sorghum and cowpea/jugo bean; and in Asia rice/millet and soybean/pigeon pea (Sauer, 1993). The paradigm of cereal-legume rotation was later altered with the Green Revolution. Improvement in agricultural tractive force, high-yielding varieties of cereal grains, synthetic N fertilizers, and pesticides and herbicides made the use of legume crops avoidable and cereal monocultures often more lucrative. Simultaneously, citizens from industrialized countries started to increase their meat consumption as a protein source causing a decrease in leguminous grains demand (Davies, 2003).

In the Canadian Prairies, farming also started with wheat production. The first recorded harvest was in 1814 and the first exportation in 1868 with 857 bushels (48,000 kg) of Red Fife wheat (Hubner, 1998). Fallow-wheat system was the initial practice in the Canadian Prairies. The fallow was used to retain moisture and hasten soil organic matter (SOM) mineralization, providing extra N for the subsequent crops. However, at the beginning of the twentieth century, soil studies indicated that this practice was causing weed infestations, loss of humus, and loss of N. To solve this problem, crop rotations with legumes were suggested by Frank T Shutt in 1910. In order to test the viability of this proposal, numerous crop rotation studies were established and rotations with legumes resulted in better N status. The inclusion of legumes in the rotations was then formally promoted in the prairies but the use of summer-fallow persisted to preserve moisture (Janzen, 2001). In the 1950's, mass commercialization of potent herbicides, pesticides

and inexpensive synthetic N fertilizer made legume-wheat rotation less profitable than continuous wheat (Larney et al., 2004). In Manitoba during World War II, *Brassica napus L*-wheat rotation was initiated, but *Brassica napus L* culture was not extended until the 1970's when varieties low in erucic acid and glucosinolates were developed (and named canola). In the last 15 years, herbicide resistant canola varieties and demand for canola for the manufacture of biodiesel has increased canola production area by 250% in Canada (Johnston et al., 2002b; Canola Council of Canada, 2011).

Canola-wheat rotation and continuous wheat represent unidirectional farming practices where high inputs of synthetic fertilizers, pesticides, herbicides and on-farm fuel are applied at the beginning of production and maximum yield is exported out of the agro-ecosystem (Pearson, 2006). This practice has raised health, agronomic, environmental and economic concerns during the last decades. On several occasions since the 1990's, extreme weather events have worsened economic loss to farmers who were practicing monoculture (Lotter et al., 2003; Akinremi et al., 1999). Nitrogen fertilizer derived from the Haber-Bosch reaction is highly endothermic and requires large amounts of energy. Approximately 60% of the energy requirement for conventional grain production is for fertilizer production and application (Zentner et al., 2004). As well, N fertilizer prices are indirectly based on oil barrel value, which partly explains the recent world food price increase (Fyksen, 2007; FAO, 2011). Herbicide resistance is of increasing concern in the Canadian Prairies. Weeds are becoming resistant to herbicides forcing farmers to use more potent agrochemicals (Larney et al., 2004) and heavy use of pesticides and herbicides has been connected with several human diseases and malign neoplasms (Goldman, 2007; Gilden et al., 2010). On the environmental side, intense use of synthetic N fertilizers has been linked to acid rain, eutrophication of water bodies and emission of nitrous oxide (Galloway et al., 2004).

To mitigate these problems, engineering development on crop rotation with legumes was incentivized. Technological improvements for seeding, harvesting and weed control have improved legume cultivation and reduced production costs (Smith and Young, 2003). In addition, rotation with legumes spreads out labour and equipment requirements due to different maturity dates (Zentner et al., 2002). For these reasons and because rotation with legumes reduces disease prevalence, weed infestations, and reduces N fertilizer need, legume production has been

increasing and reached more than 5 million ha per year since 1997 in the Canadian Prairies (Agriculture and Agri-Food Canada, 2000; Hemantaranjan, 2007; Griffiths, 2009). The main Fabaceae species currently cultivated in Canada are: lentil (*Lens culinaris*), field pea (*Pisum sativum*), chickpea (*Cicer arietinum*), alfalfa (*Medicago sativa*) and dry bean (*Phaseolus vulgaris*) (Agriculture and Agri-Food Canada, 2000; Curtin et al., 2000; Johnston et al. 2002a).

Legumes lead to several environmentally positive benefits. Novel research on energy balance has shown that energy efficiency (kg of grain per joule) is significantly higher in legume-wheat rotations compared with continuous wheat (Hoeppner et al., 2006). Therefore, because fossil fuels supply 86% of the world's energy (International Energy Outlook, 2011), rotation with legumes indirectly reduces CO_2 emission. Another study (Smith et al., 2001) reported that the inclusion of legumes (alfalfa) in crop rotations in the Prairies increases soil C an average of 0.44 Mg C ha^{-1} yr^{-1} which also reduces CO_2 emissions. With legumes, N fertilizer need is reduced so there is less leaching of nitrates, which helps control surface and ground water quality. As well, legumes in crop rotation increase mycorrhizal diversity and enhance microbial activity (Miller et al., 2002; Welsh et al., 2006; Nayyar et al., 2009).

The overall positive effect of legume crops has been known since the beginning of civilization; however, recently new questions have been raised related to the inclusion of legumes crops in rotation. It is not yet known why legume crops in rotation with wheat have shown to have statistically the same SOC status as continuous wheat since legume crops are producing less straw biomass than wheat (Lemke et al., 2007). As a result, many questions about the comparative root biomass production of legume crops versus non-legume and the decomposition dynamic of legume versus non-legume crop residues have been brought up. As well, the effect of legume crops on N gross mineralization is still not fully understood. The main objective of this study was to evaluate the impacts of incorporating lentil and pea in rotation with wheat on the fate of the C fixed during a single growing season in soil and on SON mineralization. This work was part of the *cropping systems* module of Pulse Research Network (PURENet), which has the overall aim to address questions about the use of pulse crops in sustainable cropping.

2. Literature Review

2.1. C and N dynamics

Carbon (C), hydrogen (H), oxygen (O) and nitrogen (N) are the four main elements in living organisms. Carbon and N are currently of particular interest because C is the main component of soil organic matter (SOM), which is central in soil fertility. Soil organic matter is beneficial to plants through physical (e.g. soil structure, aggregation, porosity, moisture retention) and chemical properties (e.g. ion exchange, buffering capacity, nutrient storage and release) (Schulten and Schnitzer, 1998; Janzen, 2006). Carbon makes the skeleton of all organic compounds and therefore is the principal component of SOM. Nitrogen is a central part of proteins and nucleotides and thereby central to all living organisms. Furthermore, N is commonly the most limiting element in agricultural production. Nitrogen, although usually only one-tenth of the C content, is also an important component of SOM; about 75% of this is in the form of proteinaceous and heterocyclic rings (Knicker and Ludeman, 1995).

Most C enters into agroecosystems via photosynthesis by plants. Carbon dioxide is fixed by RuBisCo and transformed into glyceraldehyde ($C_3H_6O_3$) in the Calvin cycle. A fraction of this C is directly respired to produce adenosine-5'-triphosphate (ATP) and the other fraction is synthesized into organic molecules. Some of these C-containing compounds are harvested with the crop and the remainder is added to the soil as plant residues (Janzen et al., 1998). Then, a portion of these fresh organic compounds is respired by organisms and another portion is converted into SOM by the process of humification (Janzen, 2006; Lal, 2005). When the amount of new organic residues added to the soil is greater than the C lost by SOM decomposition, SOM content increases (Ellert and Bettany, 1995).

2.1.1. Nitrogen cycle

Very few rocks and minerals contain N. Atmospheric N usually enters into the pedosphere by N_2 fixation (biological and anthropogenic). Nitrogen is generally the most limiting nutrient in agricultural production. In soil, approximately 95% of the N is in the organic form and 5% inorganic, mainly as nitrate and ammonium. Although plants can directly uptake amino acids and other small biomolecules, this organic N does not contribute significant amounts

of N to plants (Nasholm et al., 2008). Therefore, organic N has to be mineralized into inorganic forms to be available to plants.

In undisturbed environments, N enters into the ecosystem via dinitrogen gas (N_2) fixation by the means of diazotrophs (bacteria and archaea that fix N_2). Nitrogenase is the only known enzyme capable of transforming N_2 into ammonium (NH_4^+) (Schrauzer, 2003). Subsequently the NH_4^+ reacts with one aldehyde in a nucleophilic addition to produce amino acids, nucleic acids and other N-biomolecules. However, today the Haber–Bosch process industrially fixes a large proportion of the N used in agriculture. An estimated 40% of the Earth's population depends on industrially fixed N for protein, but the extra N cycling in the agricultural systems is leached and causes contamination and eutrophication of water bodies (FAO, 2004; Robertson and Vitousek, 2009).

The decomposition of SOM into inorganic molecules through chemical and biochemical reactions is called mineralization (Gregorich et al., 2001). The mineralization of SOM is mainly done by heterotrophic soil microorganisms and releases nutrients for plant uptake (Janzen, 2006). The principal products of the SOM mineralization process are CO_2, CH_4, NH_4^+, and NO_3^- (Hopkins, 2008). The C and N mineralization rates typically depend on a number of factors such as aeration status, temperature, moisture content, and microbial activity and population characteristics. The main soil properties that affect mineralization are the ratio of C:N, lignin:N and polyphenol:N, pH, texture, and clay (Booth et al., 2005; Brady and Weil, 2008; Ha et al., 2008). After that organic N is transformed into NH_4^+ by ammonifiers, nitrifying bacteria convert NH_4^+ into NO_3^- (nitrification). Nitrification generally involves two steps: first, nitrosomonas transforms NH_4^+ into NO_2^- and then nitrobacter converts NO_2^- into NO_3^-. Subsequently the NO_3^- that is neither assimilated nor leached is reduced to N_2 by denitrifying bacteria (Zehr and Kudela, 2011).

Sugars and simple proteins are decomposed readily, hemicelluloses, cellulose and lipids at a moderate rate, and lignins and phenolic compounds most slowly. Lignins and phenolic molecules have strong and varied structures with methoxyl groups, which means that only select fungi can break them down (Christian et al., 2005). It is thought that the phenol rings play a key role in the synthesis of stable SOM (Huang et al., 2008). Some allelopathic molecules (alkaloids and other secondary plant compounds) also inhibit decomposition by decreasing microbial

activity and forming resistant complexes with proteins (Levin, 1976; Clapp et al., 2005). In many ecosystems, the accumulation of SOM is explained by the production of recalcitrant plant residues (Cadisch and Giller, 1997; Gentile et al., 2011a). Legume residues tend to have a low C:N ratio, which is generally associated with more rapid mineralization rates, but in some legumes the phenol and alkaloid contents are high, diminishing mineralization rates (Franzluebbers and Hill, 2005). Singh et al. (2007) compared the instantaneous decay rate constants of wheat straw and shoot residues from a green manure legume (*Sesbania sp*) in a dry tropical climate. Their results showed that *Sesbania sp* residues decomposed faster than wheat straw. In contrast, Mungai and Motavalli (2006) have shown that Fabaceae does not always have a decay rate constant higher than Poaceae. In their study, under temperate conditions in Missouri, bluegrass (*Poa trivilis*) had a slightly higher decomposition rate than soybean (*Glycine max*).

2.1.2. Soil carbon and soil nitrogen relationships

Carbon enters the bio-pedosphere through photosynthesis and N mainly through fixation (biological and industrial). As well, in some environments, singular microorganisms use inorganic molecules as energy sources instead of photosynthesis in order to transform CO_2 into organic molecules. Nitrifiers are chemoautotrophic microbes that use CO_2 as their C supply for growth and NH_3 as their energy source. Carbon escapes through aerobic respiration and N through nitrification/denitrification and leaching. Yet, C and N cycles are strongly linked; plant residues with high C:N ratios increase net N immobilization and therefore decrease the net N mineralization. This situation usually stimulates the process of biological nitrogen fixation. Low C:N ratio residues, on the other hand, promote mineralization and nitrification/denitrification while decreasing immobilization. In turn, low amounts of soil inorganic N limit plant growth and therefore decrease photosynthesis (Recours et al., 2000; Gärdenäs et al., 2011). Nitrogen accessibility also influences microbial catabolic capacity and turnover rates of the SOC. Therefore, the size and characteristic of the SOC pools are also impacted by the mechanisms that affect N dynamics (Knicker, 2011). Different plant genera produce different quantities of residues with variation in their N content, amount of lignin and phenol. Consequently, to understand SOM formation and to evaluate the potential of some crop rotations to increase SOC storage, it is essential to study both C and N processes.

2.2. Pulse crops

2.2.1. Pulse crops and Fabaceae

Lentil and pea are pulse crops, defined by Food and Agriculture Organization (FAO) to include 11 annual legume crops. Fabaceae family appears to have diversified 65 million years ago in the early Tertiary (Herendeen et al., 1992). This family is cosmopolitan in distribution and many genera are found in arid, temperate and tropical ecosystems (Rundel, 1989). The flowers are bisexual with a single superior carpel and the fruit (legumes) are pods, which typically are dry and dehiscent (Polhill, 1994). World pulse production in 2002 stood at 54.4 million tonnes (FAO, 2011). In 2009 Canadian pulse production was 5.6 million tonnes (Pulse Canada, 2011). Legume is one of the few taxa that are able to form symbiotic relationships; they form symbioses with rhizobacterium and fix N_2. The benefits to growing legume crops are two-fold: the seeds contain high amounts of amino acids, essential to balanced nutrition, and root nodules produce an excess of NH_4^+, which adds to the supply of organic N in soil and enhances the growth of the crops following in rotation. Yield of cereal crops following pulse crops have been found to be 12 to 20% greater than the yields of cereals following cereals (Evans et al., 1989; Smiley et al., 1991). The amount of N in the grain is also 17 to 22% higher in wheat following a legume than continuous wheat (Wright, 1990). Wheat following a legume crop has been estimated to contain up to 50 kg ha^{-1} more N in the grain than continuous wheat (Evans et al., 1989). However, there is large variability in the effect of pulse crops on soil N, ranging from losses (-32 kg N ha^{-1}) to substantial gains (up to 96 kg N ha^{-1}), with the variability considered a consequence of the inhibitory effect of NH_4^+ and NO_3^- on nitrogenase activity (Bremer et al., 1988). Variances in N fixation rates are also caused by photosynthesis rates, water availability and energy availability (ATP) (Cowell et al., 1989). In addition to the N benefits, legume crops generate many other positive effects on subsequent crops such as: decreased root and shoot diseases, diminished weed populations, enhanced P, K and S availability, improved soil structure and exudation of beneficial compound such as auxins, gibberelins, cytokinins, and ethylene (Bullock, 1992; Smiley et al., 1991; Bashan and de-Bashan, 2005).

Greenhouse experiments have shown that roots exude up to 46% of the N_2 fixed by legume-rhizobium symbiosis and all these exudates are often unaccounted for in root biomass

estimation (Sawatsky and Soper, 1991). From the total N produced by legume crops, a fraction has been reported to be unavailable to the immediately succeeding crop (Armstrong et al., 1994). Therefore, a positive N balance in soil after a legume crop does not necessarily produce an immediate benefit to the next crop; instead it represents an N accumulation that may eventually be used by subsequent crops or lost by leaching and denitrification (Jensen, 1996). Bremer and van Kessel (1992) have shown that only 2 to 14% of the N in legume residues is recovered by the subsequent crop. Approximately 20% of the N in lentil residues was found in the microbial biomass 10 days after incorporation into the soil in fall, and 80% of the lentil N remained in the soil in the following spring. As well, a fraction of the legume and cereal N residues are incorporated into a recalcitrant fraction of the SOM after having been assimilated by soil microorganisms (Bremer and Van Kessel, 1992). In a study on pulse residue dynamics, Jensen (1996) found that from six months to three years after incorporating pea residues into the soil, N leaching was estimated to increase from 16 to 34% and a large amount of N was also lost by denitrification. Comparing crop rotations in the Canadian Prairies, Campbell et al. (1992) found that soils under a lentil-wheat rotation had SOM mineralization rates 250% greater than soils under continuous wheat.

