

THE INFLUENCE OF FIELD PEA ON CARBON AND NITROGEN DYNAMICS
AND GREENHOUSE GAS EMISSIONS

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By

Amy Sangster

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ABSTRACT

Pulse crops have been long associated with biological dinitrogen fixation and therefore improve the sustainability of cropping systems when included in rotation. However, studies indicate there may be additional benefits of including pulse crops in rotation. To quantify these potential benefits, soil processes and properties related to nitrogen (N) and carbon (C) cycling were examined in five crop rotations with and without field pea (*Pisum sativum* L.) in Scott, Saskatchewan. Gross mineralization and nitrification rates were determined using the ^{15}N isotope dilution technique in intact soil cores. To estimate the proportion of nitrous oxide (N_2O) emissions derived from nitrification related processes rather than denitrification processes tracer techniques using ^{15}N were used. Field incubations were performed in 2008 at seeding (May 13), anthesis (July 8) and just after harvest (October 8). Mean mineralization and nitrification rates were not significantly different among rotations on any date and there was no significant difference in mean N_2O emissions among rotations. From labeled $^{15}\text{NO}_3^-$ cores, it was determined that nitrification-related processes were the major contributors to N_2O emissions. There was no difference among the rotations in microbial biomass carbon (MB-C) or microbial biomass N (MB-N) with the exception of MB-C in the continuous field pea (FP) and the canola (*Brassica napus* L.)-wheat (*Triticum aestivum* L.)-field pea (CNL-W-FP) rotation at anthesis. There was no effect of rotation on dissolved organic carbon (DOC) and only seasonal differences were observed with DOC levels being lower before seeding than at anthesis and post-harvest. Based on the results obtained from a single growing season, our results show that N benefits of including field pea in rotation, beyond dinitrogen fixation, were not detectable and that the immediate N benefit of including field pea in rotation may be due simply to the direct effects of biological dinitrogen (N_2) fixation. However, there have been reports of pulse crop benefits to succeeding crops in rotation. As a result, we investigated both the quantity and quality of crop residues, which can have an impact on soil properties and processes. Plants enriched with isotopic tracers can be used to trace crop residue decomposition to various C pools

but only if the tracer is homogeneously distributed throughout the plant. In order to determine if repeat-pulse labeling could be used to trace crop residue decomposition, this method was followed using $^{13}\text{CO}_2$ to enrich plant material of field pea and canola plants in a controlled environment. The distribution of ^{13}C throughout the plant parts (roots, stem, leaves, and pod) and biochemical fractions [acid detergent fiber (ADF) and acid detergent lignin (ADL)] were determined. It was found that ^{13}C was not homogeneously distributed throughout the plant parts or biochemical fractions. The pod fraction in particular was much less enriched in comparison to the other fractions. The ADL fraction was less enriched than the ADF fraction. Because of the heterogeneity of the label throughout the plant, modifications of the method are needed and ^{13}C distribution throughout the plant needs to be assessed before the repeat-pulse method can be used to trace C residue through various C pools. Nevertheless, root contributions to below-ground C were successfully determined from the enriched root material and the resulting enriched soil. It was found that canola contributed more above- and below-ground residues than field pea, however canola was also higher in ADF and ADL fractions indicating a more recalcitrant residue. Research should continue to better define the impact of pulse crop residues on C and N cycling and subsequent crops in rotation.

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LIST OF ABBREVIATIONS

ADF - Acid detergent fiber
ADL - Acid detergent lignin
ANOVA - Analysis of variance
BBCH - BASF–Bayer–Ciba-Geigy–Hoechst phenological development scale
C – Carbon
CNL – continuous canola rotation
CNL-W-FP - canola-wheat-field pea rotation in the field pea phase
DOC - Dissolved organic carbon
DOM - Dissolved organic matter
IRGA - infrared gas analyzer
FP – continuous field pea rotation
ITC - isothiocyanates
MB - microbial biomass
MB-C - microbial biomass carbon
MB-N - microbial biomass nitrogen
MDCD - minimal detectable concentration difference
N- Nitrogen
PURENET - Pulse Research Network
PVC - polyvinyl chloride
REGWQ - Ryan-Einot-Gabriel-Welsh multiple range test
SOC - soil organic carbon
SOM - soil organic matter
WSOC - water-soluble organic carbon
WFPS - water filled pore space
W-CNL - wheat-canola rotation on the canola phase
W-FP - wheat-field pea rotation in the field pea phase

1. INTRODUCTION

Since the advent of farming on the Northern Great Plains, cropping sequence decisions have been influenced by production factors such as crop synergism, nutrient cycling, and soil water, and by external factors, such as weather, markets, and government programs (Tanaka et al., 2007). The effects of these cropping sequence decisions on C and N cycling are of great interest from both an agronomic and an environmental viewpoint.

Perhaps one of the most profound changes that influenced crop sequence decisions was the advent of the Haber-Bosch process and the subsequent availability of a synthetic N source (Peoples and Craswell, 1992). Despite the apparent agronomic advantage of applying N-fertilizer derived from the Haber-Bosch process, concerns have been raised regarding the sustainability of agricultural systems that are reliant on this endothermic reaction. Currently, N fertilizer prices reflect increasing fossil fuel prices (Fykse, 2007) therefore, increasing N use efficiency on farm is paramount. However, application of N fertilizer is often only practical at or just following seeding resulting in N losses, including N₂O emissions, as plant demands are not well matched with N availability (Crews and People, 2004). On the other hand, a good N fertility program has the potential to increase soil organic carbon (SOC) (VandenBygaart et al., 2003), which is important in terms of the health and quality of soils.

Agriculture has been identified as a significant source of greenhouse gasses including CO₂, CH₄ and N₂O (Cole et al., 1997). However, cropping systems in semi-arid regions have been suggested to be potential net sinks of CH₄ (Paustian et al., 1995). Although there are large annual exchanges of CO₂ between agricultural lands and the atmosphere, global net fluxes of CO₂ from agricultural lands are estimated to be only 0.04 Gt CO₂ yr⁻¹ (Smith et al., 2007). Because of this balance of CO₂ fluxes, there is much interest in the potential of these soils to sequester C by increasing the photosynthetic input of C and slowing the release of CO₂ from soil C stores (Lal, 2004). Nitrous oxide is a potent greenhouse gas with a global warming potential 296 times that of CO₂ (IPCC, 2001). Atmospheric concentrations of N₂O have grown by 50% since

1970 (11% since 1990), mainly due to the increased use of fertilizer and the aggregate growth of agriculture (Smith et al., 2007). Mitigations of these gasses from agricultural ecosystems can be facilitated by more efficient management of C and N flows (Smith et al., 2007).

Cropping sequences on the Canadian Prairies have become specialized based on the most readily marketable and profitable crops, which has resulted in the intensification of cropping sequences that include only a few crops (Johnston et al., 2005). Saskatchewan has 43 million acres of arable cropland and in 2006 had the largest production area in the country for wheat, oat (*Avena sativa* L.), rye (*Secale cereale* L.), canola, flaxseed (*Linum usitatissimum* L.), dry pea, chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medic.), mustard seed (*Sinapis alba* L. and *Brassica juncea* L.), canary seed (*Phalaris canariensis* L.) and caraway seed (*Carum carvi* L.) (Statistics Canada, 2006). Canola production area has increased by 26.9% since 2001 (Statistics Canada, 2006). Traditional cereal- and oilseed-based crop rotations in the Canadian Prairies are heavily dependent on the input of non-renewable energy (Zentner et al., 2004) of which fertilizer, combined with on-farm fuel use, represent over 80% of the total energy input of traditional grain production systems (Zentner et al., 1989). Currently, cropping sequences in the Northern Great Plains are supported by a heavy reliance on N fertilizer inputs. In Saskatchewan, the reliance on N fertilizer inputs has been magnified by the recent growth in biofuel crop production. Because of market incentives to grow high-input crops such as canola for biofuel feedstock (Zilberman and Rajagopal, 2007), cropping inputs must be carefully considered so that the production of biofuel crops does not negate any potential environmental credits of replacing conventional petroleum-based fuels.

One possible strategy for Saskatchewan farmers to improve the sustainability of cropping systems is to increase the frequency of pulse crops in rotation. The potential environmental and agronomic benefits of pulse crops are significant (Miller et al., 2003b; Johnston et al., 2005). Research has shown that a more diversified regime of crop types and species will result in increased overall productivity (Bullock, 1992). Including pulse crops, such as field pea, in rotation can benefit subsequent crops by the additional N mineralized from their residues compared to the residues of non-legumes (Stevenson and van Kessel, 1996b; Beckie and Brandt, 1997). There has been speculation that the

presence of legumes in rotation may result in more efficient conversion of residue C to SOC compared with monoculture wheat (Drinkwater et al., 1998). In attempting to improve soil organic matter (SOM) levels, a more efficient conversion of pulse residue C would help mitigate SOC loss (Lemke et al., 2007).

This study falls under the cropping systems module of a larger study through the Pulse Research Network (PURENet) which involves over 50 scientists whose vision is “to achieve a thriving and prosperous Canadian bioeconomy, driven by innovative scientific solutions, to deliver sustainable, environmentally friendly, and functionally superior food, thereby contributing to the well being of all Canadians” (Agriculture and Agri-Food Canada, 2009). This particular study is designed to provide estimates of below-ground C and N contributions of field pea and will provide necessary information to complete a life cycle analysis of rotations that include pulse crops.

2. LITERATURE REVIEW

2.1. Nitrogen Dynamics in Rotations in the Northern Great Plains

2.1.1. Dinitrogen fixation of field pea and N contribution to soil

The requirement for and dependence on synthetic N inputs can be reduced through the biological N-fixing ability of field pea (Soon and Arshad, 2004). Contributions of N via biological fixation from pulse crops have been well documented for the Saskatchewan area (Cowell et al., 1989; Androssoff et al., 1995; Stevenson and van Kessel, 1996a; Beckie et al., 1997; Matus et al., 1997). However, there is much variability between estimates of N fixation ranging from 0 to 90% of total plant N derived from atmosphere (Walley et al., 2007). Biological dinitrogen fixation has been found to be largely dependent on existing soil N levels, increasing when soil N availability is low (Carlsson and Huss-Danell, 2003).

The benefits of including field pea in a rotation are greater than simply reducing N inputs in the year of pea production. Cereals produced following legumes in rotation frequently have a greater yield than those following non-legume crops (Johnston et al., 2005). This greater production has been attributed to a combination of a rotational effect and a residual N effect (Soon and Arshad, 2004). The benefit of the rotational effect includes a break in the disease (Pedersen and Hughes, 1992) and weed cycle (Blackshaw et al., 1994) of the succeeding crop. Walley et al. (2007) summarized results from studies in the Northern Great Plains and found that not all pulse crops are associated with N additions to soil. However, they did find that field pea has been associated with N additions ranging from 18 to 103 kg ha⁻¹ among sites in Saskatchewan and despite the variability, are likely to contribute to an overall accumulation of N over the long term (i.e., residual N). This was attributed to the mean N derived from air (N_{dfa}) values of field pea (>59.9%) resulting in N additions.

Most measures of pulse N contribution are made by measuring soil N pools. For example, Soon and Arshad (2004) reported net inorganic N soil levels were higher in rotations following legumes than cereal crops. Although net mineralization provides an

index of plant available N, it does little to explain the total amount of N cycling between organic matter and soil inorganic N (Robinson, 2001). Gross measurements on the other hand, provide estimates of the total release of mineral N from a given pool (Wang et al., 2001).

2.1.2. Gross mineralization and nitrification

Gross processes of mineralization, nitrification and immobilization occur simultaneously in the soil, and their relative magnitudes will determine whether there is a net release of N into the soil (Recous et al., 1999; Murphy et al., 2003). Estimates of gross N fluxes may help in predicting N availability in the soil. In order to predict the amount of N availability in soil, mineralization-immobilization-turnover rates and the factors that control these rates, such as soil C, also need to be quantified (Murphy et al., 1998). Although the C and N cycles in soil are usually considered to be closely linked, there is little data available where both C and N pools and gross N fluxes have been measured seasonally (Murphy et al., 2007).

The ^{15}N isotope technique is the only tool for independent estimations of gross mineralization and immobilization (Recous et al., 1999). Booth et al. (2005) reviewed gross mineralization and nitrification data from 100 studies conducted in forest, shrubland, grassland and agricultural systems. In semi-arid agricultural regions, gross mineralization rates range from 0.4 to 7 mg NH_4^+ -N kg^{-1} soil d^{-1} and gross nitrification rates were 0.2 mg NO_3^- -N kg^{-1} soil d^{-1} . Nitrification appears to be controlled primarily by the mineralization rate and resulting availability of NH_4^+ ; however, competition for NH_4^+ , which in turn reduces the amount of NH_4^+ available as a substrate for nitrification, can also affect nitrification rates (Booth et al., 2005).

2.1.3. Nitrogen losses as N_2O emissions

Nitrogen losses as N_2O emissions result in both economic and environmental costs. Nitrous oxide is a potent greenhouse gas that absorbs solar infrared wavelengths in the lower atmosphere, which warms the earth's surface (Sylvia et al., 2005). Of global anthropogenic emissions in 2005, agriculture accounts for about 60% of N_2O (Smith et al., 2007) and has a global warming potential 296 times that of CO_2 and an atmospheric life time of 114 years (IPCC, 2001). Because of its long persistence, it can be transported

into the stratosphere, where it contributes to ozone destruction (Mosier, 2001). Agricultural N_2O emissions are projected to increase by 35 to 60% up to 2030 due to increased N fertilizer use and increased animal manure production (FAO, 2003).

There are several processes that can produce N_2O : nitrification; denitrification; coupled nitrification-denitrification; chemodenitrification; and nitrifier denitrification (Wrage et al., 2001) (Fig. 2.1). However, of all of these processes, denitrification has been suggested to be a main source of N_2O (Ambus, 1998). Nitrous oxide from denitrification can be released in large quantities in low oxygen environments with sufficient NO_3^- and metabolizable organic C (Wrage et al., 2001).

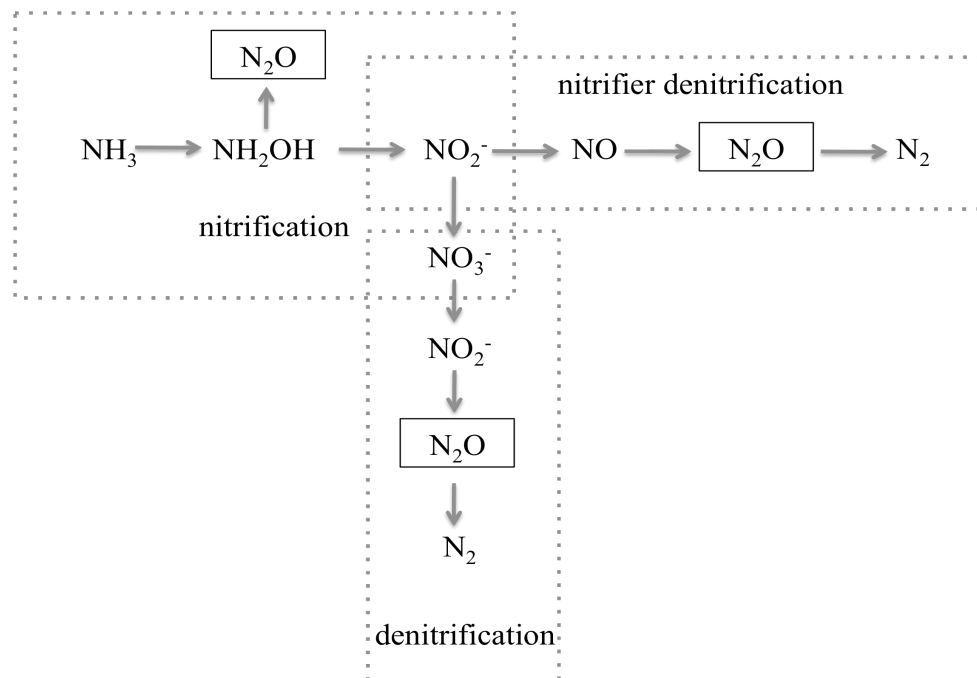


Fig. 2.1. Overview of the pathways of N_2O production from nitrification, nitrifier denitrification and denitrification. Adapted from Wrage et al. (2001).

Nitrous oxide emissions are increased by environmental factors such as increased rainfall, temperature, snowmelt, and freeze-thaw cycles (Smith et al., 2004b) and have the potential to be decreased by management practices that improve synchrony between crop nutrient use and available plant N (Mosier et al., 2005). The dominant control of N_2O emissions within a landscape is the spatial differences in water-filled pore space (WFPS) (Pennock and Corre, 2001). In the Parkland regions of Alberta, Lemke et al.

(1998) found that denitrification occurs when soils reach a WFPS of greater than 60%. Bateman and Baggs (2005) found that all of the N₂O produced in a Cambisol (FAO classification) at 70% moisture was produced by denitrification. They further found that nitrification was the main process producing N₂O at 25 to 60% WFPS. Because of the arid climate in Saskatchewan, N₂O emissions from nitrification could contribute significantly to agricultural greenhouse gas emissions. At the St. Denis National Wildlife Area in central Saskatchewan, Bedard-Haughn et al. (2006) found a strong correlation between N₂O emissions and WFPS ($r^2 = 0.66$, $p < 0.01$) but did not find any correlation between N₂O emissions and nitrification rates. They suggested that these findings support importance of nitrifier-denitrification in the ephemeral cultivated wetland soils as sole contributions from nitrification would have resulted in a positive correlation of N₂O to nitrification rates. In this study nitrification related processes contributed 30 to 70% of total N₂O emissions. With soils from the same site, Ma et al. (2008) found that nitrification related processes contributed 99% of N₂O emitted from cultivated soils with 50% WFPS.

2.2. Soil Organic Matter Dynamics in the Northern Great Plains

Soil organic matter is a key factor in sustainable agricultural systems. The primary additions to SOM in agricultural systems are crop residues. Crop management practices can alter the quantity, quality and placement of crop residues in soil, thereby influencing C and N cycling (Drinkwater et al., 1998). Nitrogen mineralization and immobilization are closely related to C cycling due to the simultaneous assimilation of C and N by heterotrophic microorganisms during decomposition of SOM (Recous et al., 2000). Because decomposition is relatively slow in drylands, information is limited on long term cropping effects on SOC and total soil N in the Northern Great Plains (Sainju et al., 2006). Soil organic matter can be fractionated into various pools depending on its inherent characteristics. The organic matter portion of soil can be characterized in terms of a stable humus portion and a labile portion. The labile portion is made up of readily decomposable organic matter and of living organisms while the stable portion shows little change over time and is virtually unaffected by management practices (Janzen et al., 1997).

2.2.1. Crop rotational effects on labile soil organic matter pools

Soil organic matter that changes slowly over time may not be influenced by crop rotation (Campbell, 1992). However, labile organic matter pools can be considered as indicators of soil quality that are much more sensitive to changes in soil management practice (Haynes, 2005).

Dissolved organic matter (DOM) represents less than 0.2% of the total organic matter (Zsolnay, 1996) but because of its mobile and labile nature, it is perceived as being the most active SOM pool (Chantigny et al., 2008). Yet, even within this fraction there are varying degrees of lability (Zsolnay and Steindl, 1991). Dissolved organic matter is defined as the organic matter in solution that can pass through a 0.45 μm filter (Thurman, 1985; von Lützow et al., 2007). Plant litter, root exudates, SOM, and microbial biomass (MB) all serve as sources of inputs for DOM and the release of DOM into soil solution is assumed to be largely due to biotic processes and characteristics such as high microbial activity, high fungal abundance and any conditions that enhance mineralization, which increases DOM concentrations (Kalbitz et al. 2000). Reviews by Chantigny (2003) and Kalbitz et al. (2000) indicate that most work done on DOM has primarily taken place in forest soils. In forest situations it is well documented that substrate quality and quantity play an essential role in determining DOM concentrations. Chantigny (2003) noted a few agricultural studies (Mazzarino et al., 1993; Chantigny et al., 1997; Campbell et al., 1999) which reported that water extractable organic carbon was higher in rotations that included legumes. He suggested that this difference might be due to different root exudation patterns among crop species, further hypothesizing that legumes exude significantly more soluble molecules to signal their presence to *Rhizobia*.

The biologically active fractions of SOM, such as MB-C and MB-N, also change rapidly with time and can provide an early indication of slow changes in organic matter content as affected by crop additions (Bremner and van Kessel, 1992). In a review paper, von Lützow et al. (2007) found that MB turnover times were one to five years. In agricultural topsoil, soil microbial carbon ranges around 0.3 to 4% of SOC (Wardle, 1992). Chloroform fumigation extraction was developed to address the limitations of the chloroform incubation method (Vance et al., 1987) and gave the possibility of analyzing for microbial N as well as for C. In agricultural soils, MB-C is often within the range of

200-to 1000 $\mu\text{g biomass C g}^{-1}$ soil (Martens, 1995). Some authors have used the MB C:N ratio as an indication of whether the soil is predominantly colonized by bacteria or fungi. Ratios of 3:1 and 5:1 have been associated with bacterial communities and ratios of 4.5:1 to 15:1 have been associated with fungi (Paul and Clark, 1996).

Roots release considerable amounts of organic materials that can support growth of microbes in the rhizosphere by providing substrate for microbial biosynthesis and energy supply (van Veen et al., 1989). Because different plant species release different amounts and qualities of organic materials from their roots (Nguyen, 2003; Wichern et al., 2007), it is possible that different microbial groups and numbers would be associated with each plant species. However, the effect of crop rotation on MB and diversity is somewhat inconsistent. For example, on a Gray Luvisol at Breton, Alberta, a rotation of wheat-oat-barley (*Hordeum vulgare* L.) -forage-forage contained 117% more MB-N than a rotation of wheat-fallow (McGill et al., 1986). Yet, in Swift Current, SK, Campbell et al. (1999) reported that there was no effect of crop rotation on MB estimates and at the same site, Hamel et al. (2006) concluded that changes in MB estimates stem from dramatic events such as heavy moisture additions to a dry soil.

Effects of legumes in rotation on MB are equally inconsistent. Again, on a Gray Luvisol at Breton, Alberta, it was found that MB-C was generally higher in oat plots than in alfalfa (*Medicago sativa* L.) (Fyles et al., 1988). Similarly, an Orthic Brown Chernozem at Swift Current, Saskatchewan under continuous wheat had higher MB-C and MB-N content than the same soil with a wheat-lentil rotation (Biederbeck et al., 1994). At the same study site, Sainju et al. (2009) found that the inclusion of legumes, such as field pea, in wheat rotations also did not increase MB-N compared with other treatments, except for a spring-tilled wheat-fallow treatment. In contrast, Chang and Juma (1996) found that although MB-C content was not significantly affected by crop rotation, MB-N was significantly higher under barley following fababean (*Vicia faba* L.) than continuous barley. They also found a significant residual effect of fababean root residues from the previous year resulting in an increase in MB-C and MB-N at the spring sampling date.

2.2.2. Residue quantity and quality

Soil organic carbon status is strongly related to the quantity of crop residues returned to the field (Campbell et al., 2000); therefore, quantifying crop residue inputs will be crucial to our understanding of residue impact on SOM characteristics. Carbon returns to the soil also vary with different crop types. Sainju et al. (2006) found that the inclusion of lentil and field pea in rotation did not increase annual biomass returns to the soil; the residue amount was greater in rotations without pea and lentil. This was attributed to a lower amount of biomass being returned to the soil with pea and lentil followed by their rapid decomposition. Above-ground residue C returns are estimated to be 16 to 50% lower for pulses compared with spring wheat (Lemke et al., 2007).

The quality of crop residues from pulse crops may also vary substantially from cereal crops, thus influencing the amount of residue C stabilized as SOC (Lemke et al., 2007). Gunnarsson and Marstorp (2002) showed that by combining different qualities of residue it was possible to change the total amount of N mineralized and the time-course of mineralization during decomposition. The C:N ratio of crop residues strongly influences their decomposition rates and, in turn, N mineralization rates (Booth et al., 2005).

Residues with narrower C:N ratios are believed decompose more rapidly in soil and therefore promote mineralization (Ha et al., 2008). Typically the C:N ratio for pulse crop above-ground residues ranges from 25:1 to 40:1 while cereal is 70:1 to 100:1 (Stevenson and van Kessel, 1996a). A wide range of C:N ratios have been reported for canola above-ground residues ranging from 71:1 (Soon and Arshad, 2002) to 132:1 (Bhupinderpal-Singh et al., 2006). Despite reported differences in C:N ratios, Lupwayi et al. (2005) found that the N concentration of field pea above-ground residues after harvest was similar to canola and wheat residues, indicating that most of the N was removed with the field pea grain. Below-ground residues are a major proportion of crop residue additions to soil and also be taken into consideration. Although little information is available on the below-ground residue quality of crops, Soon and Arshad (2002) found that field pea root had more than twice the N concentration of canola and wheat roots. They also found that the dry matter loss from buried nylon bags of the pea roots was similar to the wheat and canola roots. Campbell et al. (2001) determined that there was

little difference in the rate of SOC decomposition among crops on the Canadian prairies but there have been suggestions that SOC loss may be mitigated by inclusion of pulses due to the efficient conversion of pulse residue C to SOC (Lemke et al., 2007). This suggestion was illustrated by Drinkwater et al. (1998) who concluded that the quantity of C inputs was not the major factor affecting soil C storage and despite having lower C inputs from above-ground sources, increases in soil C occurred in the legume based system compared to the conventional fertilizer based system. In summary, soil C additions from above- and below-ground residues are driven by the combined effects of both quantity and quality of specific crops. Although legume crops have been associated with increases in SOC, there are inconsistencies in reports regarding the potential of field pea to contribute to SOC.

Measuring residue quantity in annual cropping systems is often done solely on the measurement of above-ground residue. However, the main sources of C are rhizodeposition and crop residues including both above- and below-ground contributions (Recous et al., 1999). Although above-ground residue contributions are important, it has been suggested that the below-ground concentration of root residues should not be underestimated (van Kessel and Hartley, 2000). Root biomass N contribution is often difficult to quantify and most N balance studies do not take this into consideration leading to an underestimation of the legume N contribution (Walley et al., 2007). For example, Stevenson and van Kessel (1996a) used ^{15}N labeled field pea residues to assess N contributions and found that above-ground pea residue contributed an extra 6 to 14 kg N ha⁻¹ to the succeeding wheat crop compared to wheat residue. Similarly, there are not sufficient quantitative data available to derive an estimate of root C inputs for pulse crops (Lemke et al., 2007). Few studies take into account below-ground C additions due to the difficulty of isolating root biomass. Quantification of root biomass through conventional excavation and washing has been associated with losses of up to 36% of root biomass in spring wheat (Subedi et al., 2006). The use of isotopic techniques such as continuous labeling with ^{13}C has allowed for much progress in determining below-ground C translocation (Yevdokimov et al., 2006).

2.2.3. Methodological approaches to studying crop residue effects on carbon flow dynamics using isotopic tracers

Isotopic labeling of plant material with CO₂ has traditionally been accomplished with the radioactive ¹⁴C isotope. Recently, the number of studies using the stable isotope ¹³C has increased due to its safety in handling (Bromand et al., 2001) and improvement in analytical techniques (Recous et al., 2000). Isotopic methods for atmospherically labeling plants can be broadly classified as: (1) continuous labeling; (2) pulse labeling; and (3) repeat-pulse labeling (Hanson et al., 2000).

Continuous labeling is accomplished by the assimilation of ¹³CO₂ supplied at a constant rate over the life span of a plant. Hanson et al. (2000) describes the main advantage of continuous labeling over pulse labeling as a more homogenous labeling of plant C pools. Furthermore, steady state assumptions can be applied, which simplifies calculations. Continuous labeling allows for the estimations of the amount of C transferred by the plant into the soil and the below-ground pools during labeling. However, continuous labeling in the field is difficult because it requires equipment that is both cumbersome and expensive (Meharg, 1994). It is certain that best estimates of the total production of C by roots can be made when plants are exposed throughout their entire life cycle to an atmosphere maintained at a constant enrichment (Newman, 1985). However, pulse labeling is often more feasible for field measurements and if the data are interpreted cautiously additional information on C cycling processes may be gained (Keith et al., 1986).

Pulse labeling exposes the plant to a single pulse of ¹³CO₂ at some point during the plant's growth. The fundamental difference between continuous and pulse labeling is the distribution of label within the plant (Lynch and Whipps, 1990). Continuous labeling homogeneously labels the plant, whereas with pulse labeling, recently assimilated photosynthate in labile non-structural C pools will dominate (Meharg and Killham, 1988) and no pool will be labeled homogeneously (Meharg, 1994). It is generally assumed that newly assimilated C is quickly translocated throughout the plant depending upon species and stage of plant growth (Hanson et al., 2000). Because of this rapid translocation, pulse labeling may be used to follow rapid shifts in C fluxes with changing environmental conditions (Meharg, 1994).

Repeat-pulse labeling was developed to evade some of the logistical constraints of continuous labeling (Bromand et al., 2001). Repeat-pulse labeling is a variant of pulse labeling where isotopically labeled CO₂ is administered to plants at different times during the growing season. It is assumed that if the amount of label applied is proportional to the rate of photosynthesis at each labeling period, and the pulses occur at regular intervals over the entire growing season, then the label should be uniformly distributed in the plant (Bromand et al., 2001). Pulse labeling repeated at regular intervals with ¹³C₂O₂ has also been used to approximate cumulative below-ground C input (Subedi et al., 2006), to examine the rate of plant decomposition (Thompson, 1996) and to trace crop residue movement into different C pools (Bird et al., 2003; Williams et al., 2006; Moore-Kucera and Dick, 2008).

Using repeat-pulse labeling for tracing plant residue C through various C pools imposes important constraints on the interpretation of the data and it is essential that all plant pools are sufficiently homogeneous in ¹³C enrichment. Of the ¹³C repeat-pulse labeling studies mentioned, all were successful in obtaining sufficient levels of plant enrichments, but not in obtaining homogeneous labeling. Significant differences were obtained in the enrichment of either plant parts or biochemical fractions. In drawing conclusions on C flows from ¹³C repeat-pulse labeling, the implications of heterogeneously labeled plant material need to be better defined and accounted for when summarizing data.

2.2.4. Linking carbon and nitrogen cycling

The mineralization-immobilization of N in soil is linked to the cycling of C through the decomposition of organic matter when C and N are assimilated simultaneously by heterotrophic microflora (Recous et al., 2000). In turn, this will affect subsequent soil processes by providing a substrate, as in the case of nitrification. Because agricultural crops differ in both the quantity and quality of residue applied to the soil, it is important to study both C and N cycling in order to more fully understand the effects of cropping decisions on both C and N cycling. Quantification of C and N dynamics in pulse crop rotations for Saskatchewan conditions will clarify the environmental benefits of optimized crop rotations and enable producers to make crop rotation decisions

accordingly. Stable isotope techniques allow for more accurate measurement of C and N cycling. The objectives of this study are to: (1) quantify gross mineralization, gross nitrification and nitrifier N_2O flux in crop rotations with and without field pea; (2) investigate the differences in residue quantity and quality and the contribution to soil C as influenced by field pea versus canola; and (3) test the homogeneity of ^{13}C label within field pea and canola roots, stem, leaf and pod fractions as well as biochemical fractions by the repeat-pulse label method. These objectives were pursued by addressing the following hypothesis: Including field pea in the long-term crop rotation increases environmental and agronomic benefits compared to other rotations by increasing plant available N and decreasing greenhouse gas emissions.