2.2.2. Shoot and root morphology

Through evolution plants became separated into root and shoot (stems, leaves and flowers). The roots anchor plants to the soil and take up nutrients and water from the soil. The shoots sustain the plant in the air and carry on photosynthesis and reproduction. Roots and shoots are morphologically and biochemically different and significant differences exist among plant species (Raven and Edwards, 2001; Vergara-Silva, 2003).

Canola, lentil and pea are eudicot plants and have a taproot and vascular bundles arranged in a ring. Taproots penetrate deep into the soil and have many smaller lateral roots. The centre of root steles is occupied by a strong star-shaped region of xylem. Eudicots have pith in the young stems but not in the roots. The pith often gets replaced by lignified xylem in older branches and stems. Fabaceae (lentil and pea) have sieve cell plastids with protein crystal and secretory canals that can release tannins and alkaloids. Brassicaceae plants (e.g. canola) have myrosin cells that produce glucosinolates, although canola has been bred to produce low amounts of this toxic compound (Raven et al., 2005; Evert et al., 2006).

Wheat, a monocot of the Poaceae (or Gramineae) family, has a fibrous root system and scattered vascular bundles. The adventitious rooting with abundant unicellular root hairs allow optimal growth in the A horizon. Root hairs decompose quickly though, and are difficult to quantify. Pith is located in the centre of the roots pericycle stele but pith is not present in the shoots. The stele of the shoots does not form lignified rings, and instead they are scattered throughout the stem. The leaf blades are hardened with silica phytoliths. These rigid silica structures are difficult for animals to consume and digest, which reduces the degradation rate of the straw residues (Raven et al., 2005; Evert et al., 2006).

Wheat generally produces 1.2 to 2 times more straw than pulse crops (Lemke et al., 2007). Gan et al. (2009) estimated the shoot and root biomass of pea, lentil, canola and wheat in the Canadian Prairies. The shoot biomass values (kg ha^{-1}) were: pea 4100-5200, lentil 3200-4300, canola 4000-5000, and wheat 6100-6700. Root biomass at maturity (kg ha^{-1}) was: pea 460-540, lentil, 690-910, canola 950-1570, wheat 1070-1420. Soil organic matter content is usually correlated with the amount of crop residue returned to the soil. However, several papers reported that even if legumes produce fewer crop residues, with a lower C:N ratio and less lignin than wheat and canola, the SOC content is similar or higher under legume crops (Drinkwater et al., 1998; Campbell et al., 2000; Soon and Arshad, 2002; Sainju and Lenssen, 2011). Lemke et al. (2007) suggested that pulse crop residues might be more efficiently converted to SOC.

2.2.3. Residue quantity, quality and soil fertility

Some ecosystems annually produce small amounts of vegetal residues and have high levels of SOC and other ecosystems generate high quantities of residue, yet have low levels of SOC (Bohn, 1982; Eswaran et al., 1993; Milne et al., 2005). Therefore, SOC status is not always correlated to net primary production or to the quantity of crop residues returned to the soil (Drinkwater et al., 1998; Brady and Weil, 2008). In the northern Great Plains it has been recognized that the inclusion of lentil and pea in rotation with wheat decreases residue biomass production (root and shoot) and C returned to the soil (Sainju et al., 2006; Gan et al., 2009); nevertheless, recent studies have found that long-term rotation with legumes does not cause a decrease in SOC in the Canadian Prairies (Campbell et al., 2007a, 2007b; Lemke et al., 2010b).

Along with environmental conditions and soil properties, vegetal residue quality is a key factor affecting SOC status (Stevenson, 1994; Batjes, 1996). In agro-ecosystems, residues with high amounts of phenol and lignin and high C:N ratios are considered low quality resources because only a few microbial taxa are able to consume them and their mineralization is slow. Typically legumes are considered as high quality and canola and wheat as medium quality (Soon and Arshad, 2002; Brady and Weil, 2008). However, recalcitrant materials (low quality) often help explicate the accumulation of humified and passive SOM. In contrast, litter residues considered as high quality, rapidly mineralize in soil and fertilize the soil but high quality residues alone do not necessarily contribute significantly to passive SOM accumulation (Brady and Weil, 2008). Gentile et al. (2011b) found that despite low quality, the input of crop residues can provide long-term soil fertility. The combination of vegetal residues of different qualities can improve short-term nutrient dynamics while conferring the same benefits to long-term SOM contents (Gunnarsson and Marstorp, 2002; Griffith et al., 2011). Whereas several studies suggest that residue quantity and quality have a limited effect on passive SOM, the complete absence of a crop in fields (summer-fallow) significantly decreases SOC amount (Janzen et al., 1998; Lemke et al., 2010a, 2010b). Overall, residue management is important for short-term soil fertility and farm sustainability (Limon-Ortega et al., 2009; Puttaso et al., 2011).

2.3. Characterization of soil C and N

2.3.1. Root measurement methods

Below-ground plant residues (roots and exudates) are believed to be a major source of SOC; therefore, accurate measurement of root biomass is essential to make precise C budgets in croplands and to predict SOC and SON dynamics (Swinnen et al., 1994; Recous et al., 2000; van Kessel and Hartley, 2000; Ping et al., 2010; Sainju and Lenssen, 2011). Soil coring and whole plant excavation are the most widespread root biomass quantification techniques but these methods are labourious and usually underestimate below-ground C additions because of the rapid turnover of the root hairs (Livesley et al., 1999); leaching of exudates (Zobel and Zobel, 2002); loss of fine roots that pass through sieves (Ruhigwa et al., 1992; Amato and Pardo, 1994) and the damage and loss of roots during the washing process (Paustian et al., 1997). Shoot biomass is often used as a root biomass proxy but data are lacking for shoot:root ratios for pulse crops in

order to generate reliable proxies (Lemke et al., 2007) and the above/below ground ratio is usually affected by soil N availability, porosity and water availability (Bolinder et al., 2002). Accurate below-ground biomass can be generated with ^{13}N and/or ^{15}N isotopic labeling (Subedi et al., 2006; Yevdokimov et al., 2006).

2.3.2. ^{13}C labeling

Plants that have been pulse labeled with ^{13}C can be used to trace shoot and root decomposition through different SOM pools. However, the isotope must be uniformly distributed in the plant. This can be achieved when the $^{13}CO_2$ is applied at regular intervals (repeated pulse labeling) throughout all growing stages, and in direct proportion to the photosynthesis rate (Bromand et al., 2001; Moore-Kucera and Dick, 2008; Sangster et al., 2010). With ^{13}C-enriched residues of *Phacelia tanacetifolia*, Thompson (1996) found a rapid mineralization phase during the first two months after the residues were incorporated into the soil, followed by a slower decomposition phase during the next months. Williams et al. (2006) followed the fate of ^{13}C-labeled root and straw residues from a grass (*Lolium multiflorum*) and a legume (*Trifolium incarnatum*) and found that the contribution of legume straw to the soil C pool increased more rapidly than the grass during the first months of decomposition but after the winter no differences were found between legume and grass. They also found that most root C disappeared during the first months of decomposition and after nine months, the root C from the grass represented about 5.5% of total SOC, whereas the contribution of legume root derived C was about 1.5%. In a dual-labeling study on the stabilization and immobilization of ^{13}C and ^{15}N enriched crop residues, Bird et al. (2003) found divergent humification pathways for molecules rich in C versus molecules rich in N.

2.3.3. Soil organic matter characterization and fractionation

The first references to SOM date back to the earliest civilizations. The ancient Chinese book Yugong (2,500 BC) is one of the earliest references, classifying soil by colour, texture and hydrologic feature, all related to SOM (Krasilnikov et al., 2009). The lack of suitable techniques and analytical methods limited progress in SOM studies well into the 20th century (Hatcher et al., 2001). Humic and fulvic acid separation of the SOM was first carried out by Sprengel in 1826 (Schnitzer, 2000), although this fractionation became widely used only when the pH meter was

developed a century later. Subsequently, pyrolysis gas chromatography and mass spectrometers allowed the identification of literally hundreds of chemical structures including alkanes, fatty acids and esters and the identification of these compounds in the fulvic and humic acids. However, X-ray microscopy has since shown that pH and ionic strength of the alkali solutions used in the fractionation change the micro-molecular structure of the SOM, which indicates that humic/fulvic fractionation is not the optimum separation technique (Myneni et al., 1999). In addition, alkaline/acid extraction causes dissolution of silica from the mineral matrix, dissolution of fresh tissues and autoxidation of some organic molecules. Yet, alkaline extraction is still considered the most efficient SOM chemical extractant today (Schnitzer, 2000).

During the last two decades physical fractionation (size and/or density) of the SOM has become more popular. These methods are based on the premise that SOM makes functional and key associations with the primary soil particles and that these associations regulate SOM dynamics. The light (LF) and heavy fractions (HF) are the main density fractions differentiated by using a heavy liquid, most commonly a NaI solution at 1.7 g mL^{-1}. The LF floats and is believed to be a transitional pool of organic matter between fresh plant residues and stabilized SOM. The HF is bonded with minerals and is considered to be more decomposed and humified, and usually has a narrower C:N ratio than the LF (Gregorich et al., 2006; Gregorich and Beare, 2007). Using a liquid with a density of 1 g mL^{-1}(e.g. deionized water) it is possible to subdivide the LF. The fraction that floats on water (very light fraction, VLF) consists mainly of identifiable fresh plant residues and charcoal. From this soil-water solution it is also possible to extract the dissolved organic matter (or water-extractable organic matter, WEOM). The WEOM consists of dead microbes and labile organic molecules that microorganisms can use as a source of energy. Therefore, the WEOM is predominantly produced by some microbes and serve as a source of substances and energy for other microbes (Zsolnay and Steindl, 1991; von Lützow et al., 2007; Chantigny et al., 2008). A study in the Canadian Prairies reports that rotations that include legume crops produce high amounts of exudates to signal their presence to rhizobacteria which leads to higher level of WEOC under legume crops than continuous wheat (Campbell et al., 1999).

Theoretical models divide the SOM into two or three pools. The pools, although based loosely on fractions isolated after extraction, are basically conceptual. The Century Model

created by Parton et al. (1987) is one of the most used models. This model analyzes the effects of management and global change on productivity and sustainability of agro-ecosystems simulating SOM dynamics through annual cycles over time for grassland/crop, forest or savanna. The Century Model divides the SOM into an active, an intermediate and a passive pool. The active pool consists of easily decomposable (labile) compounds, with high N turnover rates. The passive pool, where the majority of the SOM is stored, is a very stable pool physically protected in clay-humus composites. The intermediate pool is thought to be composed of slowly mineralizable compounds and chemically-resistant molecules. These conceptual pools are difficult to isolate in practice. The HF and the humic acid are considered to be recalcitrant fractions, although there are easily degradable materials in these fractions (Paustian et al., 1992; Bolinder et al., 2006). Recalcitrance is not easy to determine because degradability is affected by chemical bonds, physical protection and chemical interactions with mineral surfaces. Traditionally aromatic structured molecules were thought to be the core of recalcitrant SOM, but recent theories postulate that amide functional groups can make strong bonds with clays, generating highly recalcitrant material (Knicker and Demann, 1995; Schulten and Schnitzer, 1998; Marschner et al., 2008). Nuclear magnetic resonance spectrometry demonstrated that more than 100 N compounds are present in the SOM. Proteinaceous materials and heterocyclic N rings represent approximately 75% of the total N in soil (40% and 35% respectively). Hydrocyanic acid, acetamide and hydrazoic acid are among the most common low mass N organic compounds (Schulten and Schnitzer, 1998). Canadian studies illustrated that soil management causes changes in SOM aromaticity. Intense cultivation degrades aliphatic (lipids, fatty acids, alkanes and alkenes) molecules and causes an increase in concentration in aromatic compounds (phenols + lignin monomers, alkyl-aromatics and N-components) (Schnitzer et al., 2006).

2.3.4. Nitrogen mineralization measurement

Nitrogen mineralization (also called ammonification) is the conversion of SOM and organic forms of N to NH_4^+. Nitrogen immobilization is the inverse process, it occurs when inorganic N is incorporated into the microbial biomass or the SOM. Microbes mineralize and immobilize N simultaneously and when the decomposable substrates have low N contents (high C:N ratio) immobilization is usually higher than mineralization (Ladd and Foster, 1988). As a result, mineralization is occurring yet no net N mineralization is detected. This situation causes

agronomic problems because accurate estimates of N mineralization rates are essential for accurate N fertilizer recommendation (Bedard-Haughn et al., 2003; Portl et al., 2007). Isotopic dilution techniques allow the measurement of gross N mineralization as well as net N mineralization. The Hart et al. (1994a) isotope dilution technique is an expensive and time consuming procedure but is effective for estimating gross N mineralization (Wienhold, 2007). This technique is based on the addition of ^{15}N to label the product pool of soil NH_4^+. Gross mineralization releases unlabeled (^{14}N) NH_4^+ and so dilutes the ^{15}N enrichment of the product pools, whereas consumptive processes are assumed to remove ^{15}N and ^{14}N at equal rates from the pools (Murphy et al., 2003).

2.4. Objectives

The objective of this thesis was to determine the impact of lentil and pea on the fate of recently fixed C in soil and on N mineralization. The general hypothesis of this study was that rotation with pulse crops (lentil and pea) increases gross mineralization rates and increases the amount of C remaining in different soil fractions. Research on the C and N impacts of incorporating pulse crops in rotation with wheat will allow a better understanding of the processes that control the C sequestration and flux of CO_2. These studies will help to elaborate policies to optimize cropping systems and will serve to formulate strategies for sustainable development in the sectors of agronomy and land use.

3. Lentil and Pea Effects on Soil Nitrogen Mineralization in Two Chernozemic Soils

3.1. Abstract

Understanding factors controlling gross N mineralization will lead to the development of better fertilization strategies to enhance N fertilizer efficiency. The goal of this work was to assess the effect of pulse crops, growth stage, soil type and N fertilizer on gross N mineralization in the Canadian Prairies. Fieldwork was carried out at Agriculture and Agri-Food Canada research stations located at Scott SK. and Swift Current SK. in 2009 at seeding (May) and anthesis (July). Stable ^{15}N isotope dilution was used to quantify gross mineralization. At AAFC-Scott (Dark Brown Cz) five crop rotations were sampled: canola-wheat, pea-wheat, pea-canola-wheat, continuous wheat (without N fertilizer), continuous wheat (with N fertilizer) and three at AAFC-Swift (Brown Cz): lentil-wheat, continuous wheat (with N fertilizer), and continuous wheat (without N fertilizer). All rotations were in the wheat phase at sampling time. Additionally, lentil-wheat in the lentil phase was selected at Swift Current. Overall there were no significant differences in mean gross mineralization rates between rotations. Mean mineralization rates were higher on fertilized wheat compared to unfertilized, and significantly higher at anthesis than at seeding. On average mean mineralization rates at seeding were 1.20 and 0.65 mg NH_4^+-N kg^{-1} d^{-1} for Scott and Swift Current, respectively and 3.76 and 3.33 mg NH_4^+-N kg^{-1} d^{-1} at anthesis. The results obtained show that the mineralization rates where pulses are included in crop rotation are higher only when compared to unfertilized continuous wheat.