3. THE EFFECT OF FIELD PEA IN ROTATION ON GROSS MINERALIZATION, NITRIFICATION AND NITRIFIER NITROUS OXIDE EMISSIONS

3.1. Abstract

Pulse crops in rotation improve the sustainability of cropping systems through biological N fixation, however studies indicate there may be additional benefits. To quantify these additional benefits, soil processes and properties related to N and C cycling were examined in five crop rotations with and without field pea at Scott, Saskatchewan. Stable ^{15}N isotope dilution and tracer techniques were used to quantify gross mineralization and nitrification rates and estimate the proportion of N_2O emissions derived from nitrification related processes rather than denitrification processes. Field incubations were performed in 2008 pre-seeding (May 13), anthesis (July 8) and just after harvest (October 8). Mean mineralization and nitrification rates were not significantly different between rotations on any date and ranged from 0.18 to 2.50 $\text{mg NH}_4^+\text{-N kg}^{-1} \text{d}^{-1}$ and 0.13 to 7.78 $\text{mg NO}_3^-\text{-N kg}^{-1} \text{d}^{-1}$ respectively. There were no significant differences in mean N_2O emissions among rotations on any given date although emissions at harvest were lower than emissions on the other two sampling dates. Nitrous oxide emissions ranged from 0 to 5.66 $\text{ng N}_2\text{O-N m}^2 \text{s}^{-1}$. Nitrification related processes were the main contributor to N_2O emissions, contributing 96.9 to 99.4% of total emissions from the K^{15}NO_3 labeled cores. With the exception of MB-C in the FP and canola-wheat-field pea (CNL-W-FP) rotation at anthesis, there was no effect of rotation on MB-C, MB-N and DOC. Based on the results obtained from a single growing season, our results show that the immediate benefit of including field pea in rotation in comparison to canola may be due simply to the effects of biological N_2 fixation on plant nutrition.

3.2. Introduction

Cropping systems in the Northern Great Plains have evolved considerably since the beginning of agricultural production. Management changes in these systems have a profound effect on agro-ecosystem sustainability due to the immensity of agricultural

production; Saskatchewan alone has over 26 million hectares of cropland in agricultural production (Statistics Canada, 2006).

Since there recently has been an unprecedented shift to oilseed and pulse crops in dryland cropping regions (Miller and Holmes, 2005) new interest has developed regarding the effects of these crops on soil processes, such as N cycling, and the effect of pulse crops on subsequent crops in rotation (Miller et al., 2006; Tanaka et al., 2007; Malhi et al., 2009). Depending on conditions, pulse crops such as pea (*Pisum sativum*) can biologically fix up to 90% of their total plant N from the atmosphere (Walley et al., 2007). In contrast, canola N requirements are commonly met through the use of synthetic N fertilizers at recommended application rates as high as 120 kg N ha⁻¹ for maximum seed yield (Gan et al., 2007). The reduced N input for the pea production year might not be the only benefit received from including pea in rotation. Yield benefits to crops following pea have been attributed to N mineralized from pea residue (Stevenson and van Kessel, 1996a; Beckie et al., 1997; Raun and Johnson, 1999; Johnston et al., 2005; Miller and Holmes, 2005).

For mineralization and nitrification to take place, optimum conditions relating to the population of organisms, pH, temperature, oxygen, moisture, and substrate concentration and availability are known to be important (Booth et al., 2005; Sahrawat, 2008). However, DOC is also considered to have an important role in the supply of soil-derived NH₄⁺ and thus the regulation of subsequent microbially-mediated N transformations (Murphy et al., 2000). Labile C substrates in DOC provide energy to heterotrophic microorganisms (Chantigny, 2003). Additionally, DOC has been reported to be higher in rotations that include legumes (Mazzarino et al., 1993; Chantigny et al., 1997; Campbell et al., 1999). Residues serve as an important source of water soluble C (Kalbitz et al., 2000) and residues with a low C:N ratio, such as legume crop residues, decompose more completely and may produce more DOC (Huang et al., 2004).

Higher turnover rates of SOC are also associated with microbial characteristics including population size, activity and composition (Cookson et al., 2005). There have been mixed reports regarding the effect of pulse crops on MB (Govaerts et al., 2007; Lupwayi et al., 2007; Lupwayi and Kennedy, 2007). Increases in microbial population size were measured in rotations including legumes as a green manure (Biederbeck et al.,

2005), and some researchers hypothesize that this increase should translate to an increase in MB with the inclusion of pulse crops in rotation (Lupwayi and Kennedy, 2007). However, decreased microbial populations in pulse-cereal rotations in comparison to cereal-cereal rotations have been reported (Biederbeck et al., 1994; Chan and Heenan, 1999).

Plant N availability is often controlled by net mineralization or net nitrification. Net measurements are calculated from the difference in the size of the NO_3^- or NH_4^+ pool at different times throughout the growing season. However, rates at which mineral N are released to and removed from N pools are important in regulating the nutrient supply to plants (Di et al., 2000). These rates can only be determined by stable isotope dilution techniques, which measure gross rates of NH_4^+ and NO_3^- production (Davidson et al., 1991; Hart et al., 1994; Booth et al., 2005). To our knowledge, there have been no reports of gross mineralization and gross nitrification rates as influenced by legumes in semi-arid regions of the Northern Great Plains.

Mineralization and nitrification, in addition to soil water content and temperature, influence the rate of N_2O emissions in agricultural soils (Grant et al., 2004). Nitrous oxide (N_2O) is a potent greenhouse gas with a global warming potential 296 times that of carbon dioxide (CO_2) and an atmospheric life time of 114 years (IPCC, 2001). In Canada, N_2O emissions resulting from transformations of mineral N in agricultural soils make up the largest proportion of total N_2O emissions (Rochette and McGinn, 2008). During 1990 to 2005, the Canadian cumulative mean N_2O emissions from agricultural soils were estimated to be $58.1 \text{ Gg N}_2\text{O-N yr}^{-1}$. Of these emissions, $13.7 \text{ Gg N}_2\text{O-N yr}^{-1}$ were attributed to applications of synthetic N fertilizers and equated to the largest direct source of N_2O contributions (Rochette et al., 2008). Nitrous oxide emissions from pulse crops may be lower than their fertilized counterparts (Lemke et al., 2002) likely due to decreased losses of N_2O from fertilizer applications but pulses may also affect the other factors contributing to emissions such as substrate supply as well as mineralization and nitrification of organic N.

Although N_2O emissions may be a result of several processes including nitrification, denitrification, coupled nitrification-denitrification, chemodenitrification, and nitrifier denitrification (Wrage et al., 2001), the two main processes believed to

contribute to soil surface N₂O flux in arable agriculture are denitrification and autotrophic nitrification (Pennock et al., 2006). Denitrification is associated with anaerobic soils with greater than 60% water-filled pore space, low N, and high C (Wrage et al., 2001); whereas autotrophic nitrification is associated with well-aerated soils, high N contents and low pH (Wrage et al., 2001; Booth et al., 2005). There are many reports of N₂O emissions in the semi-arid region of Canada (Corre et al., 1996; Malhi et al., 2006; Yates et al., 2006). However, there are few field experiments that report on the relative contributions of nitrification and denitrification to total N₂O emissions (Bedard-Haughn et al., 2006; Ma et al., 2008). Stable isotope techniques have enabled differentiation and quantification of N₂O produced during denitrification and nitrification (Baggs, 2008).

A greater understanding of the effect of field pea on N cycling processes such as mineralization, nitrification and N₂O production will help better delineate potential N benefits during the growing season and to succeeding crops. The objectives of this paper were to evaluate the effect of field pea in rotation on gross mineralization and nitrification rates and the relative contribution of nitrification related pathways to total N₂O emissions. In addition, rotational effects on soil MB and DOC were examined.

3.3. Experimental Procedures

3.3.1. Study site

The study was conducted at the Experimental Farm of Agriculture and Agri-Food Canada, at Scott, SK (52°13'48" N, 108°30'00" W) during the 2008 growing season. The soil type at Scott is a moderately acid Elstow loam, an Orthic Dark Brown Chernozem on glaciolacustrine parent material (Biederbeck et al., 1996). The topography at Scott is level, very gently sloping to undulating (Clayton and Schroer, 1948). The 0 to 15 cm depth had a pH of 5.5 (Soon et al., 2008). Winter precipitation (1 Nov. 2007 to 31 Mar. 2008) was 85.1 mm, which was above the normal of 77.2 mm. Precipitation from 1. Apr. 2008 to 31 Oct. 2008 was 313.4 mm which was also slightly above the normal of 281.7 for this time period (Table 3.1) (Environment Canada, 2009a). Rain fall events in the week prior to the sampling dates (May 13, July 8, October 8) were minor and included 8mm on May 11, 0.2 mm on May 12, 2.4 mm on July 6, 6.6 mm on July 7, 3.6 mm on

October 5, 6.7 mm on October 6 and 10.5 mm on October 7 (Environment Canada, 2009b).

Table 3.1. Precipitation monthly averages at Scott, Saskatchewan from 1 Apr. 2008 to 31 Oct. 2008 in comparison to the Canadian climate normals (1971-2000) (Environment Canada, 2009a; Environment Canada, 2009b).

	Apr.	May	June	July	Aug.	Sept.	Oct.	Total
Monthly average precipitation 2008 (mm)	72.4	13.0	87.0	85.8	20.8	10.0	24.4	313.4
Scott climate normals (1971-2000) (mm)	23.6	35.9	62.5	70.9	43.1	31.4	14.3	281.7

3.3.2. Rotations

The long-term experiment, established by Agriculture and Agri-Food Canada in 1998, is a randomized complete block design with four replications. Each plot measured 6.4 by 30.5 m. The design included seven different crop rotations seeded in different rotation phases. For this experiment, five of the seven rotations, in either the field pea or the canola phase of the rotation, were used for field measurements of gross nitrification, mineralization, and N₂O emissions. Rotations included continuous canola (CNL), continuous field pea (FP), wheat-canola (W-CNL), wheat-field pea (W-FP), and canola-wheat-field pea (CNL-W-FP). During the year of this study (2008), all plots were sprayed with glyphosate (Roundup Transorb[®]) at 1.85 L ha⁻¹ on May 8. Plots were seeded May 14 with ‘CDC Golden’ pea at a rate of 224 kg ha⁻¹ and ‘Invigor 5020’ canola at a rate of 6.7 kg ha⁻¹. A granular pea *Rhizobium* inoculant, ‘Nodulator[®]’, (Becker Underwood Inc., Saskatoon, SK) was banded with the seed at the recommended rate of 7.84 kg ha⁻¹. Canola received P at a rate of 15.2 kg P ha⁻¹ as mono-ammonium phosphate fertilizer (11-52-0) and N as both mono-ammonium phosphate fertilizer and urea (46-0-0) at a combined rate of 74.4 kg N ha⁻¹ which was side banded. On June 5 field pea plots were sprayed with metribuzin, (Sencor[®] 75 DF) at 190 g ha⁻¹ and 2-methyl-4-chlorophenoxyacetic acid (MCPA Sodium 300) at 0.47 L ha⁻¹. On June 6, canola plots received an application of glufosinate ammonium (Liberty[®]) at 3.33 L ha⁻¹ and clethodim (Select[®]) at 63 mL ha⁻¹. Field pea plots were sprayed again on June 13 with Quizalofop

P-Ethyl (Assure[®] II) at 0.37 L ha⁻¹. On September 3, canola was desiccated with diquat dibromide (Reglone[®]) at 1.23 L ha⁻¹.

3.3.3. Quantification of gross mineralization and nitrification

For each rotation, gross mineralization and nitrification were measured three times during the growing season: pre-seeding (May 13), anthesis (July 8) and post harvest (October 8). Specifically, at anthesis, according to the Biologische, Bundesanstalt, Bundessortenamt and Chemical (BBCH) decimal system of growth stages, canola was at stage 65 (full flowering: 50% of flowers open) and field pea was at stage 62 (20% of flowers open). In each of the five rotations, five intact soil cores (15 cm x 5 cm i.d.) were taken adjacent to one another between crop rows in order to minimize spatial variability. Crop residue was gently removed from the soil surface before core sampling. One of these soil cores was used to determine gravimetric soil moisture content, total C, DOC, background levels of NH₄⁺ and NO₃⁻ and soil microbial C and N. Microbial biomass was estimated on anthesis and post-harvest samples only.

The remaining four cores were used to determine gross mineralization and nitrification with the N isotope dilution technique (Davidson et al., 1991). Briefly, immediately after collection, two cores were injected with (¹⁵NH₄)₂SO₄ for gross mineralization measurements and two cores were injected with K¹⁵NO₃ for gross nitrification measurements. An 18 gauge side-port spinal needle (Cadence Science, Lake Success, NY) was used to inject seven 2-mL injections of ¹⁵N solution into each core (30 µg N mL⁻¹ at 99 atom % ¹⁵N). Gravimetric moisture content increased on average 4.2% above the moisture content of the unlabeled cores. Due to sampling time constraints unlabeled cores were unaltered. As well, unlabeled cores were used for measuring additional soil properties described in section 3.3.5. Within one hour of injection, one core from the (¹⁵NH₄)₂SO₄ labeled pair and one core from the K¹⁵NO₃ labeled pair was extruded and homogenized. A 30 g soil subsample was taken and extracted with 100 mL of 2 mol L⁻¹ KCl. The other core from each pair was buried to a depth of approximately 20 cm in the field for a 24 h (T₂₄) incubation before being extracted.

Subsamples of the KCl extracts were analyzed colorimetrically for NH₄⁺ and NO₃⁻ concentration using a Technicon Auto Analyzer (Technicon Industrial Systems,

1978). Extracts also were analyzed for $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ using an acidified diffusion disk procedure as described by Hart et al. (1994) with the modification of using a polytetrafluoroethylene (PTFE) encased acid disk (Sørensen and Jensen, 1991). Following diffusion, acid disks were removed and analyzed for N concentration and atom % ^{15}N using an ANCA–GSL elemental analyzer coupled to a continuous flow Tracer/20 mass spectrometer (Europa Scientific, SerCon Ltd., Cheshire, UK).

Gross mineralization and gross nitrification were calculated as described by Kirkham and Bartholomew (1955). The rate of NH_4^+ and NO_3^- turnover (i.e., mean residence time) was calculated using the ratio of NH_4^+ or NO_3^- pool size to gross mineralization or nitrification rates. All observations with negative rates of either gross mineralization rates or gross nitrification rates were omitted from the data set. There are a number of assumptions regarding the tracer kinetic equations proposed by Kirkham and Bartholomew (1955) and negative rates were likely due to a violation of these assumptions. The assumptions of the calculations include: all rate processes can be described by zero-order kinetics over the incubation period; there is no isotopic discrimination of the mineral nutrient during transformation processes in the soil; labeled mineral N immobilized over the incubation period is not remineralized; the added label is homogeneously mixed with the soil inorganic pool (Hart et al., 1994).

3.3.4. Quantification of N_2O flux

Nitrous oxide flux from nitrification related pathways, was determined by the procedure described by Bedard-Haughn et al. (2006). The T_{24} core of the K^{15}NO_3 labeled pair was placed into a 1.5L Mason jar fitted with a septa in the lid. For N_2O measurements, ~ 15 min after sealing the jar, time zero ($\text{T}_{0.25}$) headspace samples were taken from each jar using a 20 mL syringe and injected into a Labco Exetainer[®] vial (Labco, Limited, UK). The jars were then opened briefly for ~5 min to adjust the internal atmospheric pressure to ambient and to detect any N_2O flux from core disturbance, then resealed and buried for a field incubation of 24 h. After 24 h, the jars were excavated and T_{24} headspace samples were taken. Samples were analyzed for total N_2 and N_2O as well as $^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ at UC Davis Stable Isotope facility using a SerCon CryoPrep trace gas

concentration system interfaced to a PDZ Europa 20-20 mass spectrometer (SerCon Ltd., Cheshire, UK).

Ambient air samples were included as references in each analytical run to check for precision, detector drift and to calculate the minimum detectable concentration difference (MDCD). The MDCD was calculated according to Yates et al. (2006):

$$\text{MDCD} = \mu_{\text{pair diff}} + (2\sigma_{\text{pair diff}}). \quad [3.1]$$

Where:

μ = average difference between sample pairs

σ = standard deviation between sample pairs

If the N₂O flux was less than the MDCD then the flux was considered to be not significantly different than 0. The contribution of nitrification and denitrification to N₂O flux was calculated (Arah, 1997; Russow et al., 2008). An unlabeled set of soil cores was used to establish background levels of gas emissions during the incubation to determine whether there was an increase in emissions due to the method used.

3.3.5. Total carbon, dissolved organic carbon and microbial biomass

Total soil organic C was determined by dry combustion at 840°C in a LECO CR-12 Carbon System (781-600) (Leco Corporation, St. Joseph, MI), according to Skjemstad and Baldock (2008). Plant residues (pod, stem and leaf) were analyzed for total C as well as N. Above-ground canola and field pea residues were collected at harvest and analyzed for C and N using a LECO CNS-2000 analyzer (LECO Instruments Ltd., St. Joseph, MI). Dissolved organic carbon was determined using the method outlined by Chantigny et al. (2008) with the exception that the DOC fraction was defined as that organic C passing through a 45 µm filter (Thurman, 1985; von Lützow et al., 2007). Briefly, 10 mL of 5 mmol L⁻¹ CaCl₂ solution was added to 5 g soil samples and stirred for one minute. The samples were centrifuged for 20 min at 3000 rpm (Beckman Model TJ-6, Beckman Instruments Inc., Palo Alto, CA) and the supernatant then poured into a 20 cc syringe with an attached 45 µm filter. The filtrate was collected and was analyzed by combustion using a total organic carbon analyzer (Shimadzu TOC-VCPN, Shimadzu, Columbia, MD).

Field-moist stored soils (4°C) were incubated for a 10 d period at room temperature and then MB-C and MB-N were determined following the fumigation-extraction procedure (Voroney et al., 2008). Organic C and total N was determined using a Shimadzu TOC-VCPN (Shimadzu, Columbia, MD). Biomass C and N were calculated using K_{EC} factor of 0.41 (Joergensen, 1996) and a K_{EN} factor of 0.4 (Joergensen and Mueller, 1996) which represent the fraction of microbial N and C rendered extractable after biocidal (chloroform) fumigation.

3.3.6. Statistics

All data were checked for normality using the frequency distribution histogram and the Kolmogorov-Smirnov test ($p < 0.05$). Mineralization, nitrification and N_2O flux were analyzed using the non-parametric Kruskal Wallis Test and differences between individual rotations were tested for statistical significance using the Mann-Whitney-U procedure in SPSS version 17.0 for Windows (SPSS INC., 2007). One-way ANOVA was used to identify treatment differences for MB, DOC, NH_4^+ and NO_3^- concentrations. The Ryan-Einot-Gabriel-Welsh multiple range test (REGWQ) was used for rotation means separation. Relationships between soil properties and processes were also examined using Spearman's rank correlation (SPSS INC., 2007). Effects were declared significant at $p < 0.10$ unless otherwise specified. Because of the inherent variability of field studies, a 0.10 level of significance was chosen to increase the likelihood that differences would be detected.

3.4. Results

3.4.1. Nitrogen cycling

Mean gross mineralization rates ranged from 0.18 to 2.50 mg NH_4^+ -N $kg^{-1} d^{-1}$. Rotation had no significant effect on gross mineralization rates on any of the sampling dates ($p > 0.1$) (Fig. 3.1). At anthesis, mineralization rates for W-CNL and CNL rotations were higher than the other rotations. At post-harvest, mineralization rates increased relative to pre-seeding and anthesis for all rotations except the CNL rotation. Over the growing season, there appears to be a slight trend of increasing mineralization rates in the rotations including field pea, but not in the canola rotations. There was no correlation

between soil NH_4^+ concentrations and mineralization rates ($r_s=0.154$, $p=0.312$). The soil NH_4^+ concentrations were significantly different ($p < 0.05$) between the dates with pre-seeding < post-harvest < anthesis (Table 3.1). Ammonium turnover times range from 0.17 to 3.73 d (Table 3.1). For each rotation the NH_4^+ turnover times were most rapid at pre-seeding and slower at anthesis and post-harvest, with the exception of the CNL-W-FP rotation where the reverse was observed.

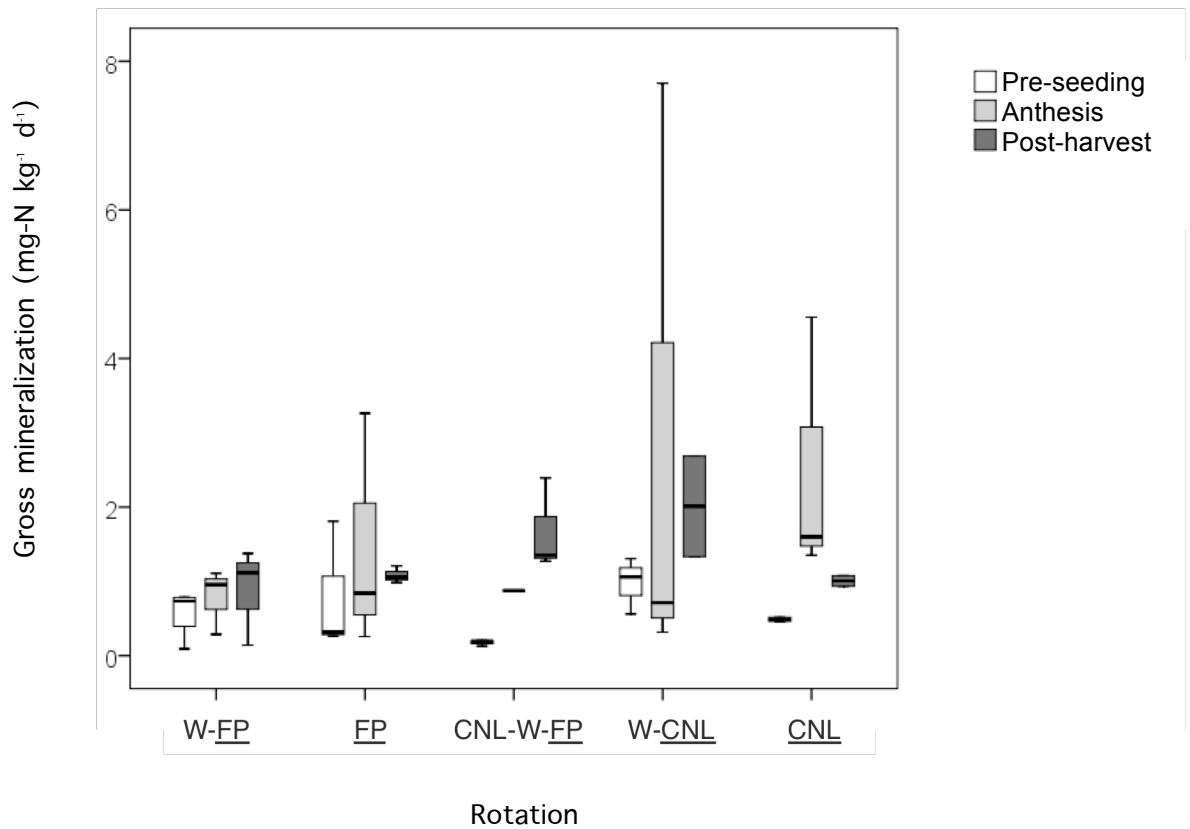


Fig. 3.1. Gross mineralization rates of rotations at pre-seeding, anthesis, and post-harvest grouped according to rotation. Boxplots indicate the median and upper and lower quartiles; the vertical bars indicate the most extreme values (Underlining indicates the crop phase sampled; W-FP=wheat-field pea, FP=continuous field pea, CNL-W-FP=canola-wheat-field pea, W-CNL=wheat-canola, CNL=continuous canola).

Table 3.2. Soil NH_4^+ levels and NH_4^+ turnover for rotations with and without field pea at pre-seeding, anthesis and post-harvest.

Date†	Rotation‡	mg NH_4^+ -N kg^{-1} soil		NH_4^+ turnover (d)	
		n	Mean§	n	Mean
Pre-seeding	<u>W-FP</u>	3	0.60 (0.09)	4	1.23 (0.95)
	<u>FP</u>	4	1.16 (0.20)	4	1.02 (0.50)
	CNL-W- <u>FP</u>	4	0.99 (0.38)	3	3.73 (1.45)
	W- <u>CNL</u>	4	0.98 (0.33)	3	0.17 (0.05)
	<u>CNL</u>	4	0.67 (0.31)	3	0.23 (0.22)
Anthesis	<u>W-FP</u>	4	2.24 (0.95)	3	3.43 (1.61)
	<u>FP</u>	4	1.90 (0.45)	3	3.60 (2.57)
	CNL-W- <u>FP</u>	2	2.44 (1.92)	1	1.18
	W- <u>CNL</u>	4	1.65 (0.53)	4	3.31 (0.92)
	<u>CNL</u>	4	2.05 (0.35)	3	1.29 (0.30)
Post-harvest	<u>W-FP</u>	4	0.91 (0.33)	4	2.21 (0.95)
	<u>FP</u>	3	0.84 (0.27)	3	1.17 (0.44)
	CNL-W- <u>FP</u>	4	0.90 (0.31)	3	1.60 (0.56)
	W- <u>CNL</u>	4	1.47 (0.52)	2	1.31 (0.35)
	<u>CNL</u>	3	1.27 (0.61)	2	1.31 (0.40)

†Pre-seeding = May 13, Anthesis = July 8, Post-harvest = October 8

‡ Underlining indicates the crop phase sampled; W-FP=wheat-field pea, FP=continuous field pea, CNL-W-FP=canola-wheat-field pea, W-CNL=wheat-canola, CNL=continuous canola

§Mean (SE) values for each rotation on a given date

Gross nitrification rates ranged from 0.13 to 7.78 mg N kg^{-1} d^{-1} (Fig. 3.2). Similar to mineralization, rotation had no significant effect on gross nitrification rates on any of the sampling dates ($p > 0.1$). As well, sampling date had no significant effect on nitrification rate ($p > 0.1$); however, the CNL rotation had a higher mean nitrification rate and higher variability at anthesis than the other rotations.

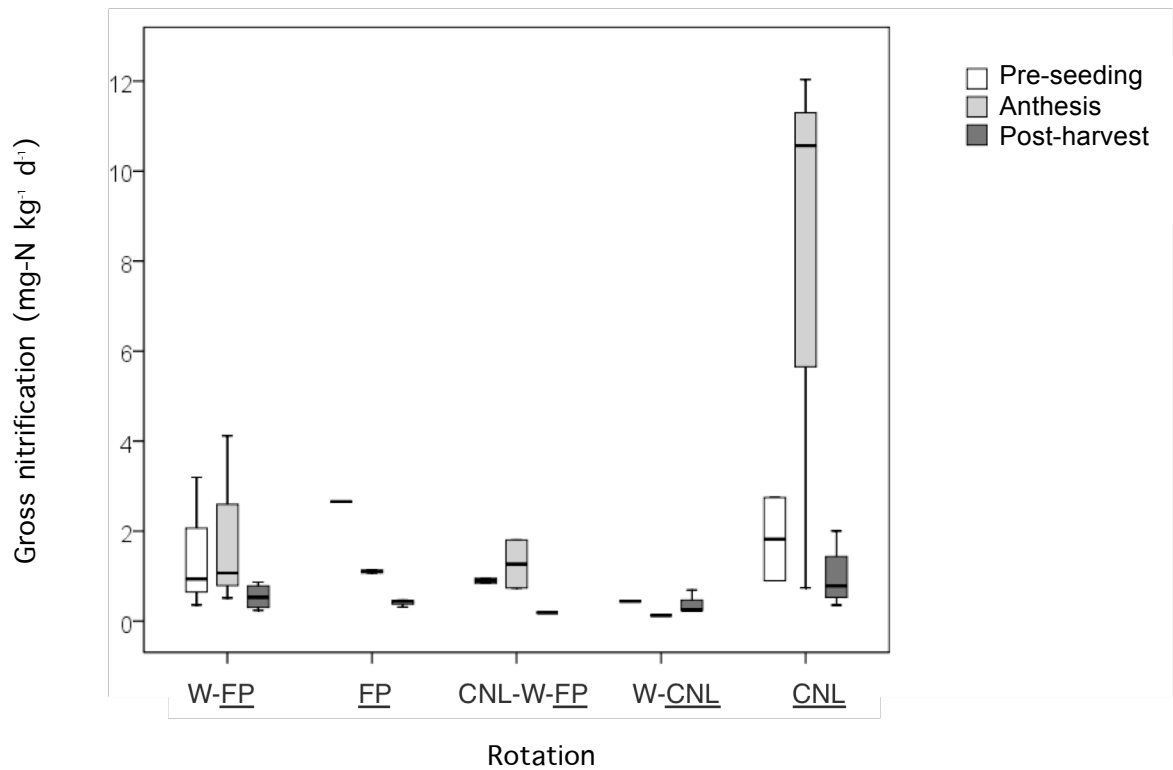


Fig. 3.2. Gross nitrification rates of rotations at pre-seeding, anthesis, and post-harvest grouped according to rotation. Boxplots indicate the median and upper and lower quartiles; the vertical bars indicate the most extreme values (Underlining indicates the crop phase sampled; W-FP=wheat-field pea, FP=continuous field pea, CNL-W-FP=canola-wheat-field pea, W-CNL=wheat-canola, CNL=continuous canola).

The only significant difference in NO_3^- concentration was in the CNL-W-FP rotation at anthesis (Table 3.2). The mean NO_3^- soil concentration at pre-seeding and post-harvest were somewhat higher in the rotations including field pea but this pattern was not reflected in the nitrification rates. There was no correlation between NO_3^- soil concentration and gross nitrification rate ($r_s=0.086$, $p=0.61$). Turnover times were longer at post-harvest than on the other sampling dates (Table 3.2). Overall turnover times ranged from 0.7 to 22.1 days.

Table 3.3. Soil NO₃⁻ levels and NO₃⁻ turnover for rotations with and without field pea at pre-seeding, anthesis, and post-harvest.