3.2. Introduction

Limited understanding of factors that control SON decomposition into plant accessible forms (N mineralization) limits the predictability of how much ammonium (NH_4^+) and nitrate (NO_3^-) will be released from the soil to sustain crops in time and space (Cassman et al., 2002). Walley et al. (2002) established that current soil N testing practices failed to explain more than 50% of the yield variability and as a result, over application of N fertilizers is often made to secure lucrative yields (Jackson et al., 2008). In the last decade, world synthetic N demand grew

by 17%. Currently, the amount of N fertilizer used annually is 0.65 Tg N in Saskatchewan, 14.5 Tg N in North America and 85-100 Tg N worldwide (FAO, 2008; Ministry of Government Services, 2010; Liu et al., 2010). Nitrogen fertilizer cost is linked to methane cost and fossil fuel costs. Consequently, the increase in food price during the last decade was partly due to the increase in fossil fuel value that caused a fertilizer cost increase of 172% (The Fertilizer Institute, 2011). Paradoxically, in North American agricultural systems, on average only 17% of N used as fertilizer is consumed by people and losses can be as high as 45% in some environments (Robertson and Vitousek, 2009; Liu et al., 2010). Thus, the quantity of N in terrestrial ecosystems has more than doubled in the last century due to industrial N fixation (Jackson et al., 2008). Eutrophication of water bodies, acid rain and greenhouse gas emissions are some of the side effects of N fertilizer excess (Galloway et al., 2004).

Progress in the understanding of factors that affect gross N mineralization could potentially improve fertilization strategies and enhance N fertilizer efficiency. Gross N mineralization rate is the velocity of the transformation of organic N to NH_4^+ during decomposition of organic matter before N immobilization by plants and microorganisms (Li et al., 2004). Gross N mineralization can be estimated with the ^{15}N isotope dilution technique (Hart et al., 1994b). Since the enhancement of combustion mass spectrometry, isotope dilution studies are more frequently reported in the scientific literature, yet only a few studies have been done in prairie agroecosystems (Booth et al., 2005; Robertson and Vitousek, 2009). Hence, the effect of many environmental and anthropogenic variables on N mineralization is still unclear in the Canadian Prairies. The objective of this chapter was to evaluate the effect of pulse crops, growth stage, soil type and N fertilizer on gross N mineralization in the Canadian Prairies.

3.3. Method

This study was carried out at Agriculture and Agri-Food Canada research facilities at Scott (AAFC-Scott) and Swift Current (AAFC-Swift), Saskatchewan, Canada during the 2009 growing season. Table 3.2 shows the weather data for the spring-summer 2009 and the 30 y monthly average respectively for both sites. The weather of the 2009 summer at Scott and Swift Current was in the normal range of climate variation. At AAFC-Scott five crop rotations were sampled: canola-wheat (*Brassica napus-Triticum aestivum* cv. Lillian), pea-wheat (*Pisum*

sativum-Triticum aestivum cv. Lillian), pea-canola-wheat, continuous wheat (without N fertilizer), continuous wheat (with N fertilizer) and three at AAFC-Swift: lentil-wheat (*Lens culinaris-Triticum aestivum* cv. Lillian), continuous wheat (without N fertilizer), and continuous wheat (with N fertilizer). All rotations were in the wheat phase at sampling time. Additionally, lentil-wheat in the lentil phase was selected at AAFC Swift. At AAFC-Swift wheat-lentil rotations were established in 1979 and continuous wheat in 1967. Three plots (field replicas) were taken for each crop rotation at AAFC-Swift. At AAFC-Scott the crop rotation wheat-pea, wheat-canola and wheat-pea-canola were established in 1997 and continuous wheat with N fertilizer in 2007 and without N in 2008. Four plots (field replicas) were sampled for each crop rotation at AAFC-Scott.

Table 3.1. Soil and environmental characteristics at AAFC-Scott and AAFC-Swift Current (Ayres et al., 1985).

Sites	Location	Ecoregion	Soil type†	Soil texture	$pH_{(1:2\ H2O)}$	C	D_b
				----------0-10 cm----------			
						%	g cm^{-3}
AAFC Scott	52°23'N 108.50'W	Moist Mixed Grassland	O.DBCz	loam	5.7	3.4	1.16
AAFC Swift	50°12'N 107°24'W	Mixed Grassland	O.BCZ	sandy loam	6.4	2.0	1.16

† O.DBCz, Orthic Dark Brown Chernozem; O.BCz, Orthic Brown Chernozem; D_b, bulk density.

Table 3.2. Precipitation and temperature monthly averages at Scott and Swift Current, Saskatchewan from 1 Apr. to 31 Aug. 2009 and 30 y averages (Environment Canada, 2009a; Environment Canada, 2009b).

	Apr	May	June	July	Aug.	Total	Average
Scott							
Precipitation 2009 (mm)	5.0	10.0	81.5	58.5	90.5	245.5	
Precipitation 30 y averages (mm)	23.	35.9	63.0	70.9	43.1	236.5	
Temperature 2009 (°C)	3.0	8.8	14.9	15.8	16.0		11.7
Temperature 30 y averages (°C)	3.6	10.9	15.0	17.0	16.3		12.5
Swift Current							
Precipitation 2009 (mm)	14.	19.2	30.3	44.9	57.0	166.2	
Precipitation 30 y averages (mm)	22.	49.5	66.0	52.0	39.9	229.7	
Temperature 2009 (°C)	4.0	9.9	14.5	16.9	16.7		12.4
Temperature 30 y averages (°C)	4.9	11.1	16.0	18.1	17.9		13.6

Each of the selected crop rotations was sampled twice during the 2009 growing season: immediately prior to seeding (May) and a few days before anthesis (July). In the text below the sampling made in May is referred as growth stage seeding which would be equivalent to growth stage 0 and the sampling made in July is referred as growth stage anthesis which would be equivalent to growth stage 10.5 in the Large (1954) scale. In each plot, three intact soil cores (15 cm height by 5 cm diameter) were extracted between plant rows. Any surface litter was removed before sampling in order to analyze only the mineral A horizon. Following the Davidson et al. (1991) procedures, the NH_4^+ mineralization samples consisted of three adjacent soil cores: two of these cores were labeled with $(^{15}NH_4)_2SO_4$ solution (30 µg N ml^{-1} at 98% ^{15}N) and the third was used as a blank (no injection). In the field immediately after core extraction, seven 2 mL injections were made into each of the two labeled cores with an 18-gauge side-port spinal needle (Cadence Science, Lake Success, NY). The first core of each was extracted after 15 min of incubation (T=0 reference), and the second (labeled) and third (unlabeled) core were incubated in the soil on site for 24 h before they were extracted. For each soil core, a sub-sample of soil (30-50 g) was put into a bottle with 100 mL 2*M* KCl solution. The KCl extractions were used to assess the concentration of inorganic N and to determine the amount of $^{15}NH_4^+$ in the solution according to Bedard-Haughn et al. (2006). The mineralization rate was calculated by determining the difference of the total N concentration and ^{15}N between time at zero with 24h incubation (Kirkham and Bartholomew, 1954; Hart et al., 1994b) (equation 3.1).

$$Gross\ N\ mineralization = \frac{[NH_4^+]_{t0} - [NH_4^+]_{t1}}{t1} \times \frac{log\ \frac{APE_{t0}}{APE_{t1}}}{log\ \frac{[NH_4^+]_{t0}}{[NH_4^+]_{t1}}} \quad [3.1]$$

where *t* is the time (hours), and APE is the atom % ^{15}N excess

An analysis of variance test (ANOVA) with N mineralization rates as the response variable and crop rotations, growth stage (seeding/anthesis) and sites (Brown Cz / Dark Brown

Cz) as factors was run with the statistical program R Foundation for Statistical Computing version 2.8.1 (R Development Core Team, 2008) with the function linear model (lm). As well, a 2-way ANOVA was performed with the N mineralization rate, growth stage, and continuous wheat with/without N fertilizer. Another 2-way ANOVA was run with the N mineralization rate, growth stage and phase of lentil-wheat rotation. Theoretically negative gross mineralization rates are impossible. Negative values close to zero were included in the statistical analysis but two datum with values lower than -5 (-9.57 and -8.51) were rejected because they noticeably violated the isotope dilution method assumption that $^{15}NH_4$ must not be immobilized and re-mineralized into the substrate pool (Bjarnason, 1988).

3.4. Results

The average mineralization rate at seeding was 0.96 mg NH_4^+-N kg^{-1} d^{-1} with a standard error (SE) of 0.74. The average at anthesis was 3.57 mg NH_4^+-N kg^{-1} d^{-1} with a SE of 0.79. The N mineralization rate was significantly higher at anthesis than at seeding (P=0.002) but no overall significant differences were found among crop rotations (P=0.23). The mineralization rates were slightly lower on the Brown Cz than the Dark Brown Cz but this difference was not statistically significant (P=0.49). As well, no significant interaction effect was found between the growth stage and the crops (P=0.13 to 0.71). In the continuous wheat rotations, the average N mineralization rates were higher in the soil that had received N fertilizer (P=0.07) (Fig. 3.1). On average the lentil phase of the wheat-lentil rotation had a higher N mineralization than the wheat phase (Fig. 3.2), but due to high variance in the data no statistical differences were found (P=0.36).

Table 3.3. Gross mineralization rates of the different crop rotations at seeding and anthesis.

	Mineralization†					
	Seeding			Anthesis		
	----------mg N kg^{-1} d^{-1}----------					
Brown Cz‡	n	Mean#		n	Mean	
wheat-Lentil§	3	0.68	(0.14)	3	2.2	(1.57)
Wheat-lentil	2	-0.84	(0.12)	3	1.3	(0.24)
cont. Wheat (+N)¶	2	1.32	(0.27)	3	8.7	(6.14)
cont. Wheat (-N)	2	1.46	(0.39)	2	1.0	(<0.01)
Dark Brown Cz						
pea-Wheat	4	0.98	(0.49)	4	2.5	(1.03)
pea-canola-Wheat	4	1.09	(0.18)	4	5.5	(1.83)
canola-Wheat	4	1.17	(0.36)	4	3.2	(1.81)
cont. Wheat (+ N)	4	1.82	(0.63)	3	5.4	(3.44)
cont. Wheat (- N)	4	0.96	(0.28)	4	2.0	(0.41)

Summary of ANOVA analysis between growth stage and crop ††

	df	F value	*P* value
Growth stage	1	10.40	0.002
Crop	8	1.38	0.23
Growth stage X Crop	8	0.622	0.75
Residuals	45		

Summary of ANOVA analysis between with/without N fertilizer and growth stage

	df	F value	*P* value
With/without N fertilizer	1	3.61	0.07
Growth stage	1	3.71	0.06
N fertilizer X Growth stage	1	1.84	0.18
Residuals	2		

Summary of ANOVA analysis between site comparing wheat crop only

	df	F value	*P* value
Site	1	0.62	0.43
Residuals	22		

† Gross mineralization rates were calculated with Kirkham and Bartholomew (1954) equation.
‡ Cz, Chernozem; df, degree of freedom.
§ Capital letter of the crop indicates the crop phase sampled.
¶ +N indicates with N fertilizer and –N indicates without N fertilizer.
# Mean (SE) values for each rotation.
†† ANOVAs were run with the statistical program R, with the function aov

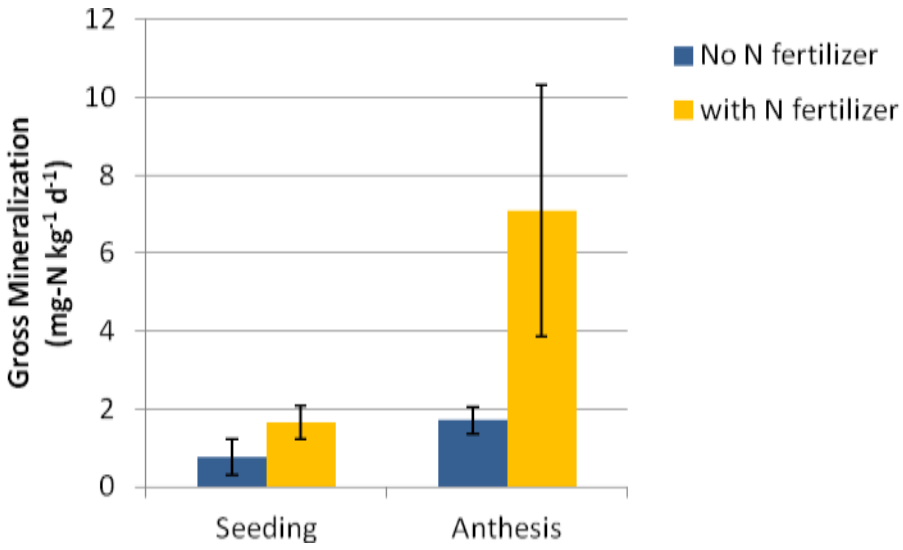

Fig. 3.1. Gross mineralization rates of rotations at seeding and anthesis (average of both sites) under continuous wheat with and without N fertilizer applied. Means values with SE.

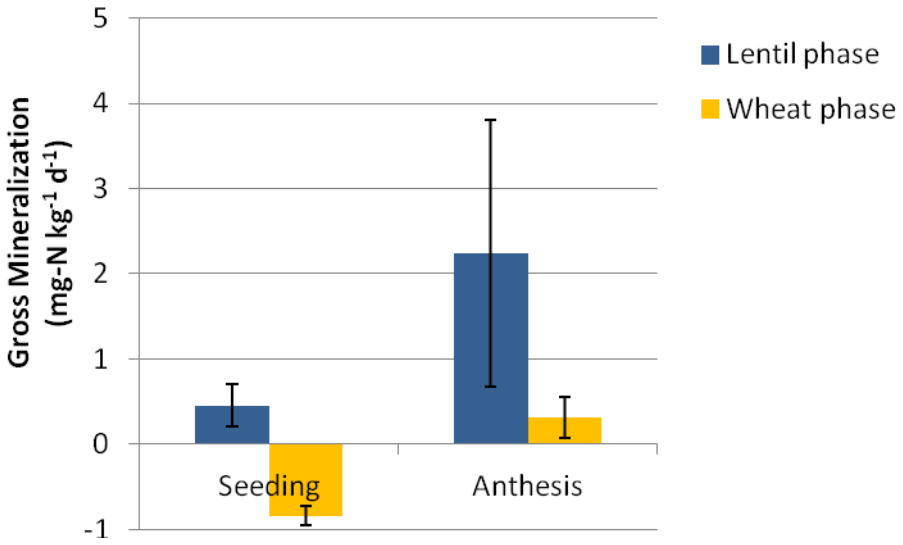

Fig. 3.2. Gross mineralization rates of rotations at seeding and anthesis in the lentil vs. wheat phases of the lentil-wheat rotation at AAFC Swift Current (Brown Cz). Means values with SE.

3.5. Discussion

Nitrogen fertilization is known to increase soil microbial activity and to accelerate SON cycling (Zahir et al., 2010; Katkar et al., 2011). Higher N mineralization rates on the plots with N fertilizer were expected and were detected. Nonetheless, more samples are needed to statistically corroborate this trend. One of the weaknesses of the Davidson et al. (1991) technique is that it does not discriminate among N mineralization from decomposition of plant residues, microorganisms, labile and recalcitrant soil fractions and N exuded from the roots. The higher N mineralization found under N fertilized fields could be due to higher N content in fertilized crop residues or could be from the decomposition of microbes that had ingested the N fertilizer.

The principal difference between the Orthic Dark Brown Cz (AAFC-Scott) and the Orthic Brown Cz (AAFC-Swift) is the percentage of C (3.4% and 2% respectively). Booth et al. (2005) reported a significant positive regression between %C and N mineralization. However, Booth et al. (2005) meta-analysis compared more than 100 samples with %C from ~0 to >10% and a noteworthy variation was present in this regression. Despite not statistically significant, analogically to Booth et al. (2005) this study found a trend of higher N mineralization rates on the Dark Brown Cz than the Brown Cz.