Date†	Rotation‡	mg NO ₃ ⁻ -N kg ⁻¹ soil		NO ₃ ⁻ turnover (d)	
		n	Mean§	n	Mean
Pre-seeding	W- <u>FP</u>	4	0.8 (0.1)	3	5.5 (1.4)
	<u>FP</u>	3	0.9 (0.2)	1	6.7
	CNL-W- <u>FP</u>	4	0.8 (0.1)	2	5.4 (0.5)
	W- <u>CNL</u>	4	0.6 (0.1)	2	3.9 (2.7)
	<u>CNL</u>	3	0.6 (0.2)	2	3.1 (0.4)
Anthesis	W- <u>FP</u>	4	0.4 (0.1)	3	2.7 (2.0)
	<u>FP</u>	4	1.0 (0.2)	2	1.6 (0.8)
	CNL-W- <u>FP</u>	4	2.6 (1.1)	2	4.2 (2.8)
	W- <u>CNL</u>	4	0.4 (0.2)	1	4.8
	<u>CNL</u>	4	1.4 (0.4)	3	0.7 (0.3)
Post-harvest	W- <u>FP</u>	4	1.1 (0.5)	4	11.9 (3.6)
	<u>FP</u>	4	1.6 (0.4)	3	15.8 (4.5)
	CNL-W- <u>FP</u>	4	2.0 (0.6)	1	22.1
	W- <u>CNL</u>	4	0.8 (0.1)	3	17.1 (5.2)
	<u>CNL</u>	4	1.0 (0.0)	4	6.5 (1.6)

†Pre-seeding = May 13, Anthesis = July 8, Post-harvest = October 8

‡ Underlining indicates the crop phase sampled; W-FP=wheat-field pea, FP=continuous field pea, CNL-W-FP=canola-wheat-field pea, W-CNL=wheat-canola, CNL=continuous canola

§Mean (SE) values for each rotation on a given date

The mean N₂O emissions after 24 h incubation were low for all sampling dates and ranged from 0 to 5.7 ng N₂O-N m² s⁻¹ (Table 3.3). At each sampling date, there was no significant difference in N₂O emissions between the rotations. There was, however, a significant effect of the added label which caused a flush of N₂O emissions above and beyond the background levels for all dates and sampling times (T₂₄ and T_{0.25}) except for T_{0.25} at pre-seeding. Moisture content at pre-seeding was 10.7 and 12.1% higher than at anthesis and post-harvest, respectively, which is why there might not have been an immediate effect of the added enriched solution at pre-seeding for the T_{0.25} samples. Mean WFPS of the T₂₄ K¹⁵NO₃ labeled cores at pre-seeding, anthesis and post-harvest was 39.9%, 24.6% and 23.1% respectively. There were significant correlations between soil NH₄⁺ concentrations and the N₂O flux from the labeled cores at T_{0.25} (r_s=0.455, p=0.001), the non-labeled cores at T_{0.25} (r_s=0.546, p=0.0001), and the non-labeled cores at T₂₄

($r_s=0.372$, $p=0.005$). Overall, nitrification related processes contributed 96.9-99.4 % of the N₂O flux.

Table 3.4. Nitrous oxide (N₂O) emissions from intact soil cores with (labeled) and without (background = BG) added K¹⁵NO₃ (n=4). Gas samples were taken at time zero (T_{0.25}) and after a 24 h field incubation (T₂₄). The proportion of the N₂O flux attributed to nitrification related processes is shown (% Nit).

Date†	Rotation‡	T _{0.25} N ₂ O Flux (ng N ₂ O-N m ² s ⁻¹) §			T ₂₄ N ₂ O Flux (ng N ₂ O-N m ² s ⁻¹)		
		BG	Labeled	% Nit	BG	Labeled	% Nit
Pre-seeding	W- <u>FP</u>	0 ¶	0	99.6 (0.2)	0.4 (0.4)	1.6 (0.6)	97.7 (1.1)
	<u>FP</u>	0	0	99.6 (0.0)	0.4 (0.4)	5.7 (3.3)	96.9 (2.3)
	CNL-W- <u>FP</u>	15.1 (15.1)	0	99.5 (0.0)	0.5 (0.5)	3.4 (1.9)	98.7 (0.4)
	W- <u>CNL</u>	0	0	99.4 (0.1)	0.4 (0.3)	2.0 (0.7)	98.0 (0.8)
	<u>CNL</u>	0	0	99.6 (0.0)	0.9 (0.9)	1.5 (0.6)	98.0 (0.6)
Anthesis	W- <u>FP</u>	10.7 (3.6)	79.6 (31.9)	99.5 (0.0)	0.3 (0.1)	1.8 (0.4)	99.4 (0.0)
	<u>FP</u>	9.6 (4.3)	65.8 (8.6)	99.5 (0.0)	0.3 (0.1)	1.6 (0.3)	99.3 (0.1)
	CNL-W- <u>FP</u>	6.8 (4.9)	91.3 (30.6)	99.5 (0.0)	3.8 (2.0)	3.2 (0.5)	98.8 (0.6)
	W- <u>CNL</u>	3.6 (3.6)	80.0 (29.3)	99.5 (0.1)	0.8 (0.5)	2.1 (0.5)	99.0 (0.4)
	<u>CNL</u>	17.2 (5.8)	79.8 (15.2)	99.4 (0.1)	1.0 (0.4)	4.2 (2.1)	99.0 (0.4)
Post-harvest	W- <u>FP</u>	0	28.0 (11.3)	99.5 (0.0)	0.3 (0.3)	0.5 (0.3)	98.8 (0.3)
	<u>FP</u>	0	51.6 (10.3)	99.6 (0.0)	0	0	99.4 (0.0)
	CNL-W- <u>FP</u>	0.4 (0.2)	14.2 (8.2)	99.5 (0.0)	0	0.4 (0.2)	98.6 (0.6)
	W- <u>CNL</u>	0	23.2 (13.4)	99.5 (0.0)	0	0.4 (0.2)	98.9 (0.2)
	<u>CNL</u>	0	41.8 (6.1)	99.5 (0.0)	0	0.2 (0.2)	99.2 (0.1)

† Pre-seeding = May 13, Anthesis = July 8, Post-harvest = October 8

‡ Underlining indicates the crop phase sampled; W-FP=wheat-field pea, FP=continuous field pea, CNL-W-FP=canola-wheat-field pea, W-CNL=wheat-canola, CNL=continuous canola

§ Mean (SE) values for each rotation on a given date

¶ The 0 values for N₂O flux represent the values where emissions were < MDCD.

3.4.2. Total carbon, dissolved organic carbon and microbial biomass

There was no difference among the rotations for total SOC. Total SOC averaged for all rotations was 2.78%. The above-ground C:N ratio of field pea and canola residues (pod, stem, and leaves) was 58:1 and 144:1, respectively.

Mean MB-N values for each rotation at each sampling date ranged from 10.9 to 42.8 µg N g⁻¹ soil (Table 3.4). Although mean MB-N appeared slightly higher for the FP and W-FP rotations at post-harvest, there was no significant difference in MB-N among the rotations at either date. The only significant difference in MB-C was between FP and

CNL-W-FP at anthesis. Overall, mean values for MB-C ranged from 140 to 200 $\mu\text{g C g}^{-1}$ soil. Although there were no significant differences in MB C:N, all ratios increased from anthesis to post-harvest except for the FP (Table 3.4).

Table 3.5. Soil microbial biomass C and N pool sizes at anthesis and post-harvest as determined by fumigation-extraction, using a K_{EC} factor of 0.41 and a K_{EN} factor of 0.4.

Date†	Rotation‡	MB-C ($\mu\text{g g}^{-1}$ soil)		MB-N ($\mu\text{g g}^{-1}$ soil)		C:N	
		n	Mean	n	Mean	n	Mean
Anthesis	W- <u>FP</u>	4	191.7 (35.4)	4	19.1 (9.4)	4	11.9 (6.1)
	<u>FP</u>	4	200.1 (53.8)	4	27.6 (17.8)	4	8.4 (2.7)
	CNL-W- <u>FP</u>	3	130.4 (29.3)	4	10.9 (9.8)	3	8.9 (8.1)
	W- <u>CNL</u>	4	140.0 (23.3)	3	19.3 (11.0)	3	7.6 (4.5)
	<u>CNL</u>	4	155.3 (42.7)	4	17.3 (3.4)	4	9.2 (2.9)
Post-harvest	W- <u>FP</u>	4	221.1 (68.4)	3	42.8 (16.2)	3	6.0 (1.0)
	<u>FP</u>	4	142.5 (55.0)	3	32.3 (13.3)	3	5.5 (1.4)
	CNL-W- <u>FP</u>	4	166.5 (63.3)	3	31.8 (27.1)	3	10.6 (9.4)
	W- <u>CNL</u>	4	164.4 (86.8)	3	29.1 (19.8)	3	6.6 (2.8)
	<u>CNL</u>	4	202.9 (70.5)	3	30.6 (24.1)	3	12.3 (11.8)

† Anthesis = July 8, Post-harvest = October 8

‡ Underlining indicates the crop phase sampled; W-FP=wheat-field pea, FP=continuous field pea, CNL-W-FP=canola-wheat-field pea, W-CNL=wheat-canola, CNL=continuous canola

§Mean (SE) values for each rotation on a given date

There was no significant difference among rotations for DOC levels. However, a seasonal effect was observed. Mean DOC at pre-seeding was 19.6 (\pm 1.2) mg DOC kg^{-1} soil and was significantly lower ($p = 0.01$) than DOC levels at anthesis and post-harvest which averaged 41.7 (\pm 2.4) mg DOC kg^{-1} soil and 45.6 (\pm 3.7) mg DOC kg^{-1} soil, respectively.

3.5. Discussion

3.5.1. Gross mineralization and nitrification

There is a general acceptance that including legumes in rotation will result in N benefits that extend beyond N fixation including reduced inorganic N-uptake by legumes in comparison with non-legumes, increased plant residue quality, SOM turnover, and the amount and quality of rhizodeposits (Chalk, 1998; Mayer et al., 2003; Lemke et al., 2007), all of which should be reflected in rates of N cycling. However, we found no difference among rotations with field pea versus canola in gross mineralization and

nitrification rates, although the overall rates were within the range of previously reported values for semi-arid agricultural land (Booth et al., 2005). Most reports of N benefits from legumes have been conducted based on a comparison of cereal versus pulse crops (Lemke et al., 2007). Interestingly, accumulation of mineral N has been associated with both canola and pulse crops (Kirkegaard et al., 1999; Ryan et al., 2006). Therefore the net effect of incorporating either of these crops into a rotation may result in no apparent difference during the growing season in the measured N cycling processes. However, the processes leading to the accumulation of mineral N have been speculated to be quite different between the two crops (Kirkegaard et al. 1999) and there are key trends within the data that warrant further consideration.

Mineralization rates for field pea rotations, although not significantly different, tended to increase over the growing season. Substrate availability is a major factor regulating inorganic N production. Ofosu-Budu et al. (1990) reported that during pod filling in pulse crops, large quantity of readily mineralizable N-compounds are lost from roots. Wichern et al. (2007) found that the inorganic N content at crop maturity - and the proportion derived from rhizodeposition - was much higher under pea than oat, and that during the reproductive growth phase, existing roots die and therefore contribute to rhizodeposition which could further drive mineralization. The increasing mineralization rates over the growing season seen in the field pea rotations may therefore reflect the cumulative rhizodeposition that occurs during the vegetative phase resulting in higher gross mineralization rates at anthesis. At harvest, this rhizodeposition effect would be augmented by root mortality and loss of readily mineralizable N-compounds. This slight trend of increasing mineralization rates supports claims from researchers of N benefits to crops succeeding legumes.

In addition to these rhizosphere effects on soil N status, Miller et al. (2003a) suggested that the higher residual fall soil NO_3^- -N concentrations found with pulse crops compared to wheat may be attributed to lower root density and depth of pulse crops, which may cause these plants to use N more conservatively resulting in higher soil NO_3^- -N. There have also been reports of narrower C:N ratios in pulse crop root and shoot biomass, which enhances N availability in the soil (Miller et al., 2006). However, Lupwayi and Soon (2009) found that Camry pea (a semi-leafless variety) exported most

(66%) of its N to the seed leaving the residues with a higher C:N ratio. The average C:N ratio of field pea residues in our study was 58:1 and canola was 144:1. These ratios are wider in comparison to other researchers (Soon and Arshad, 2002) but Bhupinderpal-Singh et al. (2006) reported a C:N ratio for canola of 132:1 and Miller et al. (2006) reported C:N ratios for pea to be 56. Therefore in comparison to other pulse crops, it is possible that field pea has a relatively high C:N ratio, therefore minimizing differences in gross mineralization and nitrification rates in comparison to canola. In terms of residue decomposition, Lupwayi and Soon (2009) found that only 15% of the residue N was released in the first 14 to 22 weeks of pea residue decomposition, whereas faba bean released 70% of residue N after this same time period. Therefore, field pea, relative to other pulse crops, may have less of an impact on N cycling processes with reference to cereal and oilseeds crops.

Lack of differences in gross mineralization rates and gross nitrification rates between field pea and canola rotations may also be due to the hydrolysis of glucosinolates that are contained in Brassicaceous plant tissues, such as canola. Upon hydrolysis, toxic compounds, including isothiocyanates (ITCs), are formed which have a biofumigation effect on soil organisms (Bending and Lincoln, 2000). It has been suggested this biofumigation effect results in a flush of N mineralization following the degradation of ITCs, possibly from the degraded tissues of fumigated organisms (Bending and Lincoln, 2000). In contrast, Rumberger and Marschner (2004) reported that lower ITC levels may not lead to biofumigation but may have other effects on soil biota and Ryan et al. (2006) reported that changes in soil mineral N status was unlikely due to biofumigation by ITCs. Therefore, there may be a range of factors affecting changes in soil properties that occurs after canola flowering including differences in the chemical constituents of root or shoot tissues, ITC release or other factors inducing a gradual shift in microbial populations under *Brassica* crops during the growing season. In addition, a considerable amount of shed *Brassica* leaf material may influence N cycling during anthesis (Ryan et al., 2006). Senesced leaf material, which begins to drop at the emergence of flower buds, (Hocking et al., 1997; Malagoli et al., 2005) contains 17.5 kg ha⁻¹ of N of which a proportion could be re-adsorbed by surface roots following rainfall prior to harvest (Hocking et al., 1997). Within our data, there is a wide range of gross

mineralization rates at anthesis in both the W-CNL and CNL rotations. Furthermore, gross nitrification values for the CNL rotation are also high and quite variable and had faster NO_3^- turnover at anthesis and post-harvest. It may be possible that the high and highly variable gross mineralization and nitrification rates in the CNL and W-CNL rotations and the increased turnover NO_3^- in the CNL rotations at anthesis are due to a combined effect of accumulation of ITCs and N release from senesced leaf materials after rainfall events (Table 3.1). It is difficult to assess the potential effect of these compounds in the W-CNL gross mineralization rates because of the small sample size at anthesis in this rotation.

3.5.2. Nitrous oxide emissions

The N_2O emissions in this study were in the lower range of emissions according to measurements for semi-arid agricultural systems within the Canadian Prairies, ranging from mean fluxes of 97.2 to $-0.2 \text{ ng N}_2\text{O-N m}^{-2} \text{ s}^{-1}$ (Corre et al., 1996; Lemke et al., 1998; Yates et al., 2006). Since there have been many reports of soil amended with different crops residues and the corresponding differences in N_2O emissions (Aulakh et al., 1991; McKenney et al., 1993; Shelp et al., 2000), it was unexpected that we found no significant affect of crop rotation. Lemke et al. (2002) reported that during the growing season, N_2O emissions from field pea and lentil crops were significantly lower than from fertilized cereal crops and found that emissions generally reflected N fertilizer inputs. Nitrogen fertilizer applications to agricultural soils normally increase N oxide emission (Mosier et al., 2006). In our study, fertilization of crop rotations occurred just after the initial N_2O measurement at pre-seeding so any flux attributed to applied N most likely was missed.

Negative correlations have been reported between C:N ratios of crop residues and N_2O emissions (Baggs et al., 2000; Huang et al., 2004). Pulse residues are reported to have lower C:N ratios relative to wheat and oilseed crops (Janzen and Kucey, 1988) and therefore could result in higher N_2O emissions. Although it was not significant we did see an increase in the N_2O flux at pre-seeding in the FP rotation from the labeled T_{24} cores. Ellert and Janzen (2008) suggest N_2O increases under low C:N crop residues is likely due to an increase in N available for nitrification and possibly denitrification. Hadas et al.