Due to their symbiosis with rhizobium, pulse crops are importing new nitrogen into the soil system and activating soil microbial activity (Sayyed et al., 2011). Consequently, it was thought that crop rotation including pulses would produce higher N mineralization rates; however no severe effect of pulse crop on N mineralization was found. Overall, crop rotations including pulses had no significant difference in N mineralization compared to continuous wheat with fertilizer but pulse-wheat rotations were higher than continuous wheat without N fertilizer on average. More studies are required to elucidate this effect statistically.

Comparing crop rotations with canola, pea and wheat, Sangster (2010) did not find significant differences among crops and suggested that the effect of pulse crops on N mineralization may translate into an increase in the mineralization under the following crop in rotation. In this study the crops where in wheat phase following a pulse, oilseed or wheat phase but no effect of the previous phase was detected.

The significantly higher mineralization rates at anthesis could be due to warmer soil temperatures, which would lead to greater microbial activity (Madigan and Martinko, 2006). It could also be due to higher root exudates production during anthesis. Ofosu-Budu et al. (1990) found that large quantities of easily mineralizable molecules (with high amounts of N) were lost from the root at the end of flowering in legume crops. As well, in the Northern Great Plains mid-summer when microorganisms have exhausted polysaccharides from the crop residues of the previous growing season; when microorganisms die out, high amounts of N-rich molecules are liberated (Brady and Weil, 2008).

3.6. Conclusion

The factors controlling N mineralization in agricultural soils are inadequately understood and this limits our accuracy in applying supplemental fertilizer for crop production. A large number of variables affect N mineralization; here I evaluated five: crop rotations, two moments during the growth season, two Chernozomic soils, presence/absence of N fertilizer on continuous wheat and the phase of wheat-lentil crop rotation. The main finding of this study was a higher N mineralization rate at anthesis compared to seeding. The results also show that the positive effect of pulse crop on N mineralization rates is detectable only when comparing pulse-wheat rotation with unfertilized continuous wheat crop. Finally, we believe that many more statistically significant relationships will be found when integrating these results with the other studies made at these sites. As well, at the global scale, the amassing of single studies on SON dynamic will one day lead to precise models on soil N cycling and maximize fertilizer use efficiency.

4. Following the Fate of ^{13}C-Labeled Lentil, Wheat, Canola, and Pea in Two Chernozemic Soils

4.1. Abstract

Restoring C into the soil is fundamental to improve soil fertility, increase agronomic productivity, mitigate greenhouse gas emissions and enhance several ecosystem services. The objectives of this study were to 1) determine the quantity and the fate of C captured during a single growing season of four simple crop rotations in the Prairies, 2) evaluate the quantity and fate of those original residues after a second growing season and 3) to analyze the relationship between the plant and soil C:N ratio and the fate of the newly derived C. To track the progress of C into the different SOM pools, plants were grown in intact soil cores and pulse labeled with ^{13}C in airtight chambers. On a g of C per g of SOC basis, at the end of the second growing season (approximately one year after the ^{13}C enrichment), lentil had the highest ^{13}C-derived SOC but no statistical differences were found among pea, canola and wheat. However, on a g of C per ha basis at the end of the second growing season, on Swift Current soil (Brown Cz) lentil was significantly higher than wheat and on Scott soil (Dark Brown Cz) pea was higher than wheat but statistically equal to canola. At the end of the second growing season, Swift lentil, Swift wheat, Scott canola, Scott pea and Scott wheat had 403, 262, 281, 276, and 143 g of root-derived C per ha, respectively. These same crops had 423, 281, 309, 259 and 207 g of shoot-derived C per ha, respectively. The fresh plant residues quickly decomposed into the soil, yet after one year a high proportion of the labeled C was still in the VLF and LF. Lentil and pea are more resistant to further decomposition than wheat but long-term studies with isotopic C residues are required to determine if the observed trends are transient or permanent.

4.2. Introduction

Humus, a term that dates back to the ancient Roman times, was originally used to describe soil color and soil fertility (Waksman, 1936). Humus has also been referred to as organic matter that has remained in soil for a relatively long period of time and has achieved a high level of stability (Whitehead and Tinsley, 1963). Recently, in scientific literature, the term humus has been replaced by SOM, which better describes its nature and functions (Sparks, 2001). However, SOM is a very complex substance and its genesis and composition are still not fully understood. Particularly, there are many uncertainties about the rates of SOC accumulation/mineralization in agricultural fields. Carbon is the main constituent of SOM (52-58%) (Sparks, 2003) and the total global SOC is estimated as 2,000,000 Tg (VandenBygaart and Angers, 2006). Therefore, a society concerned about rising levels of GHGs in the atmosphere needs to have accurate data on how long it takes for fresh plant residues to achieve a high level of stability in soil. Since agriculture began in Canada, 15 to 30% of the C originally present in the A horizon has been lost, estimated as 1.1Pg of C (Hengeveld, 2008). Long-term studies are effective to determine which agricultural practices can enhance or decrease the amount of SOC (Lemke et al., 2010a). However, there is a gap in the scientific literature about annual gross production of SOC under different agricultural practices. This lack of knowledge is mainly due to the fact that the new C annually fixed into the soil represents only a very small proportion of the total SOC.

Recent developments in stable isotope techniques in soil science facilitate the tracking of SOC through different SOM pools and through time (Sangster et al., 2010). These new isotopic techniques can also be used to quantify root biomass production (Subedi et al., 2006). Traditional methods to estimate root production cannot account for microscopic root fragments and rhizodeposits that are quickly mineralized into the soil. Without accurate estimation of belowground C production, accurate C flow models are troublesome to produce. Consequently, it is arduous to determine the impact of different agricultural practices on SOM status and dynamics. The removal of crop straw for biofuel and pulp paper production, which has been increasing notably since the beginning of the twenty-first century, is a practice that needs to be evaluated for impacts on SOM (Timilsina and Mevel, 2011). To assess the effect of straw

removal on SOC dynamics, accurate data of the relative contribution of roots and shoots to total SOC are required (Lal, 2005).

During the past few years several scientific questions have been raised related to SOC dynamics and the inclusion of pulse crops in rotation with wheat. Some of these are: Could the inclusion of pulse crop in rotation with wheat increase the annual gross production of SOC? Into which soil pool the new organic C going? What is the relative contribution of roots and shoots to the total SOC? And what is the relationship between the fate of the labeled C and the C:N ratio of the different SOM pools? To address these questions, the objectives of this study were to: 1) determine the quantity and the fate of C captured during a single growing season of four simple crop rotations in the Prairies (continuous wheat {*Triticum aestivum*}, wheat-canola {*T. aestivum* - *Brassica napus*}, wheat-lentil {*T. aestivum* - *Lens culinaris*}, and wheat-pea {*T. aestivum* - *Pisum sativum*}), 2) evaluate the quantity and fate of those same residues after a second growing season and 3) analyze the relationship between the plant and soil C:N ratio and the fate of the newly produced C.

4.3. Method

4.3.1. Soil collection

This greenhouse study was carried out with 12.5L intact soil cores from Agriculture and Agri-Food Canada research facilities at Scott (AAFC-Scott) and at Swift Current (AAFC-Swift) Saskatchewan, Canada (Table 4.1). Three crop rotations were selected at AAFC-Scott (continuous wheat, pea-wheat and wheat-canola) and two at AAFC-Swift (continuous wheat and lentil-wheat). All rotations had completed a wheat phase of the rotation the previous year. In the field, three plots were selected for each crop rotation (except canola which has four) and six soil cores were collected from each plot (total ninety-six soil cores). Aluminum cylinders (20 cm diameter by 39 cm deep) were pushed into the soil using a truck mounted hydraulic punch (Stumborg et al., 2007) and carefully withdrawn to preserve soil structure. Prior to core extraction wheat plots had been fertilized with 15.2 kg P ha^{-1} (11-52-0) and 74.4 kg N ha^{-1} (46-0-0). Canola plots were also fertilized with 15.2 kg P ha^{-1} (11-52-0) and 88 kg N ha^{-1} (46-0-0).

The plots at AAFC-Scott are managed in conservation tillage and the plots at AAFC-Swift in conventional tillage. In the field, parts of the crop residues were removed from the soil surface to facilitate core extraction. The cores were stored at 4°C until seeding.

Table 4.1. Soil and environmental features at AAFC-Scott and AAFC Swift-Current (Ayres et al., 1985).

Sites	Location	Ecoregion	Soil type†	Soil texture	$pH_{(1:2\ H2O)}$	C	D_b
				----------0-10 cm----------			
						%	g cm^{-3}
AAFC Scott	52°23'N 108.50'W	Moist Mixed Grassland	O.DBCz	loam	5.7	3.4	1.16
AAFC Swift	50°12'N 107°24'W	Mixed Grassland	O.BCz	sandy loam	6.4	2.0	1.16

† O.DBCz, Orthic Dark Brown Chernozem; O.BCz, Orthic Brown Chernozem; D_b, bulk density.

4.3.2. Experimental design

In July 2009, pea, lentil, canola and wheat (from Scott and Swift) were germinated and grown under a photoperiod of 18 h at an average temperature of 22°C in the College of Agriculture and Bioresources Greenhouse (University of Saskatchewan). Each crop was seeded in its respective soil cores according to rotation. Four plants were grown to maturity in each soil core, approximating seeding rates in the field (density of 125 plants / m^2). Lentil and pea were inoculated with a granular *Rhizobium leguminosarum* inoculant, 'Nodulator', (Becker Underwood Inc., Saskatoon, SK). The inoculant was banded with the seed in the soil cores at the equivalent recommended rate of 8 kg ha^{-1}. Plants were abundantly watered every second day. The soil cores were arranged into six rows. Each row had three cores of Swift lentil, Swift wheat, Scott pea and Scott wheat and four cores of Scott canola (corresponding to field plots). Rows #1, 2, 3, 4 were labeled with $^{13}CO_2$ and rows #5, 6 were not labeled and were kept in a separate room of the greenhouse to prevent ^{13}C contamination (Fig. 4.1). The official climatic data from the greenhouse reported no difference between both rooms throughout the experiment. To ensure homogeneous plant illumination, blocks of soil cores were rotated on the greenhouse bench every week.

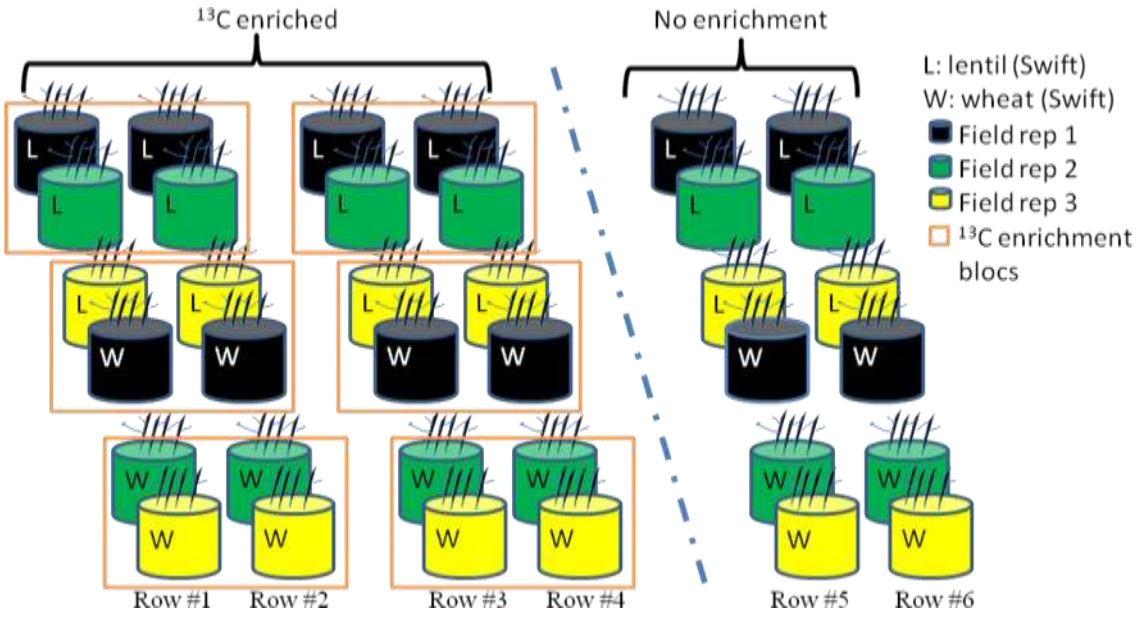

Fig. 4.1. Scheme of the experimental design in the greenhouse during the first growing season. Only cores from Swift Current (Brown Cz) are shown here. The same design was used with Scott cores (Dark Brown Cz) and the labeled cores from both sites were labeled together. The labeling was performed in blocks of four cores.

4.3.3. Chamber specifications and labeling procedure and plant and soil analysis

Following Sangster et al. (2010), labeling was accomplished, in blocks of four cores, in hermetic polymethyl methacrylate chambers (Fig. 4.2). Each block was pulse-labeled weekly for 2 h starting 20 days after germination and continuing to the end of embryogenesis for a total of eight labeling sessions. The soil surface was isolated from the enriched atmosphere during labeling by covering it and making a seal around plant stems with GLAD Press'n Seal Freezer® wrap (The Clorox Company, Oakland, CA). During the labelling sessions, in the chambers, the total CO_2 concentration was maintained around the current atmospheric concentration (380-430 ppm) and the atmospheric enrichment was 33 atom% $^{13}CO_2$. The CO_2 was devolved into the chamber by injecting a saturated solution of ^{13}C-enriched $NaHCO_3$ (33% atom ^{13}C) into a beaker

with 12*M* HCl. Total CO_2 concentration was monitored with an infrared gas analyzer (IRGA) (S151 Infrared CO_2 Analyzer, Qubit Systems, Kingston, ON).

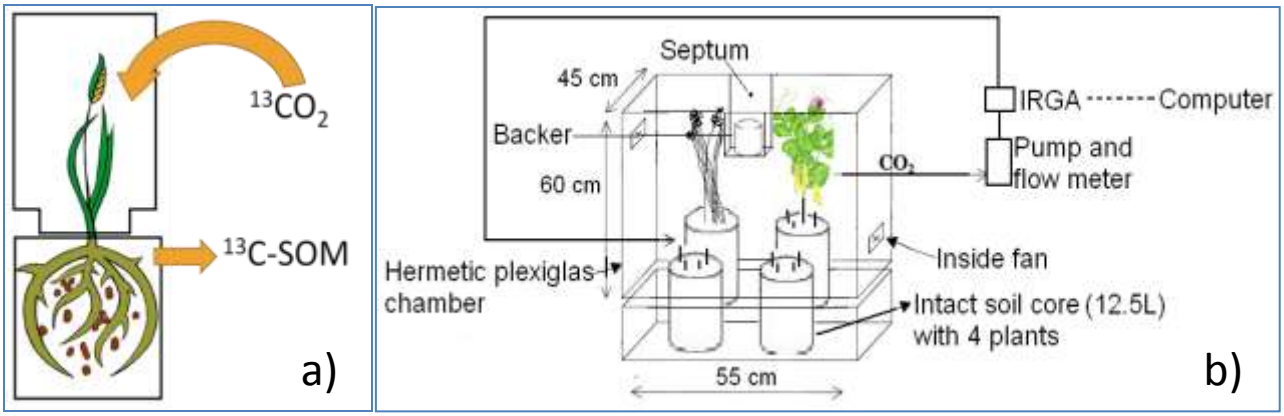

Fig. 4.2. a) Conceptual diagram of labeling and tracing process. b) Chamber design and set up for $^{13}CO_2$ atmospheric pulse labeling.