(2004) reported that lower C:N residues have higher water-soluble organic C. Therefore, the soluble C could serve as a substrate for denitrifiers and the increase in heterotrophic activity would result in a consumption of oxygen creating temporary anaerobic microsites (Ellert and Janzen, 2008). Although the proportion of N₂O from the T₂₄ cores was attributed nearly entirely to nitrification related processes it is interesting to note that while the contribution was small and insignificant, of all the rotations at each sample date, the FP rotation at pre-seeding had a higher proportion of N₂O attributed to denitrification. The higher flux at anthesis in the CNL rotation corresponds to higher rates of gross mineralization and nitrification and again may be a result of the unique influence *Brassicac*s exert on soil microbiota, although large variation in measured fluxes is not uncommon. Ellert and Janzen (2008) noted that there could be large variation of N₂O emissions among replicate plots of the same treatment or even among samples points just meters apart.

The positive correlation of NH₄⁺ to T₂₄ N₂O flux of the labeled cores supports the conclusion that 96 to 99% of emissions were from nitrification related processes (including nitrification, coupled nitrification-denitrification, or nitrifier denitrification) indicating the importance of this pathway to N₂O flux in semi-arid agricultural ecosystems. Conditions of water saturation are generally rare at this particular site so it was expected that the major contribution of N₂O flux would be from the nitrification pathway.

There appeared to be an effect of core disturbance on N₂O flux. The increase in T_{0.25} fluxes from labeled soil cores are likely due to a stimulatory effect on microbes of adding the K¹⁵NO₃ solution or simply a displacement effect where the K¹⁵NO₃ solution displaced a volume of soil gas. This stimulation and displacement effect across the treatments might have masked rotational differences. However, the T_{0.25} background cores at anthesis also show fluxes greater than the T₂₄ measurements. Ellert and Janzen (2008) also noted a chamber effect where at time zero, chamber N₂O concentrations always increased after chamber closure sometimes reaching 3.5-fold. As expected the N₂O flux is significantly lower at post-harvest in all rotations in comparison to fluxes at pre-seeding and anthesis. This is probably the result of cooling temperatures near the end of the season.

3.5.3. Dissolved organic carbon

Very few studies have directly compared the influence of crop rotations and plant species on *in situ* DOM concentrations during the growing season (Chantigny, 2003). Our DOC values at pre-seeding were comparable to values found in different wheat rotations in Saskatchewan. Campbell et al. (1999) reported water soluble organic carbon (WSOC) values, for a continuous wheat rotation in Swift Current, SK, at seeding (May), anthesis (July), and harvest (September) to be approximately 20, 23 and 15 mg WSOC kg⁻¹ soil, followed by a rapid decrease in WSOC concentration after harvest. At anthesis and post-harvest our values were two-fold higher and compare better to a canola residue decomposition incubation experiment in Western Australia where WSOC levels ranged from 40 to 1320 mg WSOC kg⁻¹ soil initially after residue incorporation but decreased within 15 d to between 20 to 40 mg WSOC kg⁻¹ soil (Bhupinderpal-Singh et al., 2006).

Campbell noted the pattern in WSOC response over the season in the wheat and wheat-fallow systems appeared to mimic the temperature trends, rising in spring and decreasing in fall (Campbell et al., 1999). Temperatures during the fall remained high at Scott, SK, which may account for the lack of seasonal differences observed here between anthesis and post-harvest. As well, the harvest of field pea and canola crops was much later than the wheat harvest so it is likely that rhizodeposition was still contributing DOC at our sample date. The higher DOC values at anthesis and post-harvest than at pre-seeding are likely due to the absence of root growth at pre-seeding. Although we found no differences among rotations in DOC concentrations, differences between plants have been observed and explained by differences in plant root exudation patterns at various growth stages (Xu and Juma, 1993).

Although we found no difference among rotations for DOC concentration, researchers have reported an effect of legumes on soluble C concentrations in soil. In a paper reviewing DOC, Chantigny (2003) cites higher WSOC concentration under legumes than under Gramineae (Poaceae) species. However, general comparisons must be made with caution when looking at soluble C. Chan and Heenan (1999) found soils that had been under different crop rotations yielded significantly different amounts of C when extracted by salt, hot water or acid extraction. Despite this, in the same study the

field pea/wheat rotations always yielded more soluble C than the canola/wheat rotation, regardless of the extraction procedure.

3.5.4. Microbial biomass

We found no effect of rotation on MB-C and -N with the exception of the FP and CNL-W-FP rotations at anthesis. Furthermore, any trends within the data were not consistent with trends for gross mineralization, nitrification or N₂O emissions. The chloroform fumigation extraction procedure measures total chloroform sensitive MB and does not provide a measurement of microbial activity (Bailey et al., 2002), which may explain why we found no correlations between the measurements in our data. Previous reports on the potential impact of pulse crops in rotation on MB are somewhat contradictory. Pulse crops have been associated with higher levels of MB when included as a green manure (Campbell et al., 1991). They also have been associated with higher levels of MB when compared to continuous crop rotations and crop rotations including summerfallow (Lupwayi et al., 1998). However, with these exceptions, literature findings regarding the effect that legumes have on MB appear to point towards lower or similar MB in rotations with legumes.

In eastern Washington and northern Idaho, Granatstein et al. (1987) found that MB differences among wheat rotations with varying frequencies of spring pea (at least 1 yr of legumes every 3 yr) were not large. They attributed this to the relative homogeneity of the treatments and that differences were likely due to the current crop in rotation (or previous crop residues) rather than an effect of the rotation itself. Chan and Heenan (1999) found that estimates of MB-C in Australia were highest in barley-wheat > canola-wheat > lupin (*Lupinus angustifolus*)-wheat > field pea-wheat rotations. In an Orthic Brown Chernozem at Swift Current, SK soil from a continuous wheat rotation had higher MB-C and MB-N content than that from a wheat-lentil rotation (Biederbeck et al., 1994). Sainju et al. (2009) found that the inclusion of legumes, such as pea, in wheat rotations also did not increase MB-N compared with other treatments, except for a spring-tilled wheat-fallow treatment. Furthermore, in a field study of canola with other crops, it was found that there was little impact of crop type on bulk soil MB estimates (Smith et al., 2004a). We found no significant difference between the two sampling dates, which is

consistent with finding of Bailey et al., (2002) who found there was little change in MB over the season with the exception of October, when fresh crop residues and rains had a strong stimulatory effect. Since we sampled directly after harvest and before any major precipitation events, any increase in MB from residue additions was likely missed.

The C:N ratios of fungi range from 4.5:1 to 15:1 while the C:N ratios of bacteria tend to be between 3:1 and 5:1 (Paul and Clark, 1996). In general, bacteria are less tolerant of acidity than fungi (Troeh and Thompson, 2005) which may explain why our C:N ratios were within a range typically associated with fungi as the pH at Scott is 5.5. At Swift Current, Saskatchewan, Biederbeck et al. (1994) found that the C:N ratio of the MB ranged from 5.0 to 8.3 and was unaffected by cropping treatment in a Brown Chernozem with a pH of 6.75. Interestingly the FP rotation had consistently the lowest MB C:N ratio, which may reflect the labile nature of the field pea residue in comparison to canola.

3.6.Summary

Although we saw no major differences in the soil processes and properties measured, following these rotations into the subsequent production year may provide further evidence regarding the effects of field pea in rotation on C and N cycling. Wheat residues would largely influence crop residue effects on C and N cycling because, in the previous year, all rotations were in the wheat phase. Although not significant, the increasing gross mineralization rates in the field pea rotations may translate into increased mineral N available to the subsequent crop in rotation. Looking at residue quantity and quality will provide further explanation for the lack of differences found in this study.

4. THE POTENTIAL USE OF $^{13}\text{CO}_2$ IN REPEAT-PULSE LABELING OF CANOLA AND FIELD PEA IN SOIL ORGANIC MATTER STUDIES

4.1. Abstract

Both the quantity and quality of crop residues can impact soil properties and processes. Plants enriched with isotopic tracers can be used to trace crop residue decomposition to various C pools but only if the tracer is homogeneously distributed throughout the plant. Continuous labeling will homogeneously label plants but is not widely accessible because elaborate equipment is needed. In order to determine if repeat-pulse labeling could be used to trace crop residue decomposition, this method was employed using $^{13}\text{CO}_2$ to enrich plant material of field pea and canola plants in a controlled environment. Plants were exposed weekly to pulses of 33 atom% $^{13}\text{CO}_2$ and grown to maturity. The distribution of the label throughout the plant parts (roots, stem, leaves, and pod) and biochemical fractions (ADF and ADL) were determined. The label was not homogeneously distributed throughout the plant; in particular, the pod fractions were less enriched than other fractions indicating the importance of continuing labeling well into plant maturity for pod producing plants. The ADL fraction also was less enriched than the ADF fraction. Because of the heterogeneity of the label throughout the plant, caution should be applied when using the method to trace C residue through various C pools. Nevertheless, root contributions to below-ground C were successfully determined from the enriched root material, as was ^{13}C enrichment of soil within the top 15 cm. Canola contributed more above- and below-ground residues than field pea; however, canola was also higher in ADF and ADL fractions indicating a more recalcitrant residue.

4.2. Introduction

Soil organic carbon plays a vital role in the health and quality of all soils. With increasing levels of atmospheric CO_2 , there has been much interest in the role of SOC in C fluxes and furthermore, the potential for the biosphere to sequester C. Agro-ecosystems are subject to intensive and continual anthropogenic disturbances and thereby serve as a

platform for investigations into whether these disturbances result in a net C sink or source. Ideally, agricultural land management policies should include information about soil C status, C sequestration and potential impacts on climate change.

To achieve a net gain in C storage, management practices must increase input from plant residues and/or decrease C loss from decomposition. In particular, above- and below-ground crop residues are considered to be critically important in building up SOM, improving sustainability and providing N and other nutrients to following crops (Jarecki and Lal, 2003; Rasse et al., 2005). However, predicting the SOC effects of changes to land management practices is not without challenges. Above-ground residues can be measured with relative ease, but root-derived sources of C, including root mucilage, sloughed off root cells, root exudates, and decomposition of the root itself, are more difficult to measure and, as a result, have not been properly quantified (Warembourg and Estelrich, 2000).

Traditionally root mass has been estimated by root excavation and washing, but this method is known to significantly underestimate below-ground production. Root losses due to the handling of root materials have been reported to be as high as 50% (Oliveira et al., 2000). Furthermore, this technique does not account for root turnover. Crawford et al. (1997) found that over the growing season, total root biomass production was 56 to 186 % greater than the maximum live-root biomass in various species.

Variations of root excavation and washing, such as sequential coring, have been developed in attempts to better account for root die-off but these methods are very labor intensive and may either over-estimate or under-estimate root biomass because of high temporal and spatial variability (Tierney and Fahey, 2007). Minirhizotrons take direct repeated observations from digital imagery of root growth along the outer surface of a tube placed in soil and have been used for below-ground estimations but again, are labor intensive and expensive (Johnson et al., 2001). Indirect methods include elemental budgets where C fluxes are measured and estimations of net below-ground production are calculated by closing the C budget (Davidson et al., 2002). However, major C fluxes may be difficult to measure, particularly separating soil respiration fluxes from root respiration (Ryan and Law, 2005).

Labeling of plant residues with isotopes (i.e., ^{13}C , ^{14}C) may offer a solution to quantifying root-related C additions because the fate of plant residue C through various soil pools can be measured directly. However, controversy exists regarding the optimum method of labeling and its subsequent interpretation. The two main methods of atmospheric labeling are continuous labeling and pulse labeling. Continuous labeling requires a sophisticated labeling system that exposes the plant to a constantly ^{13}C -enriched atmosphere, with consistent CO_2 levels, temperature, and soil moisture (Kuzyakov and Domanski, 2000). In contrast, pulse labeling is the short-term exposure to $^{13}\text{CO}_2$ at a specific plant growth stage (Bromand et al., 2001). In both techniques the plants are grown in chambers where the root atmosphere is separated from the shoot (Meharg, 1994). Shoots are then exposed to the ^{13}C -enriched atmosphere and the fate of the label within the plant/soil system may be determined.

In order to quantify root C additions, a distinction must be made between previously existing C from SOM and new C additions from fresh residues. Because plant components decompose at different rates (Trinsoutrot et al., 2000), it is required that the entire plant is homogeneously labeled including plant parts (stem, leaf, roots, pod, etc.) and biochemical fractions (cellulose, hemicellulose, lignin, etc.) (Warembourg and Kummerow, 1991). It is generally accepted that this can only be accomplished through continuous labeling (Meharg, 1994). While pulse labeling has been used to trace the fate of C assimilated into the plant during a given time period, only the plant compartment, either the plant part or biochemical fraction in question, needs to be evenly labeled and not all biochemical components and/or plant parts are evenly labeled (Warembourg and Kummerow, 1991). In this case, labile C pools (non-structural) will dominate (Meharg and Killham, 1988) and therefore this technique fails to provide reliable C budgets (Nguyen, 2003) because once the ^{13}C -enriched labile material has decomposed, distinctions between non-enriched recalcitrant plant materials and non-enriched plant materials from other compartments cannot be made.

Despite these conventions, many authors have successfully used pulse labeling with ^{14}C to approximate cumulative C budgets by assessing C allocation in the plant at different development stages after labeling (Gregory and Atwell, 1991; Jensen, 1993; Swinnen et al., 1994). Interest in using the stable isotope ^{13}C has increased due to

improved analytical techniques, and the safety of handling which is crucial for field studies (Recous et al., 2000). More recently, the use of ^{13}C in repeat-pulse labeling studies (i.e., successive periods of exposure to $^{13}\text{CO}_2$ throughout the plant growth) has been assessed and used to determine the fate of C in plant-soil interactions (Bromand et al., 2001). Bromand et al. (2001) suggested that if the label is applied in direct proportion to the photosynthetic rate at intervals that are frequent enough to represent growing season C assimilation, then it may be possible to obtain adequate homogeneity to trace plant residue decomposition through various C pools. Subedi et al. (2006) successfully used repeat-pulse labeling with ^{13}C to quantify root derived soil C from wheat and found that the ^{13}C repeat-pulse labeling technique gave a better estimation of root biomass than the conventional method of root washing. This was attributed to the greater loss of root material in washing process and better accountability of root mucilage, root exudates, and detached root tips when using the ^{13}C repeat-pulse labeling method.

Of these few studies that attempt to use repeat-pulse labeling with ^{13}C to quantify C budgets, most examine a single plant species. Yet, in an agro-ecosystem context, it is desirable to examine effects of different crop species on C cycling. It is known that in natural conditions, different plants have varying degrees of discrimination against ^{13}C (Brugnoli and Farquhar, 2000). If comparisons are going to be made between crops in a crop rotation using this technique, it is necessary to understand where the ^{13}C is deposited both in plant organs (leaf, stem, root, etc.) and biochemical fractions (cellulose, lignin, etc.). Tracing plant residue derived C through various soil C pools can only be done once the dynamics of the incorporation of ^{13}C into the various plant parts and biochemical fractions are known and proven to be adequately homogenous.

This study was initiated to examine the homogeneity of ^{13}C label within field pea and canola following a repeat-pulse labeling regime. Using the enriched plant root residue, root biomass was estimated via ^{13}C enrichment levels of root and soil in intact soil cores in comparing the below-ground carbon additions of canola and field pea. The potential application of this technique in tracing plant residue decomposition through various C pools was also considered.

4.3. Experimental Procedures

4.3.1. Soil collection

In May 2008, intact soil cores (30 cm deep by 20.3 cm i.d.) were collected from W-FP and W-CNL rotations, both having completed the wheat phase of the rotation the previous year. Intact cores were used to imitate field conditions so measurements of root derived C would more accurately depict root derived C in the field plots and each core contained similar proportions of the row and inter-row space. The cores were collected from the Experimental Farm of Agriculture and Agri-Food Canada, at Scott, SK (52°13'48" N, 108°30'00" W). The soil was an Orthic Dark Brown Chernozem on Elstow loam. The 0 to 15 cm depth had a pH of 5.5 (Soon et al., 2008). The cores were stored at 4°C until they were planted on 28 Dec. 2008.