At maturity, plants were air-dried in their soil cores and harvested at ground level. Dry weight of straw and grain were determined. The cores in rows #2, 4 and 6 were removed from the greenhouse (for analyses) and cores row #1, 3 and 5 were kept for a second growth season. Shoot residues (leaves, stems and pod/husks) from all harvested cores were ground (<0.5 mm^3) with a coffee grinder. Enriched ground plant residues from row #1 were thoroughly mixed with a spade with the 0-10 cm soil in the core in which they grew. Enriched ground plant residues from row #3 were mixed with the 0-10 cm soil of their analogical non-enriched core in row #5. Non-enriched ground plant residues from row #5 were mixed with the 0-10 cm soil of their analogical enriched core in row #3 (Fig. 4.3). Since canola produced more shoot residues than the other crops, more residues were put into the soil for canola cores in rows #1, 3 and 5 (15 g canola vs. 10 g other crops, per soil core). The standard amount of 10 g of crop shoot to be put into the soil cores for lentil, pea and wheat was chosen because this was the minimum amount produced in some cores, 15 g was chosen for canola because canola produced notably more residues than the other crops and 15 g was the minimum produced by canola.

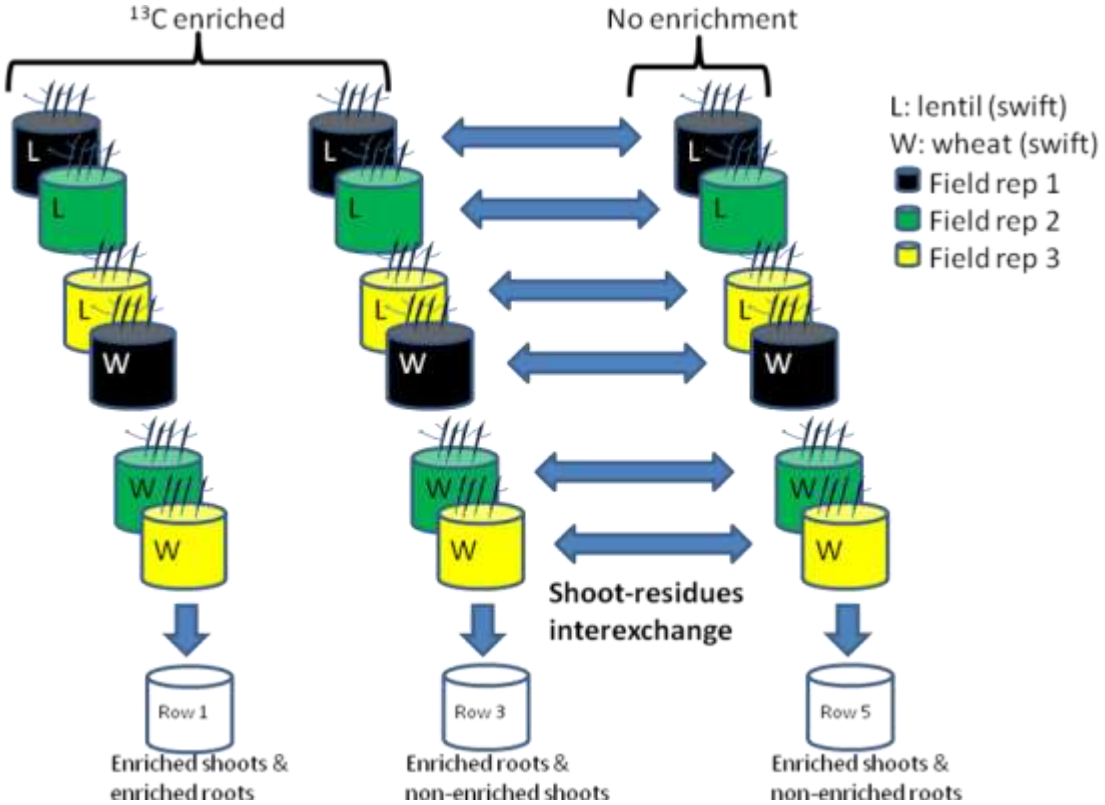

Fig. 4.3. Scheme of the experimental design in the greenhouse during the second growing season. Only cores from Swift current (Brown Cz) are show here. The same design was used with Scott cores (Dark Brown Cz).

At the end of the first growing season, samples of roots, stems, leaves, pods/husks and grains of lentil, canola, pea and wheat were oven dried at 50°C, finely ground and analyzed for %C, %N, C:N ratio and δ ^{13}C with a Costech Elemental Combustion System (Costech Analytical 191 Technologies, Inc.) coupled to a Delta V Advantage Mass Spectrometer (Thermo Fisher 192 Scientific Inc.) in the Stable Isotope Facility at the University of Saskatchewan. Also at the end of the first growing season, the 0-10cm layer of soil cores from rows #2, 4 and 6 were cross-sectioned. In each core, root samples were collected for %C and %N determination. The soil-root matrix was air dried, manually ground with a marble kitchen rolling pin, sieved at 2 mm, and thoroughly mixed. With the soil samples, following Gregorich (2007) light fraction (LF) and heavy fraction (HF) organic matter were isolated with a dense liquid (NaI) at 1.7 g mL^{-1}. Then, with deionized water the light fraction was fractionated into very light (VLF) and LF. The material that was floating on water (VLF) was mainly fresh plant residue. Very few plant

fragments were observed in the LF and none in the HF. The HF was sub-fractionated into fulvic acids (FA), humic acids (HA), humin clay (HuC) and humin sand (HuS) following Anderson and Schoenau (2008). From the bulk soil the water extractable organic matter (WEOM) was also extracted following Chantigny et al. (2008). For each soil fraction, %C, %N and $\delta^{13}C$ was analyzed with the same system as for the plant materials. Natural abundance of ^{13}C (references used in equations 4.1-4.5) in soil and plant fractions was determined from the row #6 cores. With the $\delta^{13}C$ results, the mg of derived C per g of SOC and per ha for each soil fraction was calculated adapting the Subedi et al. (2006) equation.

The proportion of SOC for each soil fraction (A) was calculated as:

$$A = \frac{\%SOC}{100} \quad [4.1]$$

The % ^{13}C of SOC for each soil fraction (B) was calculated as:

$$B = \frac{\delta_{enriched\ soil\ fraction} - \delta_{reference\ soil\ fraction}}{\delta_{enriched\ roots\ and\ /\ or\ shoots} - \delta_{reference\ roots\ and\ /\ or\ shoots}} \times 100 \quad [4.2]$$

The g of derived C per g of SOC for each fraction was calculated by:

$$C = (A \times B)/100 \quad [4.3]$$

The total SOC content per ha (0-10 cm) for each soil fraction (D) was calculated as:

$$D = weight\ of\ soil\ (0-10\ cm)\ per\ ha\ (Kg) \times \frac{\%SOC}{100} \times \frac{\%soil\ fraction}{100} \quad [4.4]$$

The derived C for each fraction was calculated by:

$$F = (D \times B)/100 \quad [4.5]$$

The root biomass in each soil fraction was calculated:

$$G = \frac{F \times 100}{mean\ \%\ C\ in\ root\ samples} \quad [4.6]$$

To simulate natural conditions, in November 2009 cores from row #1, 3 and 5 were stored outside the greenhouse and allowed to freeze. Cores were returned to the greenhouse in March 2010 and seeded with wheat (four plants per pot) in April. Prior to seeding, cores were fertilized with 15.2 kg P ha^{-1} (11-52-0) and 74.4 kg N ha^{-1} (46-0-0). At the end of the second growing season, soil from cores row #1, 3 and 5 were processed as outlined above.
The outcome of this lab experiment was:

- the derived soil carbon input from the root after 1st growing season,
- the derived soil carbon remaining after the 2nd growing season from the root,
- the derived soil carbon remaining after the 2nd growing season from the shoot,
- the derived soil carbon remaining after 2nd growing season from the root and shoot.

The decay % of derived C per ha was calculated by dividing kg of C remaining at the end of the second growing season by the kg of C remaining at the end of the first growing season.

4.3.4. Statistical Analysis

Data were checked for normality with the Shapiro-Wilk normality test. A one-way ANOVA was run to detect differences among enrichment blocks and 2-way ANOVAs were performed to detect differences in enrichment of the plant parts and crop rotations. As well, 2-way ANOVAs were run to find differences among crop rotations and soil fractions for both growing seasons. Ultimately, a factorial ANOVA with derived C per ha had a response variable and crop rotations, soil fractions and plant material enriched (roots, shoot and root + shoots) as factors was performed. Post hoc tests with the function *Pairwise T-Test* were made when the ANOVAs were detecting significant differences. Mathematical calculations and descriptive statistical analyses were made with Microsoft Excel XP®. Statistical testing was made using the statistical program R Foundation for Statistical Computing version 2.8.1 (R Development Core Team, 2008), effects were confirmed significant at $P < 0.05$.

4.4. Results

4.4.1. First growing season: $\delta^{13}C$, C:N ratios and SOM fractions

To generate assessment of derived C produced during the first growing season, an evaluation of the effectiveness of the ^{13}C enrichment technique employed was made. The labeling method was effective for enriching plants in ^{13}C and the crops that were repeat-pulse labeled had a significantly higher level of ^{13}C compared to the natural abundance plants ($P<0.01$) (Table 4.2). No statistical differences in ^{13}C enrichment were found among crops ($P=0.12$). Comparing ^{13}C enrichment among plant parts, no overall difference was found among stems, leaves and pods/husks ($P>0.05$). However, the root had significantly lower ^{13}C enrichment than the stems, leaves and pods/husks ($P<0.01$). $\delta^{13}C$ enrichment is used in equations 4.2 and 4.5.

Table 4.2. $\delta^{13}C$ of enriched and natural abundance of lentil, wheat, canola and pea plants at maturity.

	Stems	Leaves	Pods/husks	Roots
	$\delta^{13}C$ Enrichment (‰)			
Brown Cz†				
Lentil	463.2 (70.5)‡	507.4 (40.9)	611.1 (94.0)	314.1 (71.7)
Wheat	423.7 (188.7)	534.1 (148.5)	704.1 (13.9)	388.2 (80.9)
Dark Brown Cz				
Canola	466.1 (122.1)	502.2 (131.7)	530.3 (75.7)	294.1 (61.2)
Pea	483.2 (112.8)	370.3 (103.4)	568.9 (62.6)	203.7 (53.0)
Wheat	575.5 (39.1)	344.7 (25.0)	565.3 (19.4)	316.7 (120.2)
Natural abundance	-30.21 (0.85)	-31.78 (1.29)	-30.04 (2.13)	-29.25 (1.37)

Summary of ANOVA analysis between plant fraction and crop§

	df	F value	P value
Plant fraction	3	33.29	<0.01
Crop	4	3.98	0.12
Plant fraction X Crop	12	1.05	0.40
Residuals	86		

† Cz, Chernozem; df, degree of freedom.

‡ Mean values and (SE).

§ ANOVA was run with the statistical program R, with the function aov

Comparing the %C, %N and C:N ratio of the plant parts the highest % N was in the grain and the C:N ratio was lower in the grain (Table 4.3). Except for the grain, the pulse crops (lentil and pea) had lower C:N ratios than the other crops among all the plant parts. The Scott wheat had a C:N ratio in the straw notably lower than the Swift wheat. The roots and the shoots (stems, leaves and pods/husks) had %N and C:N values in the same range. The percentage of C in roots and shoots are used in equation 4.4.

The majority of the SOM was located in the HF, and the LF was higher in proportion of SOM than the VLF. The WEOM represented only approximately <0.01% of the bulk soil (Table 4.4). The bulk soil percentage of the different soil fraction was used in equation 4.4.

The C:N ratio of plant residues was lower for pea and lentil, but the soil C:N under the different crops were all in the same range at the end of the first growing season (Table 4.5). The VLF had higher %C, %N and C:N ratio than all other soil fractions. In turn, the LF had a %C, %N and C:N ratio higher than the HF. The WEOM had the lowest %C and the lowest C:N ratio. The FA, HA, HuC and HuS were fractionated from the soil HF; except for the HuS all sub-fractions had a considerably higher C:N ratio than the HF. The previous is due to the fact that on mass basis the HuS was the dominant subfraction of the HF and had a C:N ratio slightly lower than the HF which let the other subfraction to attain C:N ratio higher than the HF. The FA had the lowest %C and highest C:N ratio. The HA had the highest %C and %N (Table 4.6). The percentages of C of the different soil fractions are used in the equation 4.4. No statistical testing were performed with the %C, %N and C:N because these data were aim to explain the dynamic of the remaining derived C and the overall dynamic of the SOM only.

Table 4.3. Percentage of C, N and C:N ratio in the stems, leaves, pods/husks, roots and grains of lentil, wheat, canola and pea and the end of the 1st growing season.

	Straw			Roots	Grains
	Stems	Leaves	Pods/husks		
Brown Cz†					
Lentil	42.7/1.4, [31.2]‡	40.2/2.8, [14.2]	41.0/1.6, [25.7]	40.9/1.6, [24.8]	41.9/4.4, [9.5]
Wheat	43.0/0.5, [78.6]	37.8/0.9, [41.1]	39.1/0.9, [45.5]	41.5/1.4, [29.7]	41.6/2.8, [14.7]
Dark Brown Cz					
Canola	40.2/0.9, [45.0]	37.4/0.8, [46.4]	39.8/1.0, [39.1]	42.6/0.8, [53.7]	53.6/5.2, [10.3]
Pea	42.8/1.5, [29.4]	37.0/2.1, [17.3]	40.9/1.5, [27.8]	39.7/1.8, [22.0]	40.0/3.3, [12.1]
Wheat	42.4/1.3, [32.0]	37.3/1.3, [28.0]	40.0/1.4, [27.9]	42.3/1.3, [32.1]	39.6/3.0, [13.4]

† Cz, Chernozem.
‡ %C / %N [C:N].

Table 4.4. Percentage of the different soil fractions (on mass basis) at the end of the 1st growing season.

	WEOM†	VLF	LF	HF
	% of the bulk soil 1st growing season			
Brown Cz				
Lentil	0.01	1.5	6.1	92.9
Wheat	0.01	1.4	6.6	92.0
Dark Brown Cz				
Canola	0.01	0.8	6.6	92.8
Pea	0.01	1.0	6.2	93.6
Wheat	0.01	1.2	6.2	92.8

† WEOM, water extractable organic matter; LF, light fraction; VLF: very light fraction; HF, heavy fraction; Cz, chernozem.

end of the 1st growing season.

	WEOM†	VLF	LF	HF	Control bulk soil
	%C/%N [C:N] 1st growing season				
Brown Cz					
Lentil	1.7/0.72, [2.3] ‡	24.0/1.47, [16.3]	7.6/0.69, [11.0]	2.0/0.21, [9.9]	2.01/0.19, [10.4]
Wheat	1.6/0.53, [3.1]	21.1/1.19, [17.8]	7.5/0.61, [12.3]	1.9/0.19, [10.4]	2.46/0.23, [10.8]
Dark Brown Cz					
Canola	1.6/0.82, [1.9]	24.4/1.50, [16.3]	7.9/0.73, [10.8]	2.3/0.23, [10.0]	2.67/0.25, [10.6]
Pea	1.4/0.73, [2.0]	24.0/1.39, [17.3]	7.2/0.46, [15.6]	2.3/0.22, [10.3]	2.69/0.26, [10.5]
Wheat	1.7/0.60, [2.9]	22.3/1.44, [15.5]	5.9/0.57, [10.3]	2.7/0.25, [10.6]	2.94/0.27, [10.7]

† WEOM, water extractable organic matter; LF, light fraction; VLF: very light fraction; HF, heavy fraction; Cz, chernozem.
‡ %C / %N [C:N].

Table 4.6. Percentage of C, N and C:N in the FA, HA, HuC and HuS of lentil, wheat, canola and pea at the end of the 1st growing season and % of the subfractions in the HF

	FA†	HA	HuC	HuS	FA	HA	HuC	HuS
	%C/%N [C:N] 1st growing season				% of the HF			
Brown Cz								
Lentil	2.1/0.11, [18.0]‡	28.3/1.78, [15.9]	3.1/0.22, [14.2]	0.3/0.03, [9.6]	22.6	1.6	21.7	54.0
Wheat	2.1/0.06, [35.3]	31.3/2.33, [13.4]	3.2/0.26, [12.3]	0.2/0.03, [8.5]	25.0	1.3	17.3	56.4
Dark Brown Cz								
Canola	2.2/0.09, [24.6]	29.8/1.56, [19.1]	3.3/0.28, [11.9]	0.2/0.02, [10.4]	23.4	2.3	16.5	57.8
Pea	2.0/0.08, [26.2]	43.1/2.48, [17.4]	2.6/0.25, [10.7]	0.2/0.02, [9.8]	21.1	1.9	16.4	60.7
Wheat	2.1/0.08, [26.4]	28.8/2.08, [13.8]	3.3/0.20, [16.4]	0.3/0.03, [10.3]	21.3	2.3	18.2	58.2

† FA, Fulvic acid; HA, Humic acid; HuC, Humin clay; HuS, Humin sand; Cz, Chernozem.
‡ %C / %N [C:N].

4.4.2. First growing season: Fate of ^{13}C-labeled plant material

The proportion of root-derived C remaining in the SOC at the end of the first growing season is shown in Table 4.7. The VLF had the highest derived mg of C per g of SOC (P<0.01) but no significant differences were found among WEOM, LF and HF. The WEOM, VLF, LF and HF had average mg of derived C per g of SOC of 1.2, 18.0, 2.2, and 0.2 respectively. Comparing crops, the VLF of Swift lentil had a significantly higher mg of derived C per g of SOC than the VLF of the other crops (P<0.01). Comparing the proportional C remaining in the SOC from the roots at the end of the first growing season, the HA was significantly higher than the FA, HuC and HuS. On the other hand, the HuS was significantly lower than the HA, FA and HuC (Table 4.8). The FA, HA, HuC and HuS were subfrations of the HF and on mass basis the HuS was the dominant subfraction which let the HA and HuC reach amounts of derived C per g of SOC notably higher than the HF. Overall, no significant differences were found among crops (Table 4.8).

Table 4.7. Root-derived C remaining in the SOC at the end of the 1st growing season in the WEOM, LF, VLF and HF.

	WEOM†	VLF	LF	HF
	---------- mg of derived C per g of SOC ----------			
Brown Cz				
Lentil	1.78 (0.26)‡	28.45 (5.06)	3.20 (1.45)	0.23 (0.04)
Wheat	1.22 (0.35)	15.69 (4.60)	2.37 (0.83)	0.21 (0.03)
Dark Brown Cz				
Canola	0.99 (0.20)	14.87 (2.29)	2.71 (0.46)	0.23 (0.02)
Pea	1.22 (0.12)	11.53 (2.42)	2.04 (0.95)	0.16 (0.02)
Wheat	1.11 (0.37)	19.51 (4.52)	0.87 (0.16)	0.15 (0.01)

Summary of ANOVA analysis between soil fraction and crop§

	df	F value	P value
Soil fraction	3	76.97	<0.01
Crop	4	3.86	0.01
Soil fraction X Crop	12	2.21	0.01
Residuals	90		

† WEOM, water extractable organic matter; VLF: very light fraction; LF, light fraction; HF, heavy fraction; Cz, chernozem; df, degree of freedom.

‡ Mean values and (SE).

§ ANOVA was run with the statistical program R, with the function aov

Table 4.8. Root-derived C remaining in the SOC at the end of the 1st growing season in the FA, HA, HuC and HuS.

	FA†	HA	HuC	HuS
	---------- mg of derived C per g of SOC ----------			
Brown Cz				
Lentil	0.35 (0.13)‡	2.27 (0.35)	0.49 (0.06)	0.06 (0.02)
Wheat	0.35 (0.14)	2.66 (0.38)	0.48 (0.04)	0.05 (<0.01)
Dark Brown Cz				
Canola	0.38 (0.13)	1.81 (0.20)	0.50 (0.04)	0.05 (<0.01)
Pea	0.50 (0.21)	1.33 (0.30)	0.49 (0.12)	0.05 (0.01)
Wheat	0.40 (0.20)	1.04 (0.26)	0.34 (0.03)	0.04 (0.01)

Summary of ANOVA analysis between soil fraction and crop§

	df	F value	*P* value
Soil fraction	3	3.46	0.75
Crop	4	74.20	<0.01
Soil fraction X Crop	12	2.72	<0.01
Residuals	83		

† FA, Fulvic acid; HA, Humic acid; HuC, Humin clay; HuS, Humin sand; Cz, Chernozem; df, degree of freedom.

‡ Mean values and (SE).

§ ANOVA was run with the statistical program R, with the function aov

The derived below-ground biomass of each soil fraction of lentil, wheat, canola and pea was compared with the above ground biomass (Table 4.9). The total root biomass production was calculated as the sum of root biomass in each soil fraction. Multiplying the total root biomass per core per 322,000 (1 ha = 322,000 cores) an estimation of root biomass per ha (0-10 cm) was produced. Lentil, canola, pea and wheat had the following value of root biomass: 2405, 1423, 1172 and 1391 kg ha^{-1} respectively. Grain production ranged from 9.48 g $core^{-1}$ (lentil) to 19.23 g $core^{-1}$ (pea). Wheat on Scott soil (Dark Brown Cz) and Swift Current soil (Brown Cz) had similar grain production (11.74 to 13.68 g $core^{-1}$). No significant differences were found in straw production (stem + leaves + pods/husks) among lentil, wheat and pea. The shoot:root ratio was calculated in the 0-10cm layer of soil only. Canola produced three times more straw residues than the other crops and the shoot:root ratio had significantly higher for canola (P<0.01). On the other hand, lentil had root biomass production (0-10 cm) significantly higher than the other crops and had the lowest shoot:root ratio (P<0.01). Root biomass production was higher on the Brown Cz (lentil and wheat) than on the Dark Brown Cz (pea and wheat) (P<0.01) (Table 4.9).

Table 4.9. Above-ground and below-ground biomass (0-10 cm) of lentil, wheat canola and pea in the soil cores at the end of the 1st growing season.

	Grain†	Straw	Root biomass				Shoot:root ratio
			VLF‡	LF	HF	Total	
			g core^{-1}				
Brown Cz							
Lentil	9.48 (0.79)§	18.95 (1.17)	3.83 (0.68)	1.74 (0.79)	1.90 (0.37)	7.47A¶§	2.54c
Wheat	11.74 (0.90)	16.65 (1.17)	1.92 (0.56)	1.37 (0.48)	1.74 (0.23)	5.03A	3.31bc
Dark Brown Cz							
Canola	13.68 (0.91)	58.65 (2.96)	1.01 (0.16)	1.55 (0.26)	1.86 (0.14)	4.42A	13.27a
Pea	19.23 (1.20)	16.63 (0.98)	1.09 (0.23)	1.16 (0.54)	1.39 (0.17)	3.64B	4.57b
Wheat	12.67 (0.63)	18.52 (0.92)	1.96 (0.45)	0.47 (0.09)	1.18 (0.10)	3.61B	5.13b

† Biomass weight of grain and straw were calculated with air dried samples.

‡ VLF, very light fraction; LF, light fraction; HF, heavy fraction; Cz, Chernozem.

§ Mean values (SE).

¶ Numbers followed by different letters indicates significant difference among crops at $P< 0.05$ by the post hoc *pairwise.t.test* in The R Foundation for Statistical Computing. Capital letters are used for differences in the total root biomass and lowercase letters for shoot:root ratio.

No statistics are available for the root biomass in the different soil fraction because the values are derived from carbon mass data presented in Fig. 4.7.

§ ANOVA was run with the statistical program R, with the function aov

The relative C allocation coefficient in grain, straw, VLF, LF and HF was calculated as the amount of C in grain, straw and root divided by the total C mass (Table 4.10). Total below ground allocation was calculated as the sum of the relative C allocation coefficients in soil fraction (VLF, LF and HF). The straw had the highest relative C allocation coefficients (ranged between 0.579 for pea to 0.783 for canola), followed by the grain (ranged between 0.141 for canola to 0.307 for pea), and lowest for the total below ground allocation (ranged between 0.076 for canola and 0.194 for lentil) (Table 4.10).

Table 4.10. Relative plant C allocation for lentil, wheat canola and pea at AAFC-Swift Current (Brown Cz) and AAFC-Scott (Dark Brown Cz) at the end of the 1st growing season.

	Relative C allocation coefficient at the harvest†					Total allocation	
	Grain‡	Straw	VLF§	LF	HF	Above ground	Below ground
Brown Cz							
Lentil	0.196	0.610	0.081	0.013	0.100	0.806	0.194
Wheat	0.248	0.613	0.040	0.013	0.086	0.861	0.139
Dark Brown Cz							
Canola	0.141	0.783	0.007	0.010	0.059	0.924	0.076
Pea	0.307	0.579	0.016	0.007	0.091	0.886	0.114
Wheat	0.245	0.608	0.045	0.006	0.095	0.854	0.146

† The sum of the coefficients without total below ground = 1; very light, light, heavy fraction were measured in the 0–10 cm depth. No statistics were made because the values are derived from plant biomass data presented in Table 4.4.
‡ Biomass weight of grain and straw were calculated with air dried samples.
§ VLF, very light fraction; LF, light fraction; HF, heavy fraction; Cz, Chernozem.

4.4.3. Second growing season: Soil C:N ratios and SOM fractions

Assessment of the weight of the different soil fractions at the end of the second growing season showed similar results as those in the first growing season, the majority of the SOM was located in the HF and that the LF has a higher proportion than the VLF. However, at the end of the second growing season the LF was higher than at the end of the first growing season (Table 4.11).

Table 4.11. Percentage of the different soil fractions (on mass basis) at the end of the 2nd growing season.

	WEOM†	VLF	LF	HF
	% of the bulk soil			
Brown Cz				
Lentil	0.01	1.1	20.1	78.8
Wheat	0.01	0.9	13.5	85.6
Dark Brown Cz				
Canola	0.01	1.5	19.2	79.3
Pea	0.01	4.1	16.1	79.8
Wheat	0.01	1.6	19.4	78.9

† WEOM, water extractable organic matter; VLF: very light fraction; LF, light fraction; HF, heavy fraction; Cz, chernozem.

No significant differences were found in the %C, %N and C:N ratio among the soil cores with labeled root, shoot, or root+shoot. Table 4.12 shows the means values of %C, %N and C:N ratio by rotation. Except for the WEOM, the %C in the different soil fractions at the end of the second growing season was lower than at the end of the first growing season. As a result, the C:N ratios were lower as well for all soil fractions except WEOM. The WEOM %C and C:N ratio increased markedly by the end of the second growing season. Differences in C:N ratio between the first and second growing season show that the grinding of the shoot residues at the end of the first growing season might have caused an acceleration of the C mineralization. As well, the removal of the straw before core extraction in the field might have induced to some extent the decrease in the %C.

Table 4.12. Percentage of C, N and C:N ratio of lentil, wheat, canola and pea in the different soil fractions at the end of the 2nd growing season.

	WEOM†	VLF	LF	HF
	2nd growing season			
Brown Cz				
Lentil	14.1/1.4, [10.3]‡	4.2/0.8, [5.2]	2.9/0.4, [7.1]	1.6/0.2, [9.5]
Wheat	14.1/1.5, [9.9]	4.2/1.0, [4.4]	3.0/0.5, [6.4]	1.5/0.2, [9.5]
Dark Brown Cz				
Canola	15.4/1.5, [10.7]	3.8/0.9, [4.4]	2.8/0.5, [5.3]	1.9/0.2, [9.9]
Pea	14.2/1.4, [10.6]	3.6/0.9, [4.2]	3.0/0.5, [5.7]	1.9/0.2, [9.9]
Wheat	14.2/2.2, [7.1]	3.8/0.9, [4.3]	3.0/0.6, [5.4]	2.0/0.2, [10.0]

† WEOM, water extractable organic matter; VLF: very light fraction; LF, light fraction; HF, heavy fraction; Cz, chernozem.
‡ %C / %N [C:N].

4.4.4. Second growing season: Fate of ^{13}C-labeled plant material

The results presented in Table 4.2, 4.3, 4.11 and 4.12 were used to calculate the fate of the ^{13}C-labeled plant material in soil at the end of the second growing season following Subedi et al. (2006). The WEOM, VLF, LF and HF derived exclusively from the root at the end of the second growing season had averages of derived mg of C per g of SOC of 3.8, 1.4, 0.5, and 0.1 respectively. No significant differences were found among crops in the mg of C per g of SOC at

the end of the second growing season. Comparing the different soil fraction I found that the WEOM was higher than all other fractions (P<0.01), the VLF was higher than the LF and the HF (*P*=0.02) but no statistical differences were found between the LF and HF (P=0.32) (Table 4.13).

Table 4.13. Root-derived C remaining in the SOC at the end of the 2nd growing season in the WEOM, LF, VLF and HF.

	WEOM†	VLF	LF	HF
	---------- mg of derived C per g of SOC ----------			
Brown Cz				
Lentil	4.92 (0.95)‡	2.42 (0.52)	0.75 (0.13)	0.22 (<0.01)
Wheat	5.10 (0.64)	1.98 (0.52)	0.51 (0.02)	0.16 (0.03)
Dark Brown Cz				
Canola	3.60 (0.73)	1.04 (0.13)	0.46 (0.07)	0.17 (0.02)
Pea	1.70 (0.26)	0.92 (0.59)	0.62 (0.34)	0.13 (0.02)
Wheat	3.66 (1.08)	0.73 (0.12)	0.20 (0.04)	0.09 (0.02)

Summary of ANOVA analysis between soil fraction and crop§

	df	F value	*P* value
Soil fraction	4	65.61	<0.01
Crop	3	5.06	0.10
Soil fraction X Crop	12	2.10	0.03
Residuals	42		

† WEOM, water extractable organic matter; VLF: very light fraction; LF, light fraction; HF, heavy fraction; Cz, chernozem; df, degree of freedom.
‡ Mean values and (SE).
§ ANOVA was run with the statistical program R, with the function aov

At the end of the second growing season, no overall differences were found in the remaining mg of C per g of SOC between the soil cores with enriched root material only (row #3) and enriched shoot material only (row #5). The shoot-derived WEOM, VLF, LF and HF at the end of the second growing season had mean derived mg of C per g of SOC of 4.5, 4.0, 0.5, and 0.1 respectively. No differences were found among crops. No significant differences were found between the WEOM and the VLF (*P*=0.68) or between the LF and HF (*P*=0.68), but significant differences were found between WEOM and LF-HF (*P*<0.01) and between VLF and LF-HF (*P*<0.01) (Table. 4.14).

Table 4.14. Shoot-derived mg of C remaining in the SOC at the end of the 2nd growing season in the WEOM, LF, VLF and HF.