4.3.2. Experimental design

The intact soil cores were arranged in four blocks of four cores with each block containing the same rotation (Fig. 4.1.) Control cores were arranged in a similar fashion in a separate growth chamber. Eight field pea seeds and eight canola seeds were planted per pot on 28 Dec. 2008. Field pea seeds were inoculated with *Rhizobium leguminosarum* bv *viceae* (Nitrastik-C, EMD Crop BioScience, Milwaukee, Wisconsin). Plant density was four plants per intact core, which reflected recommended pre-seeding rates of field pea (224 kg ha⁻¹) and canola (6.7 kg ha⁻¹). The plants were grown in a growth chamber (22°C, 18 h of light per day) and watered daily to soil field capacity (Geisel et al., 2008) which was measured periodically during the experiment with a 5TE soil moisture sensor (Decagon Devices Inc. Pullman, WA). The soil had been fertilized in the field prior to core collection [canola: 15.2 kg P ha⁻¹ as mono-ammonium phosphate (11-52-0) and 74.4 kg N ha⁻¹ as mono-ammonium phosphate and urea (46-0-0)] so no additional fertilizer was applied to the cores. Each block of four cores was then inserted into a chamber as outlined below (Fig. 4.1.).

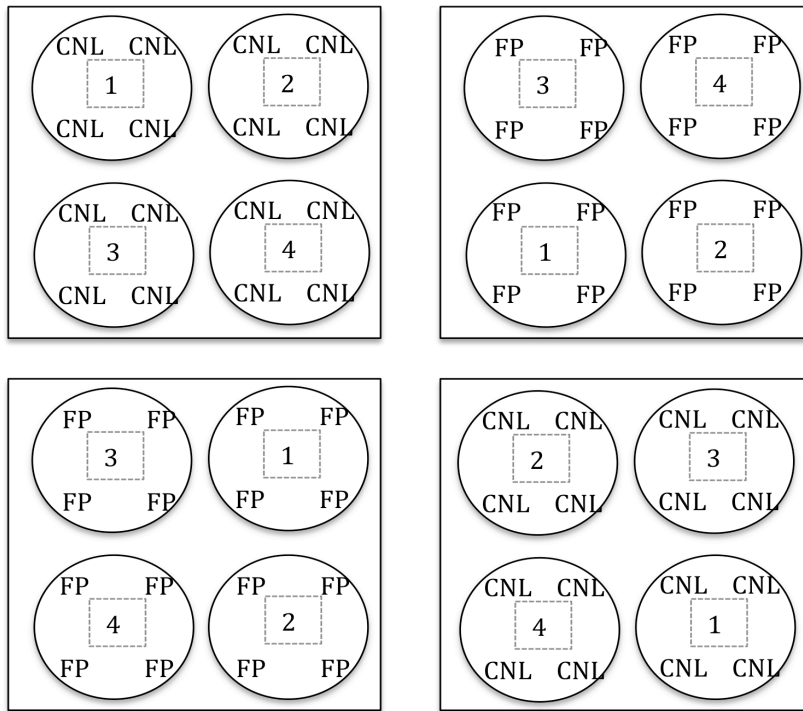


Fig. 4.1. Labeling chamber experimental design. Enriched and control plants were contained in separate growth chambers under identical environmental conditions. The boxed number indicates the plot repetition from which the core was collected. FP: soil core collected from the wheat-field pea rotation seeded with field pea; CNL: soil core collected from the wheat-canola rotation seeded with canola.

4.3.3. Chamber specifications and labeling procedure

Poly(methyl methacrylate) chambers (45cm x 55cm x 60cm) were constructed to facilitate atmospheric ^{13}C labeling of plants (Fig. 4.2). Two 12V DC fans (10 cm^2) were installed in opposite ends of the chamber to ensure air circulation during the labeling events. An injection port with septum was fitted on the top of each chamber. Each chamber was equipped with an infrared gas analyzer (IRGA) (S151 Infrared CO_2 Analyzer, Qubit Systems, Kingston, ON). The IRGA only provided an estimate of CO_2 levels due to the differing wavelengths of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ that are detected by the IRGA; it read 75% of the actual concentration of $^{13}\text{CO}_2$ within the chamber. Polyvinyl chloride (PVC) tubing was connected to the chamber, which created an airflow circuit that was connected to a pump (G100 AC Gas Pump, Qubit Systems, Kingston, Ontario), a flow meter (G265 Flow Controller and Monitor Qubit Systems, Kingston, Ontario), and

the IRGA. A flow rate of 250 mL min^{-1} was maintained during labeling. When the plants were in their first stages of growth, the rate of soil respiration exceeded the rate of photosynthesis, so the airflow circuitry also included an alternate pathway that diverted the airflow through a soda lime column that helped to decrease the CO_2 levels in the labeling chambers. The chamber fit into a 1 cm groove in a Plexiglas base, which was sealed by filling the excess space in the groove with water. Four holes (25 cm i.d.) were cut out of the base to fit over the intact soil cores. Silicon sealant was used to seal around each core. Gas leakage was assessed before the experiment began by increasing the partial pressure inside the chamber above the ambient atmospheric pressure and monitoring any changes over a 3 h period.

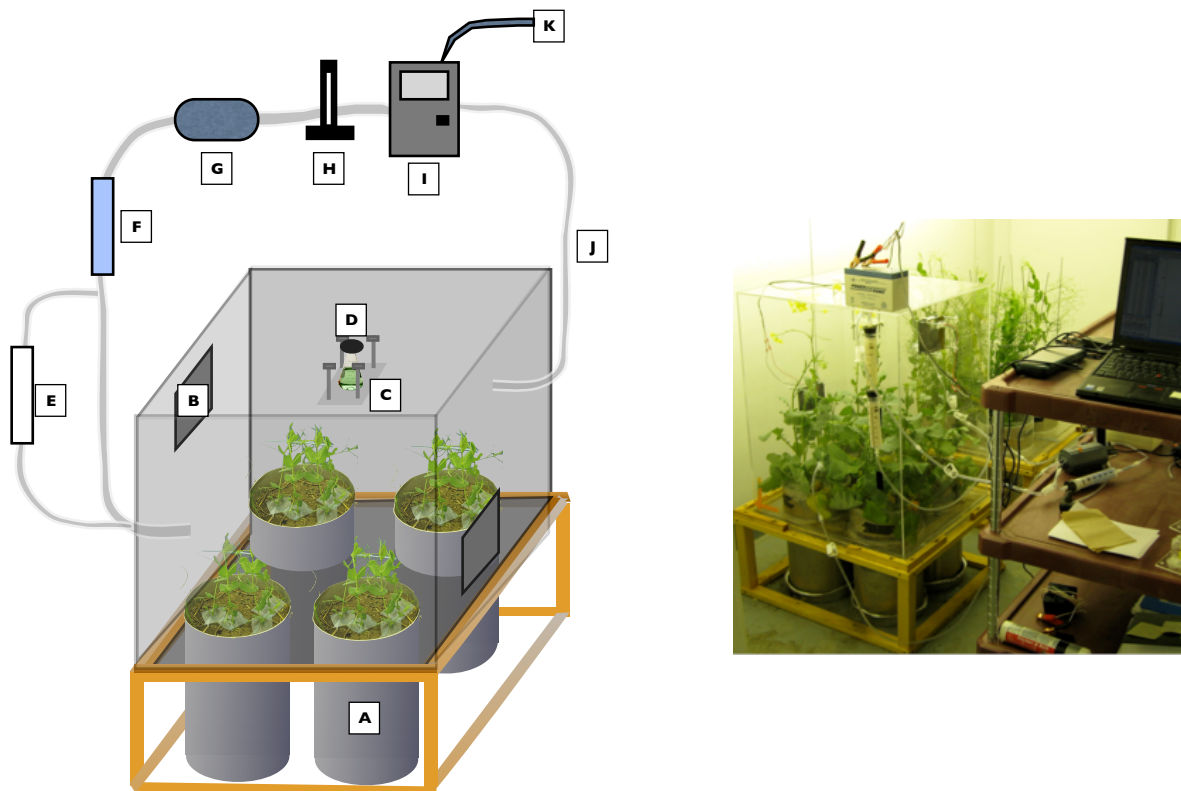


Fig. 4.2. Schematic and photograph of chamber design and set up for $^{13}\text{CO}_2$ atmospheric labeling. Components are (A) intact soil core, (B) circulation fan, (C) suspended vial for $^{13}\text{CO}_2$ production, (D) septum, (E) soda lime column, (F) Drierite® column, (G) pump, (H) flow meter, (I) IRGA reader, (J) PVC tubing, (K) data logger connection.

Labeling commenced on January 13, 2009, 16 d after planting, and occurred on weekly intervals thereafter. The duration of the labeling period was 1.5 h. Because canola matured faster than field pea, canola was labeled for five consecutive weeks and field pea was labeled for six consecutive weeks. The soil surface was isolated from the enriched atmosphere during labeling by using GLAD Press'n Seal Freezer® wrap (The Clorox Company, Oakland, CA). Gas permeability was tested by sealing a glass Erlenmeyer flask containing elevated CO₂ levels with the GLAD Press'n Seal Freezer® wrap. The CO₂ levels were monitored over a 4 h period to detect any changes. No changes in CO₂ concentration were observed [data not shown]. Dalton's Law of Partial Pressures was used to calculate the total amount of CO₂ gas in the chamber at ambient levels. The chamber was placed on the Plexiglas base slowly so that the pressure inside the chamber was not initially increased. The soda lime columns were then used to decrease the amount of CO₂ in the chamber to approximately 66% of its original value and then ¹³CO₂ replaced the removed ¹²CO₂ by injecting 2 mol L⁻¹ HCl through the chamber port into a glass vial suspended directly under the port containing 0.0723 g of NaH¹³CO₃ (99 % ¹³C). The ¹³CO₂ produced raised the CO₂ levels approximately back to ambient (430 ppm) and were monitored by the IRGA for a 1.5 h labeling period.

Throughout the labeling period an atmospheric enrichment of 33% ¹³CO₂ was maintained. When the IRGA readings dropped approximately 80 μmol mol⁻¹ (360 μmol mol⁻¹) a NaH¹³CO₃ solution (33% NaH¹³CO₃ and 66% NaH¹²CO₃) was injected into the glass vial that contained an excess amount of HCl. The solution was continually added as the CO₂ levels dropped to maintain a total concentration of CO₂ in the chamber between 360 and 430 μmol mol⁻¹. During labeling, a data logger (Logger Pro, Qubit Systems) logged CO₂ concentrations every 20 s for an indication of photosynthetic rate. Canola plants were exposed to a total of 1.2 g NaH¹³CO₃ (over five labeling intervals) and field pea plants were exposed to a total of 1.4 g NaH¹³CO₃ (over six labeling intervals). The difference in amounts is due to differences in photosynthetic rates as well as differences in maturity. Field pea received one additional labeling episode because it was slower to reach physiological maturity. For both crops, labeling ceased upon development of the seed [BASF-Bayer-Ciba-Geigy-Hoechst (BBCH) growth stage 79] (Lancashire et al., 1991).

4.3.4. Plant Analysis

Upon maturity, plants were harvested, dried for 10 d at 30°C, and then separated into shoot, leaf, and pod fractions. Plants and their individual fractions were weighed, and the fractions were ground first with a coffee grinder and then with a ball mill.

In each block, soil from two cores was collected from the upper 15 cm and weighed to determine gravimetric soil moisture content. The roots were separated from the soil with a 5 mm sieve, washed, dried, weighed and ground. A sub-sample of the sieved soil was taken and ground finely on a ball mill in preparation for mass spectrometer analysis. The remaining two cores in each block were sampled by taking a core (15 x 5 cm i.d.) that was centered on the remainder of the previously harvested shoot. This ensured that the root material collected was identifiable to an individual plant. Below-ground biomass was calculated following the procedures by Subedi et al. (2006).

The total SOC content (g) in each pot (A) was calculated as:

$$A = \text{dry weight soil per pot (g)} \times \frac{\%SOC}{100}$$

[4.1]

The % ¹³C of SOC (B) was calculated as:

$$B = \frac{\delta^{13}C_{\text{enriched soil}} - \delta^{13}C_{\text{reference soil}}}{\delta^{13}C_{\text{enriched plant root}} - \delta^{13}C_{\text{reference plant root}}} \times 100$$
 [4.2]

The root-derived carbon (C) was calculated by:

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

The total root-biomass (D) was derived from:

$$D = \frac{C \times 100}{\text{mean \% C in root samples}}$$
 [4.4]

Total root material from two cores in each chamber, and the corresponding leaves, shoot and pod samples, were prepared for biochemical analysis. Ground samples of approximately 1 mm particle length were sent to Analytical Services, Department of Animal and Poultry Science, University of Saskatchewan for ADF and ADL fractionation using the Association of Official Analytical Chemists (AOAC) method 973.18 (AOAC

International, 2000) modified by using the ANKOM automated system using filter bags (ANKOM Technology Corp. Fairport, N.Y.). Acid detergent fiber consists of cellulose and lignin portions and ADL is the portion remaining after the cellulose is removed. The ADF and ADL residues were then finely ground using a SPEX Sample Prep 6670 freezer mill (SPEX CertiPrep® Group, NJ, USA). Plant parts and ADF and ADL fractions were analyzed for $\delta^{13}\text{C}$ using a Costech Elemental Combustion System coupled to a Delta V Advantage Mass spectrometer (Isomass Scientific Inc. Calgary, AB).

4.3.5. Statistics

All data were checked for normality using visual observation of a frequency distribution histogram as well as the Kolmogorov-Smirnov test ($p < 0.05$). One-way ANOVA was used to detect differences in enrichments of the plant parts and a nested ANOVA was used to determine whether there was an effect of the chamber and pot location because the chambers and pots remained stationary during the experiment. Post hoc tests used were either REGWQ or Games-Howell depending on whether the variance was homogeneous or not (Levene's statistic). Differences in enrichment of biochemical fractions of field pea and canola among plant parts were determined using a three-factor ANOVA followed by REGWQ post hoc test. A Pearson correlation test was used to determine linear relationships between plant part C content, $\delta^{13}\text{C}$ enrichment and weight. All statistical tests were performed using SPSS version 17.0 for Windows (SPSS INC., 2007). Effects were declared significant at $p < 0.05$ unless otherwise specified.

4.4. Results

4.4.1. Effect of experimental design

The location of the chamber and pot had little effect on the enrichment levels of the plant parts (Appendix A). There was no significant effect of the chamber on the enrichment of canola or field pea root, pod, and leaf. There was an effect of the chamber on the enrichment of the field pea stem. Field pea ^{13}C enrichments of roots and leaves were not affected by pot, within or between chambers, but there was a significant difference between two of the pots for the pod and stem fraction ($p < 0.05$). There was no

significant difference in ^{13}C enrichment among the pots for canola stem, leaf, pod or roots.

4.4.2. $\delta^{13}\text{C}$ of enriched plant parts

All plants that received the repeat-pulse labeling were highly enriched in ^{13}C compared to the natural abundance plants (Table 4.1). In assessing the homogeneity of the label among the plant parts (stem, leaf, pod and root) it was found that there was no significant difference between the enrichment of field pea stem and leaves but the pod and root were significantly less enriched than all other parts. The pod had the lowest level of enrichment (Table 4.1). Canola roots and stems were not significantly different but canola pod and leaves were different from all other parts. Similar to field pea, the canola leaves were the most highly enriched plant part and the pods were the least enriched. There was no significant difference in the enrichment of the roots of canola compared to field pea ($p = 0.103$) but all other canola parts in comparison to field pea were different ($p < 0.05$).