	WEOM†	VLF	LF	HF
	---------- mg of derived C per g of SOC ----------			
Brown Cz				
Lentil	5.27 (0.46)‡	5.54 (2.28)	0.83 (0.14)	0.17 (0.02)
Wheat	4.26 (1.81)	5.25 (1.02)	0.73 (0.14)	0.11 (0.03)
Dark Brown Cz				
Canola	5.96 (1.48)	4.46 (0.72)	0.43 (0.05)	0.15 (0.03)
Pea	4.03 (1.26)	2.04 (0.35)	0.29 (0.03)	0.12 (0.02)
Wheat	3.15 (0.50)	3.03 (0.32)	0.26 (0.03)	0.10 (0.01)

Summary of ANOVA analysis between soil fraction and crop§

	df	F value	*P* value
Soil fraction	4	39.14	<0.01
Crop	3	2.73	0.05
Soil fraction : Crop	10	0.84	0.58
Residuals	46		

† WEOM, water extractable organic matter; VLF: very light fraction; LF, light fraction; HF, heavy fraction; Cz, chernozem; df, degree of freedom.
‡ Mean values and (SE).
§ ANOVA was run with the statistical program R, with the function aov

Because the different soil fractions have a different %C and were not equally represented in the bulk soil, the derived C per ha was not directly proportional to the derived C per g of SOC. The derived C per kg of C per ha was calculated based on the %C of each soil fraction in the bulk soil. The results showed that on a basis of mg of C per g of SOC the HF had the lowest amount of remaining C (Table 4.12-4.14) but on per ha basis the HF contributed to high amount of derived remaining C (Table 4.15) due to the predominance of the HF in the bulk soil. The WEOC was not presented in table 4.15 because on per ha basis this fraction has insignificant values compared to the other fractions. The factorial ANOVA among crop rotation, plant material enriched and soil fraction showed no overall significant difference on the average derived C per ha between VLF, LF and HF (P=0.14). Comparing the crops at the end of the first growing season, only lentil on Brown Cz was higher than the others (P=0.05). At the end of the second growing season, on Swift Current soil (Brown Cz) lentil was significantly higher than wheat and on Scott soil (Dark Brown Cz) pea was higher than wheat but statistically equal to canola. From the first to the second growing season, root-derived remaining C of the VLF had a

91% decrease (258 to 25 g of derived C per ha), the LF a 61% decrease (165 to 103), and the HF a 60% decrease (211 to 144). At the end of the second growing season lentil, wheat (Brown Cz), canola, pea and wheat (Dark Brown Cz) had 404, 262, 281, 276, and 143 kg of root-derived C per ha, respectively. Also at the end of the second growing season, these crops had 423, 281, 309, 259 and 207 kg of shoot-derived C per ha respectively. The amount of derived C per ha of the cores with dual enriched residues (shoot and root) should theoretically have been equal to the sum of the amount of derived C per ha of the root and shoot separately but this did not occur. This is most likely due to non-homogeneous $\delta^{13}C$ labeling between the root and shoot materials. The Subedi et al. (2006) equation uses only one value of $\delta^{13}C$ for the enriched plant materials; therefore, accurate estimates of derived C per ha are hard to produce in soil cores enriched with both root and shoot residues when these residues have different $\delta^{13}C$ and different biomass production.

Table 4.15. Root, shoot and root+shoot-derived C remaining per ha at the end of the 1st and 2nd growing season in the. LF, VLF and HF.

	VLF†	LF	HF	Total	VLF	LF	HF	Total	VLF	LF	HF	Total	VLF	LF	HF	Total
	Root end 1st				Root end of 2nd				Shoot end of 2nd				Root and shoot end 2nd			
	g of derived C (kg per ha)															
Brown Cz																
Lentil	498	227	247	972	31	173	199	403	71	193	159	423	46	333	236	614
	(89)‡	(103)	(48)		(7)	(30)	(1)		(29)	(32)	(20)		(7)	(114)	(45)	
Wheat	254	181	229	664	20	80	162	262	52	115	113	280	118	277	404	799
	(74)	(63)	(30)		(5)	(4)	(25)		(10)	(21)	(25)		(27)	(4)	(20)	
Dark Brown Cz																
Canola	136	209	250	594	18	103	160	281	77	96	136	308	115	201	229	546
	(21)	(35)	(19)		(2)	(15)	(14)		(12)	(11)	(31)		(14)	(37)	(39)	
Pea	138	147	176	461	44	116	116	276	98	54	106	258	361	212	289	863
	(29)	(68)	(22)		(28)	(64)	(20)		(17)	(5)	(15)		(157)	(62)	(72)	
Wheat	267	62	156	485	14	45	83	142	57	59	91	207	118	292	223	632
	(62)	(12)	(13)		(2)	(8)	(20)		(6)	(6)	(13)		(34)	(17)	(27)	

Summary of ANOVA analysis between crop, soil fraction and growth season-shoot/root§

	df	F value	*P* value
Crop	4	5.34	<0.01
Soil fraction	2	4.37	0.01
Growth season	3	27.61	<0.01
Crop X Soil fraction	8	1.27	0.26
Crop X growth season	12	2.55	<0.01
Residuals	175		

† VLF, very light fraction; LF, light fraction; HF, heavy fraction; Cz, chernozem; df, degree of freedom.

‡ Mean values and (SE).

§ ANOVA was run with the statistical program R, with the function aov

4.5. Discussion

4.5.1. Fate of derived C at the end of the first growing season

In a prairie agro-ecosystem, Gan et al. (2009b) estimated root biomass of lentil, canola, pea and wheat through conventional root quantification technique. This study also estimated root biomass of lentil, canola, pea and wheat but with ^{13}C enriched plants, following the Subedi et al. (2006) method. Gan et al. (2009b) estimates of root biomass (0-20 cm) for lentil, canola, pea and wheat were: 349, 758, 298 and 930 kg ha^{-1} respectively. The estimated total root biomass (0-10 cm) produced in this thesis were for lentil, canola, pea and wheat: 2405, 1423, 1172 and 1391 kg ha^{-1} respectively. In this study, VLF corresponds to the soil fraction where plant fragments were visible and except for lentil, the derived biomass in the VLF had a similar weight per ha to the root biomass quantification made by Gan et al. (2009b). The method used by those authors probably underestimated microscopic and decomposed root materials explaining why this thesis total root biomass estimates are drastically higher that those made by Gan et al. (2009b). Differences could also have been caused by variations in greenhouse environment versus field conditions. As well, differences in seeding density are likely to have caused divergences in the results between both studies. Gan et al. (2009b) used seeding densities of: lentil 120 / m^2, pea 70 / m^2, canola 80 / m^2 and wheat 200 / m^2. In contrast, this study used a standard seeding density of 125 plants / m^2 for all the crops. Accordingly, these results of root relative allocation coefficient presented in Table 4.10 are higher than the allocation coefficients for roots reported by Gan et al. (2009a). To generate optimum root biomass/rhizodeposits estimation the Subedi et al. (2006) method should be carried on under field conditions.

The C:N ratio of crop residues is important for agricultural productivity and SOM dynamics. Analogically to Gan et al. (2011b) this study determined the C:N ratio of the root, shoot and seeds for lentil, canola, pea and wheat. Table 4.16 summarizes both C:N results. Although not perfectly equal both results are in similar ranges and follow similar patterns. Differences might be due to differences in fertilization rate, illumination, soil moisture and temperature. Unexpectedly, the wheat straw on Scott soil (Dark Brown Cz) had a C:N ratio

notably higher than the wheat straw on Swift Current soil (Brown Cz) but the wheat grain C:N and the wheat root C:N were similar between both sites. No explanation was found for these differences.

Table 4.16. C:N ratio comparison of lentil, canola, pea and wheat made by Gan et al. (2011b) and this study.

	Root		Shoot		Grain	
	This study†	Gan et al. 2011‡	This study	Gan et al. 2011	This study	Gan et al. 2011
Lentil	24.8	27.8	23.7	17.4	9.5	7.3
Canola	53.7	33.6	43.5	45.4	10.3	7.2
Pea	22.0	16.0	24.8	14.3	12.1	6.5
Wheat	30.9	43.3	42.2	55.1	14.0	17.1

† Results originally from Tables 4.7 and 4.9

‡ Gan et al. results were obtained from rainfall plus irrigated fields

Gan et al. (2009a) reported straw production of canola, pea, lentil and wheat of 3200, 1600, 2000 and 3500 kg ha^{-1} respectively. The extrapolation of our greenhouse yields gives the following straw production in kg per hectare: lentil 6100, canola 18800, pea 5600 and wheat 5300 (Table 4.9). The differences between Gan et al. (2009a) results and my results are likely to be due to better growing conditions in the greenhouse than in the field and due to Gan et al. (2009a) biomass weight were dried at 50°C and mine were air dried. As well, in this study the seeding density standardized (4 plants per soil cores) under field conditions plant density vary considerably among crops and farming practices.

In Sangster's (2010) repeat pulse labeling experiment, plants were labeled for 1.5 hour a week for six weeks resulting in plant material $\delta^{13}C$ of around 130‰ and non-homogeneous labeling among stem, leaves, pod and roots. In this experiment plants were labeled for eight weeks (2h/week) and I achieved $\delta^{13}C$ of around 500‰ and homogeneous labeling among stem, leaves, pod/husk but non-homogeneous between shoot and root (Table 4.2). I began the labeling sessions 20 days after germination and labeled until embryogenesis (fruit formation). The non-homogeneous labeling between root and shoot is likely to be due to faster root growth in the initial phases of plant development. An earlier start in labeling would enhance the labeling homogeneity (Bromand et al., 2001) but would represent a major challenge to make a seal

around the young plant stems. Despite the expense, continuous labeling with relatively high level of ^{13}C atmospheric enrichment would be the optimum way to achieve homogeneous labeling.

4.5.2. Fate of derived C at the end of the second growing season

In the root and shoot at the end of the second growing season, Swift Current (Brown Cz) lentil had more derived C per ha^{-1} than wheat and Scott (Dark Brown Cz) canola and pea had more than wheat. The Poaceae (grass family) possess silica phytoliths in the wheat leaves that makes the residues hard to digest for soil biota (Judd et al., 1999), but all the monocots (e.g. wheat) have a particular stele system that leads to a low lignin content and a relatively low recalcitrance. Therefore, lentil, canola and pea would be slightly more resistant to further decomposition than wheat in the Canadian Prairie agricultural fields. It is also important to note that even after the first growing season, when more canola shoot residues were put into the soil than the other crops (15 g canola versus 10 g other crops, per soil core), canola did not produce more shoot-derived C than lentil or pea (Table 4.15). This shows that the properties of the plant residues are as important as the quantity of plant residues to produce SOM and that pulse crop may convert more efficiently residues C to SOC. Our results are in accordance with Liang et al. (2003) who found a significant positive pulse crop impact on the LF organic C in the Brown and Dark Brown Chernozemic soil zones of Saskatchewan. Our results are also in accordance with Lemke et al. (2007), who found that even if pulse crops are producing significantly less above ground residues than canola and wheat, there is no significant difference in the amount of SOC per ha between pulse crop, oilseeds and wheat in long-term rotations at AAFC Swift Current (Brown Cz).

Comparing the root-derived C decomposition (2^{nd} growing season divided by 1^{st} growing season) of the different crops, this study found that pea had the slowest root VLF decay and lentil had VLF decay statistically equal to wheat. A higher decrease in the VLF of the wheat was expected because the taproot of the dicoteledon (lentil, canola, pea) is known to be stronger than the adventitious roots of the monocot (Evert et al., 2006). On the other hand it has been reported that pulse crop residues have a higher rate of mineralization than non-legumes (Campbell et al., 1992).

Evaluating the root-derived C and shoot-derived C at the end of the second growing season, the amount of remaining derived C was statistically equal in all the crops. However, higher amounts were expected for shoot since the shoot produced three to ten times more residues than the root (Table 4.15) and at the end of the second growing season the labeled roots had been in contact with the soil for two seasons versus only one season for the labeled shoots. This lower recalcitrance of the shoot could have been caused by the grinding and/or due to morpho-molecular differences between shoot and root, with the root having a strong region of xylem in the center (Judd et al., 1999).

Comparing both sites (Swift Current and Scott) throughout the experiment, the HF of Scott (Dark Brown Cz) maintained a %C higher than Swift Current (Brown Cz) but no significant difference was found in the other soil fractions. At the end of the second growing season, lentil on Brown Cz had a higher derived C than pea on Dark Brown Cz and wheat on Brown Cz higher derived C than on wheat Dark Brown Cz for both root and shoot (Table 4.15). This might be due to the fact that in the greenhouse the temperature and moisture was the same for the soil cores of both sites (in contrast to normal field conditions) and because Brown Cz had lower initial %C it has greater potential for relative increase.

The soil C:N ratio is a property that is commonly used as an indicator of soil fertility (Adebayo et al., 2011). In this study, per site, I did not find important differences in soil C:N ratio among the different crops at the end of either growing season (Tables 4.5 and 4.12). The C:N ratio of the bulk soils were around 10.5 which is typical for this ecosystem (Stewart, 1989; Cheng et al., 2011). The C:N ratio of the HF maintained values close to the bulk soil throughout the experiment. However, C:N ratio of WEOM, VLF and LF were different at the end of the first and second growing season. Environmental conditions in the greenhouse were the same during both growing season. Therefore, differences may have been caused, in part, by the removal of the crop residues at the time of core extraction in the field, which reduced the amount of crop residues decomposed during the first growing season. Also, differences are likely due to the grinding and mixing of the straw residues with the soil in the cores before the second growing season which accelerated the mineralization rate compared with field conditions where the soil is not deeply tilled. Craswell and Waring (1972) demonstrated that mineralization rates increase when residues are ground. Brady and Weil (2008) acknowledge that fungi dominate the

microbial activity on the soil surface whereas fast multiplying bacteria play a larger role when the substrate is mixed into the soil, and that bacteria are quicker than fungi to decompose labile residues.

The increase in the C:N ratio of the WEOM from the end of the first growing season to the end of the second (Tables 4.5 and 4.12) could indicate a shift in the soil microbial population from the predominance of fungi (C:N = 4) to the predominance of bacteria (C:N = 9). As well, at the end of the first growing season the WEOM had likely been composed of exudates, and roots sloughing while at the end of the second growing season it included more standing root stock and above ground residues that had been thoroughly mixed with the soil. This stimulates microbial activitya slightly higher amount of exomolecules with relatively high C:N ratio (possibly polysaccharides) dissolved in the soil solution (Brady and Weil, 2008). On the other hand, the very low C:N ratio in VLF and LF in all the crops at the end of the second growing season indicate that the cellulose, lignin and lignin subunits have been either almost completely decomposed or fixed into the HF. It is important to note that the crop residues of lentil and pea had an initial C:N ratio markedly lower than canola and wheat (Table 4.3) yet at the end of the second growing season no significant differences were found and all the crops had a C:N ratio around 5 in the VLF and LF (Table 4.12). This suggests that the C:N ratio of the crop residues has no influence on the C:N ratio of the different soil fractions. However, the C:N ratio of the crop residues would affect mineralization rates. Our data also suggest that the C:N ratio of the crop residues strongly influence the amount of SOC produced. That is, low amount of crop residues with low C:N ratio could produce more SOC than high amount of crop residues with high C:N ratio. The previous explain why pulse residues could be converted more efficiently into SOC than non-legume crops.

The strong changes in the remaining derived C and the C:N ratio of the WEOM, VLF and LF during the second growing season shows these fractions are highly labile. As well, at the end of the second growing season the HF had lost 60% of its root-derived C mass (Table 4.15), pointing out that a major portion of the HF is also highly labile. Within the HF sub-fractions, the HA had the highest %C and most of the derived C was found in this fraction (Table 4.6, 4.8) indicating that the HA is probably not as highly a recalcitrant pool as it is thought to be (Qualls, 2004). The most recalcitrant part of the HF is possibly contained in the HuC since in this sub-

fraction a considerable amount of derived C still remains bound to the mineral matrix even after the very low pH (pH 1.5) of the humic-fulvic fractionation method. The HuC sub-fraction also has %C, %N and C:N ratio close to the bulk soil (Table 4.6). This is in line with Anderson and Paul (1984) who found that the humin fraction of the soil and the non-hydrolyzable organic carbon had an equivalent age older than HA and FA. Analogical research carried for more than 2 years would be required to model the dynamic of the recalcitrant SOM since in this study the dynamics of recalcitrant SOM was masked by the labile SOM.

4.5.3. Relationship between C:N ratio and fate of derived C

Results of this study showed that in the VLF and LF, the mineralization of C and N were not synchronized. At the end of the second growing season, the %C and remaining labeled C had diminished drastically but not the %N causing the C:N ratio to decrease to levels notably lower than the bulk soil (Table 4.12). Therefore, during the experiment in the VLF and LF, the rate of C respiration and N immobilization were higher than N losses (gaseous or leaching). Thus, at the end of the second growing season, most of the high C:N ratio molecules (carbohydrates and lipids) must have been respired and/or humified into the HF or transferred into the WEOM. Richnow et al. (1997) and Johnson et al. (2005) reported that in soil during organic C degradation the microbial metabolites with high C:N ratio (e.g. acetic acid, ethanol, glycerol, 5' guanylic acid) generally possess higher polarity than the substrate compounds and those metabolites are often found covalently bounded to the recalcitrant SOM (i.e. HF). As well, between the first and second growing season the WEOM had a noteworthy increase in %C and C:N ratio.

The asynchronous C and N mineralization observed in this study can be explained with the r/K selection theory applied for soil ecology with a few adaptations for a prairie agro-ecosystem (Andren et al., 1995; Brady and Weil, 2008). In spring, the availability of fresh plant residues recently incorporated into the soil stimulates a prompt increase in microbial metabolism (Glenn et al., 2011). The generalist and fast-multiplying microorganisms (r-strategist) overtake the slow-growing and specialized microbes (K-strategist) and start to decompose the easily decomposable molecules such as sugars and starches (Bastian et al., 2009). Thus, microbial numbers increase exponentially and synthesize new exocellular organic compounds, which

would have caused the increase in the derived C in the WEOM at the end of the second growing season (Table 4.14). When microbial activity reaches a peak of intensity, CO_2 is being emitted in large quantities and amino sugar and amino acid concentrations increase in the soil as litter decomposition progresses, which causes a decrease in the C:N ratio of the VLF and LF (Amelung et al., 2001; Nicolardot et al., 2001; Kampfl et al., 2007; Xie Jin-sheng et al., 2008). Mid-summer, when the easily decomposable compounds are exhausted, r-strategist bacterial and fungal populations plummet due to starvation (Brady and Weil, 2008; Bastian et al., 2009). The dead microbial cells trigger the mineralization of simple inorganic products such as ammonium and nitrates (Batlle-Aguilar et al., 2011). Then, the low amount of easily mineralizable C and high amount of organic and inorganic N in the system boosts the nitrifiers and denitrifiers, which would act as K-strategists. Nitrifers are chemoautotrophs that use CO_2 as C source and inorganic N as source of energy (N_2O and N_2 emissions). Therefore, the nitrifier and denitrifier activity would increase the C:N ratio of the soil until a new equilibrium is reached.

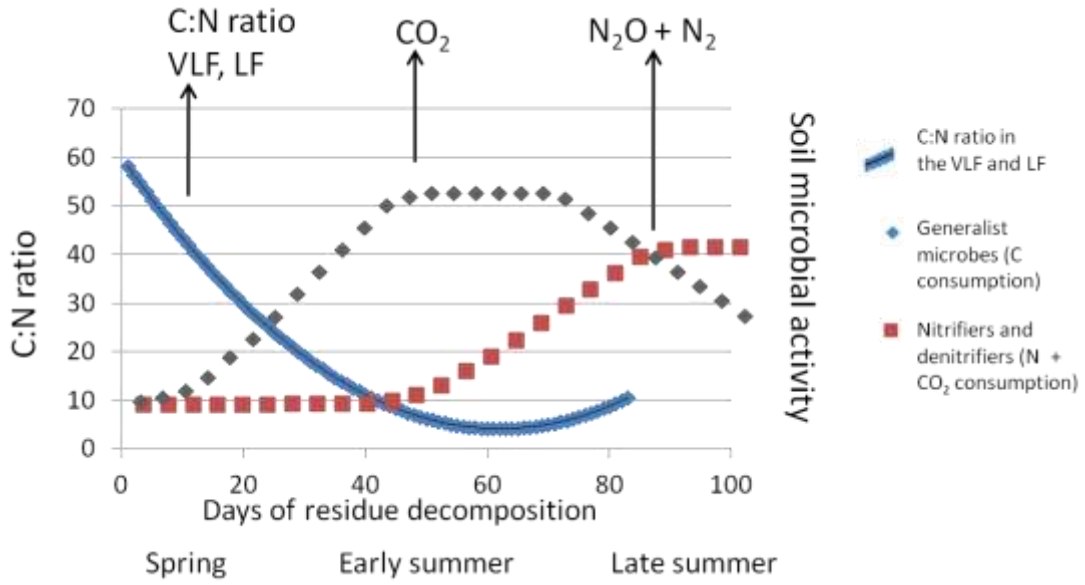

Fig 4.4. Conceptual model of the evolution of the C:N ratio in the VLF and LF in relationship with the microbial activity of generalist microbes, which consume C-organic, and nitrifier/denitrifier, which consume CO_2+N. In this model, at the beginning of the spring the VLF and LF of the fresh crop residues have a C:N ratio of around 60. Then the C:N ratio (continuous line) decrease in a proportion inverse to the generalist microbes growth (black polygons). When the C:N ratio reach value lower than the bulk soil (C:N ~10) the generalist microbes enter in a plateau stage and the nitrifier-denitrifiers (gray squares) increase their activity due to high availability of high nitrogen molecules. At the end of the summer, most of the original VLF and LF (crop residues) have been emitted in the gaseous

form (CO_2, NO_2 and N_2), or leached. Finally, a fraction of the remaining C and N is immobilized by the plant and the microbes and another fraction is fix into the recalcitrant SOM which has a C:N ratio around 10.

The simple conceptual model in Fig 4.4. illustrates the microbial growth curve, microbial population dynamics, and changes in plant residue decomposition as described by Novick (1955), Wrage et al. (2001) and Brady and Weil (2008). Our input to these classical models is the shift of the C:N ratio in crop residues (VLF and LF) and the consideration of nitrifiers-denitrifiers as K-strategists. With respect to this study, this interpretation explains: (1) the 91% and 61% decrease noted in the VLF and LF derived C, respectively; (2) how the C:N ratio of the VLF and LF become lower than the bulk soil while the HF maintain C:N values around 10; (3) why N mineralization is higher at anthesis than seeding. To corroborate or invalidate this conceptual model, plants would need to be labeled with ^{13}C and ^{15}N, and during the decomposition of the crop residues, assessments of $^{13}CO_2$, $^{15}N_2$, and $^{15}N_2O$ emissions and of the ^{13}C and ^{15}N remaining in the VLF, LF and HF would need to be made.

4.5.4. Fate of newly derived C

A conceptual model of the fresh organic residues decomposition in soil was produced with the results and interpretations made throughout this study (Fig. 4.5). The fresh crop residues are initially part of the VLF; then soil biota solubilize a fraction of this VLF and the partially degraded plant tissues move into the LF. Due to its major shift in %C and %N and due to its 61% decrease in derived remaining C from the first to the second growing season, the LF (density of 1-1.7 g mL^{-1}) is likely to be a highly labile and transitional pool. With further decomposition the fragmented tissues in the LF become free macromolecules and progress to the WEOM and/or are directly absorbed into the HF. In the WEOM, residues are consumed by soil microbes and some of the water soluble compounds are fixed into the mineral matrix of the soil in the HF. Throughout the experiment, the total %C and %N in the HF remained stable but this fraction lost 60% of its derived C from the first to the second growing season. Therefore, a high amount of the C newly fixed into the HF is still labile and can be extracted from the HF by microorganisms. The derived C increase in the WEOM at the end of the second growing season, when all the other soil fraction had lost derived C, suggests that several SOM pools are contributing organic C

to the WEOM (Fig. 4.5). In order to better uncover the dynamic and fate of the newly fixed C, plant residues should be labeled with several isotopic markers (as mentioned above) and instead of labeling the whole plant, labeling should be made on specific group of molecules separately (e.g. amino acids, cellulose, lignin, lipids, oligosaccharides), which could be made with different kind of antibodies. The HF (>1.7 g mL^{-1}) should be divided into several other density fractions and should also be subdivided with chemical fractionation. Finally, all sub-fractions should be analyzed with nuclear magnetic resonance spectroscopy with the purpose of clearly identifying the molecular transformations occurring during residue decomposition.

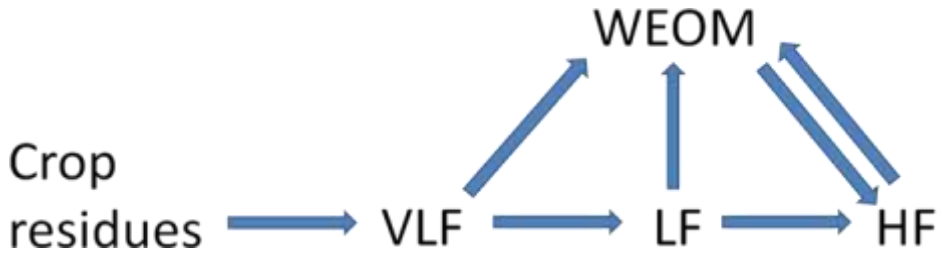

Fig 4.5. Conceptual model of the fresh organic residues dynamic in soil. VLF: Very light fraction. LF: Light fraction. HF: Heavy fraction. WEOM: Water extractable organic matter.

4.7. Conclusion

This work determined the dynamic of the C captured during a growing season of lentil, canola, pea and wheat and analyzed the relationship between the plant and soil C:N ratio and the fate of the newly derived C. Using a repeat ^{13}C pulse labeling method, density fractionation and a WEOM technique, this study established that pulse crops had more remaining derived C per ha^{-1} than wheat in all the soil fractions evaluated. As well, this study showed that in the Canadian Prairie agro-ecosystems, roots might produce significantly more biomass than what is currently estimated. Furthermore, even if roots produce notably less biomass than the shoots, roots contribute an equal amount of the total SOC to the shoot. Consequently, the removal of a portion of the crop straws for biofuel and pulp paper may not generate as negative of an impact on the total SOC level as previous estimates might imply. Finally I confirmed that the C:N ratio of the crop residues plays a central role in the dynamic of soil C and established that even if the HF is more recalcitrant than the VLF and LF, a large amount of C in the HF is from fresh crop residues. This work was the first study that used repeat ^{13}C pulse labeling method to trace the fate of crop

residue C in different SOM pools in prairie soils. As well, this study was the first to generate estimates of annual gross production of SOC for roots and shoots under different crop rotations in the Canadian Prairies. These estimation may help to generate SOC policies and strategies that could mitigate GHG emissions and enhance soil fertility. Nevertheless, to determine how long it takes for fresh plant residues to achieve a high level of stability in soil analogical studies carried on over many decades are needed. These further studies should also ideally be done *in situ* with several isotopic tracers, the HF should be sub-fractionated, and samples should be collected at several points throughout the growing season.

5. General Discussion and Conclusion

5.1. Summary of findings

This study analyzed the effects of incorporating lentil and pea in rotation with wheat on a) the fate of the recently fixed C in soil and b) SON mineralization. Several parameters of two Chernozemic soils were analyzed to determine the different N mineralization rates and to describe the fate of ^{13}C-labeled residues of lentil, wheat, canola, and pea. Various benefits of including pulse crops in rotation on the SOC and SON dynamic were found. First and foremost, I found that at the end of the second growing season, Swift Current (Brown Cz) lentil had more remaining derived C per ha^{-1} than wheat and, Scott (Dark Brown Cz) canola and pea had more than wheat. It is generally thought that grass plants were responsible for the accumulation of SOC in the Canadian Prairies but in natural prairie ecosystems a large number of leguminous plants are also present and these legumes play a vital role in SOM dynamic. Root biomass production influences SOM dynamic and in this experiment lentil produced more root biomass (0-10 cm) than canola and wheat but pea did not produce more than canola or wheat. However, legumes are not affecting in a different way all the aspects of SOM dynamic; the changes in the amount of root remaining derived C between the first and second growing season in the different soil fractions were analogical for all the crops. Also analogically for all the crops, the root residues and the shoot residues equally contributed to the SOC production (from the derived labeled C) at the end of the second growing season. As well, this study did not find significant differences among crops in the plant residues C transformation into SOC. I deduced that the fresh crop residues were found into the VLF, then decomposition transform the residues into LF, and then further mineralization converts the remaining residues into HF and all the fractions sending some C to the WEOM and the WEOM is transferring C back to the HF. Finally, even if pulse crop residues C:N ratio was lower than non-pulse, the C:N ratio of the different soil fractions was the same for the crops.

5.2. Link between C and N dynamic

In soils, C and N cycles are strongly linked; our results suggest that mineralization of C is high in fresh plant residues yet these fresh residues do not immediately activate N mineralization.

The abundant negative and near-zero gross N mineralization values at seeding indicate that N immobilization by microbes is likely to be equal or higher than N mineralization. In contrast, at anthesis gross N mineralization was significantly higher than seeding and in the greenhouse at the end of the second growing season high losses in remaining derived C, accompanied with C:N ratio notably lower than the bulk soil was found in the VLF and LF. All this indicates that plant residues decomposition is likely to occur in two phases. In the first phase, microorganisms mineralize C and immobilize N. In the second phase, molecules rich in easily mineralizable C are exhausted, and N mineralization and nitrification/denitrification are activated until the residues reach C:N values close to the bulk soil and are incorporated into the recalcitrant SOM.

Comparing crops it was found that canola and wheat straw residues had a C:N ratio markedly higher than the pulse crops, but at the end of the second growing season all the crops had C:N values around 5 in the VLF and LF. This illustrates that all the crops analyzed in this study had an analogical pattern of plant decay, but non-pulses needed to release more CO_2 than pulse to reach a C:N value of 5. It also suggests that the amount of N in plant residues would be more influential than the total residue biomass for estimates of SOC production. In this study, the same amount of straw residues for wheat and pulse crops was put into the soil (10 g per core), but at the end of the second growing season, per site, pulse crops had higher remaining derived C than wheat.

5.3. Relevancy and future work

This work represents important progress in the field of SOM dynamic in prairies soils. This study was the first to follow the fate of ^{13}C enriched crops residues into different SOM pools in Canadian Prairie soils and one of very few internationally. In this study basic soil fractionations were made and I found that the VLF and LF are highly labile. I also found that many SOM compounds are transiently bound to the HF. As well, this study confirmed that the %N in crop residues plays a central role on the dynamics of fresh SOC. The results show that even if pulse crops produce less aboveground biomass than wheat or canola, they may produce more SOC. This work also confirmed that when N fertilizer is not applied, pulse crops in rotation are beneficial on soil fertility. Thus the positive effect of pulse crops on the current environment problems is two-fold: pulse crops may help sequester C and help to decrease N fertilizer use.

Consequently, policies and strategies that promote pulse crops use should be supported because this could both mitigate GHG emissions and enhance soil fertility.

A better knowledge and understanding of root and shoot contributions to SOM is currently required since the demand for straw residues to produce biofuel and pulp paper is quickly increasing. This study was the first one to generate estimates of annual gross production of SOC for root and shoot under different crop rotations in the Canadian Prairies. I found that roots may produce significantly more biomass than what is currently estimated and that roots are likely to contribute more to the SOM than what is currently thought.

Advanced soil fractionation techniques and long-term studies with isotopically labeled plant residues will help to fully reveal the poorly understood genesis of SOM. Several density fractionations should be made within the HF and some chemical sub-fractionation should be performed as well in order to determine what is labile and what is recalcitrant in the HF. Further studies should label plant materials with ^{13}C, ^{15}N and ^{17}O simultaneously and non-simultaneously to follow the breakdown of the organic molecules into the soil. During decomposition of the plant residues, assessments of $^{13}C^{17}O_2$, $^{15}N_2$ and $^{15}N_2{}^{17}O$ emission should be made and the ^{13}C, ^{15}N and ^{17}O remaining in the different soil fraction determined. In addition, long term studies made with similar techniques could determine how long it takes for fresh residues to reach a stable state into the SOM. It would also assess if some plants are producing more recalcitrant SOM than others (e.g. legumes vs. grasses).

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