Table 4.1. $\delta^{13}\text{C}$ of enriched (n=12) and natural abundance (n=8) field pea and canola plant parts at maturity grown under controlled conditions.

	stem	leaves	pod	roots
$\delta^{13}\text{C}$ Enriched (‰)				
Field pea	132.7 (21.7)b†‡	138.0 (24.7)b	0.4 (20.0)f	73.2 (16.6)d
Canola	95.9 (13.7)c	162.8 (19.4)a	13.2 (11.3)e	84.5 (20.5)cd
$\delta^{13}\text{C}$ Natural Abundance (‰)				
Field pea	-30.4 (0.8)g	-31.7 (0.6)g	-31.9 (1.2)g	-29.6 (0.1)g
Canola	-30.4 (1.8)g	-31.0 (0.0)g	-30.5 (1.5)g	-29.6 (2.1)g

†Numbers followed by different letters indicates significant difference among plant parts and between crops at $p \leq 0.05$ by the REGWQ test

‡ Mean values and (SD) on an individual plant basis

There was variation in the dry weights of the above-ground plant parts, which was also observed visually during the growth and development of the plants throughout the experiment (Table 4.2). However, there was no correlation between above-ground plant part weight and the corresponding level of enrichment. There was a significant negative

correlation ($r = -0.419$, $p = 0.017$) between C content and $\delta^{13}\text{C}$ enrichment in the stem of field pea and canola plants.

On an individual plant basis, root biomass was lower for field pea compared to canola (Table 4.2). Total straw biomass per plant was also significantly different and lower for field pea than canola. However, there was no significant difference between the field pea straw to root ratio and the canola straw to root ratio.

Table 4.2. The above- and below-ground biomass of enriched field pea and canola plant parts expressed on a per plant basis (above-ground biomass: n=32).

	Stem	Leaves	Pod	Seed	Straw†	Root‡	H.I.§	Straw: seed	Straw:root
	(g)								
Field pea	3.59 (1.52)¶	0.67 (0.24)	1.32 (0.48)	6.60 (2.44)	5.57 (2.19)	3.23 (0.75)	54.35 (5.75)	0.86 (0.24)	1.90a#
Canola	3.41 (0.77)	0.95 (0.44)	2.92 (1.06)	2.74 (0.94)	7.28 (2.04)	4.59 (1.28)	27.00 (2.21)	2.74 (0.32)	1.57a

†straw includes all above-ground plant residues (stem, leaves and pod)

‡root biomass calculated according to Subedi et al. (2006) on a per pot basis then divided by plants/pot (n=4)

§ H.I. = harvest index = seed:total above-ground biomass x 100

¶ Mean values and (SD) on an individual plant basis

Numbers followed by the same letter indicates no significant difference at $p \leq 0.05$ by the REGWQ test

4.4.3. $\delta^{13}\text{C}$ enrichment of biochemical fractions of plant parts

The ADF content in field pea differed significantly among plant parts and was highest in the stem and lowest in the leaf (Table 4.3). The canola stem and root ADF content did not differ significantly, but both were higher than leaf or pods. The leaves were lowest in ADF content. When comparing between plants, the ADF content in canola was significantly higher for all plant parts compared to field pea. Lignin content (%ADL) in the field pea plant was highest in the stem and lowest in the leaf and there were significant differences among all parts. Canola followed a similar trend only there was no difference between the pod and the stem. Lignin was lower in field pea for all parts compared to canola, but only significantly lower in the pod and stem.

Table 4.3. Percent acid detergent fiber (% ADF) and acid detergent lignin (% ADL) for field pea and canola on a per pot basis (n=4) with control plants (n=4).

	Field pea		Canola	
	<u>enriched</u>	<u>control</u>	<u>enriched</u>	<u>control</u>
	% ADF			
Stem	60.50 (1.03)†	58.75 (3.46)	68.20 (1.96)	67.04 (2.08)
Leaf	22.74 (1.09)	21.74 (0.04)	26.07 (1.67)	26.93 (0.16)
Pod	42.64 (0.99)	38.78 (1.33)	55.59 (1.46)	53.45 (2.43)
Root	56.87 (2.24)	51.78 (2.33)	68.26 (0.67)	66.87 (1.97)
	% ADL			
Stem	12.14 (1.77)	12.35 (0.66)	16.64 (0.02)	17.35 (1.23)
Leaf	5.53 (0.87)	6.29 (0.83)	6.87 (2.15)	5.72 (2.11)
Pod	7.47 (0.48)	8.46 (0.09)	13.82 (0.15)	14.06 (0.44)
Root	18.07 (0.24)	19.22 (2.32)	19.42 (1.32)	26.74 (7.95)

† Mean values and (SD) on an individual plant basis

The ADF fraction was generally more enriched in ^{13}C than the ADL fraction but this difference was only significant for the non-enriched (natural abundance) control (Table 4.4). There was large variation in ^{13}C enrichments among the composite plant samples in each pot. In general, plant part ADF and ADL ^{13}C level of enrichment followed a similar pattern to that observed for plant parts (Table 4.1) where leaf was the most highly enriched (with the exception of field pea stem ADL) and pod was the least enriched.

Table 4.4. The biochemical fraction $\delta^{13}\text{C}$ of field pea and canola biochemical fractions (ADF, ADL) for each plant part showing enriched and control (natural abundance) plants.

† Mean values and (SD) of individual plants

	Field pea		Canola	
	<u>enriched</u>	<u>control</u>	<u>enriched</u>	<u>control</u>
	$\delta^{13}\text{C}$ ADF (‰)			
Stem	130.9 (22.3)†	-29.7 (0.4)	85.3 (26.1)	-30.6 (1.0)
Leaf	149.3 (16.3)	-30.3 (0.5)	179.0 (19.2)	-30.7 (0.2)
Pod	-5.76 (13.9)	-30.6 (1.0)	7.94 (30.3)	-30.0 (0.3)
Root	81.5 (19.2)	-29.9 (0.2)	77.1 (26.1)	-30.0 (0.9)
	$\delta^{13}\text{C}$ ADL (‰)			
Stem	127.54 (21.2)	-34.7 (0.7)	70.2 (22.3)	-33.7 (1.0)
Leaf	127.13 (16.3)	-33.3 (0.5)	164.8 (24.8)	-33.5 (2.1)
Pod	-5.36 (14.6)	-35.0 (1.0)	1.12 (28.4)	-34.2 (0.4)
Root	69.5 (24.4)	-31.8 (0.2)	70.0 (31.4)	-32.6 (0.8)

† Mean values and (SD) on an individual plant basis

4.5. Discussion

4.5.1. Analysis of labeling method

Although all plant parts were sufficiently enriched in comparison to natural abundance plants, there were significant differences in ^{13}C enrichment among plant parts. Homogeneity of ^{13}C distribution among plant parts was not obtained following the methodology used in this study. One major area of concern is the lower enrichment of the pod fraction in both the canola and field pea plant. This difference was not apparent in the control plants that were exposed to the ambient atmosphere where a constant enrichment of the naturally occurring ^{13}C isotope was maintained. This suggested that under continuous labeling with an elevated ^{13}C atmospheric enrichment, all plant parts would have similar levels of enrichment. The pod fraction contributed 24% of field pea residue and 40% of canola residue (Table 4.2). Lack of enrichment of the pod would therefore result in underestimation of the pod and overall crop residue contribution to SOM. Enriched ^{13}C levels of the pod most likely originated from enriched plant C stores rather than directly from photosynthates from the pod itself. Previous studies have suggested labeling as early as possible will improve the distribution of ^{13}C within the plant (Thompson, 1996; Bromand et al., 2001). Although we started labeling 18 d after pre-seeding, the lower enrichments of the pod fraction suggests that for pod forming plants including pulse crops and oilseed crops, ensuring adequate labeling throughout the pod forming stages is also very important in improving ^{13}C distribution. The ^{13}C enrichment of the roots was likely underestimated because the root sample was extracted from the soil after plant harvest. Labile plant fractions, even in natural conditions, are slightly more enriched with ^{13}C than recalcitrant fractions and during pulse events, labile fractions are more readily labeled (Meharg and Killham, 1988; Brugnoli and Farquhar, 2000). At this time, more labile fractions of the root were likely already decomposed and were not physically recovered. In addition, percent ADF and percent ADL may be overestimated as only the more recalcitrant material was excavated at sampling. Subedi et al. (2006) proposed that root washing could underestimate root biomass by 30% and in our case the physical recovery of roots was likely lower.

The high amount of variability among plant part enrichment was due to individual plant variation. Similar to field conditions, the plants contained within a single pot were not completely uniform with some maturing faster or having a greater biomass than other plants in the same pot. This, coupled with the physical location of the plant within the pot, may have contributed to this variation of ^{13}C enrichment. Individual plants may have had different access to the light source during labeling affecting the photosynthetic rate and therefore the amount of ^{13}C metabolized by the plant. Since there was so much variation among plants, it may be helpful in future work to use smaller pots containing one plant so that plant addition to SOM could be traced back to an individual plant with a known enrichment rather than compounding the different levels of enrichment and increasing the likelihood of misinterpretation of the data. This may however introduce a greater physical constraint in root growth. Note that there are examples of studies that also had different levels of enrichment but despite this continued to follow plant residues through SOM (Thompson, 1996; White and Rice, 2009).

It was important to also look at the enrichment of ADF and ADL fractions of the plants used in this study. Under natural atmospheric conditions, it has been shown that the ^{13}C enrichment of various plants differ and that the biochemical components within each individual plant also differ in enrichment (Benner et al., 1987). These findings are consistent with our study where the differences between the control plant enrichments of these fractions are from 1.9 to 5 ‰. These differences are slightly underestimated due to the extraction process used in this study where ADF is a chemically-defined fraction containing both lignin plus cellulose materials, and does not accurately represent the true differences between cellulose and lignin. In theory, if homogeneity of the enriched plants were achieved to the highest standard, the difference between the biochemical fractions of the enriched plant should match the relative differences between the plants grown under natural abundance conditions. However this is not the case.

The enriched plant part differences in ADF and ADL fractions are likely reflective of the stage of plant development when labeling took place and how that affected the physiological partitioning of the ^{13}C during the labeling event. For example, the ^{13}C enrichment of pod fraction for the field pea plants is very low at 0.4 ‰. Since labeling ended just as the pod was forming in the pea plant it is likely that enrichment

from past labeling was from stored carbohydrates that were relocated to the pod for structural formation. If labeling continued, more labile photosynthates in the pod would likely be more heavily enriched. So this enrichment value for the ADF (cellulose + lignin) fraction is mostly representing the enrichment for the lignin fraction. This is problematic for future studies wishing to follow the enrichment levels of various SOM fractions. Because these biochemical components differ in their susceptibility for microbial degradation, where the polysaccharide components are degraded much faster than the lignin, it would result in detritus that is more representative of the ^{13}C enrichment of the lignin-derived C (Gleixner et al., 1993). In the case of the field pea pod decomposition, the more labile biochemical components originating from the pod may be missed altogether resulting in an underestimation of the contribution of the pod to more labile fractions of SOM. In the case where the ADF fraction is much more heavily enriched than the ADL fraction, for example the field pea leaves, the contribution of leaves to more labile SOM pools may be overestimated. Therefore, it is very important to clearly specify the calculations and assumptions used when applying the repeat-pulse labeling technique to follow C flows.

Previous studies attempted to assess the homogeneity of labeling within the biochemical fractions and despite large differences between the fractions, it was concluded that pulse labeled materials could be used for C cycling studies (Williams et al., 2006; Moore-Kucera and Dick, 2008). In one study, the stem fraction of the Douglas tree seedlings had an overall enrichment 229‰, with cellulose enrichments at 455‰ and lignin enrichments of 295‰, which is a difference of 226‰ in enrichments (Moore-Kucera and Dick, 2008). Although the authors had shown that after applying the enriched materials to the soil, the bulk soil, POM fractions and humin fraction were sufficiently enriched, quantification of decomposition rates would not likely be meaningful based on the heterogeneity of the label throughout the biochemical fractions and plant parts.

4.5.2. Estimation of below-ground C contribution

The repeat-pulse labeling method does show promise in estimating below-ground contributions of root-derived C to soil. Meharg (1994) stated that pulse labeling may

result in a preferential enrichment of the labile and young pools and consequently a low enrichment of structural root compounds. However, because we are using a repeat-pulse labeling method, the effect of the preferential enrichment should be lessened. Despite the heterogeneity of label between the plant parts, we were able to estimate root biomass based on a method proposed by Subedi et al. (2006). The rationale behind using this method to quantify root biomass despite plants not being homogeneously labeled is that it may provide a better estimation of root biomass in comparison to other methods previously mentioned. Furthermore, Subedi et al. (2006) used two labeling events to calculate estimations and based on this it was proposed that the estimation would account for a proportion of root exudates where physical excavation methods could not. We feel that because our labeling events were more frequent, we were able to account for a greater proportion of root exudation and therefore gain a more accurate estimation of total below-ground C additions. For various crops grown in Swift Current, SK, Gan et al. (2009) estimated relative C allocation coefficients based on biomass dry weights of plant parts as a percentage of the total C residue contribution. They estimated below-ground C contributions using lysimeters to measure root biomass and used a rhizodeposition coefficient of 65% of the root C (Kuzyakov and Schneckenberger, 2004; Bolinder et al., 2007). If the proportions of total C for roots (based on lysimeter excavation to 100 cm depth) and rhizodeposits (calculated as a percent C of roots) are combined, the coefficients of grain:straw:root+rhizodeposits, would be 0.175:0.525:0.3 for irrigated canola and 0.262:0.531:0.206 for irrigated field pea. If we developed the same proportions for our data we would have 0.16:0.51:0.32 for canola and 0.43:0.36:0.21 for field pea. From these ratios it appears that the field pea plants grown under controlled conditions in a phytotron chamber in comparison to irrigated field conditions yielded more grain and less straw; however, the similarities between the C allocation coefficients are striking. This supports the suitability of using a repeat-pulse labeling method in determining below-ground C additions. However, because we only sampled the soil to 15 cm it is likely that the roots and rhizodeposits may be underestimated using both methods supporting the concerns of Bolinder et al. (2007) that estimates of below-ground C from rhizodeposition considered to be 65% may be conservative. Finally, using our estimate

for below-ground contributions, in terms of quantity of residues produced on a per plant basis, canola certainly appears to produce more above- and below-ground biomass.

In assessing the impact of crop residues on soil properties, both residue quantity and quality must be considered. Typically biochemical plant fractions such as ADF and ADL have been used in studies analyzing feed quality for livestock production (Reeves, 1993). Recently interest in these biochemical plant fractions has been building to use in the context of assessing crop suitability for bioproducts and biofuels (Buranov and Mazza, 2008) and soil scientists are utilizing these fractions to predict the decomposition of crop residues and the potential impact on soil (Stubbs et al., 2009). Using these fractions, we found that generally field pea would be more readily degradable because it was lower in % ADF and % ADL in comparison to canola, indicating that it would be higher in hemicellulose which is more easily degraded (Stubbs et al., 2009). However, conclusions of degradability based on biochemical fractions cannot be assessed without considering the relative volume of residues each plant produces. In this case, canola produced approximately 23% more above-ground residue and 30% more below-ground biomass in comparison to field pea. So it would appear that canola would have potential to increase C sequestration. Although field pea residues on a quality basis would decompose faster, this may not translate into increased mineralization and nitrification rates or increased bacterial communities because the canola simply has more residue. The balance between crop residue quantity and quality will ultimately dictate changes in soil processes and soil properties.

4.6. Summary

Similar to other workers (Bromand et al., 2001), we found that our repeat-pulse labeling regime resulted in heterogeneous labeling among plant parts and recommend adapting the protocols to include more frequent labeling; perhaps twice a week. The advantage of repeat-pulse labeling in comparison to a continual labeling system is that it is less expensive and can be done without an elaborate climate controlled chamber system. However, if the frequency of labeling were increased to gain a better distribution of ^{13}C , any benefit of the repeat-pulse labeling method in comparison to continual labeling may be negated as more regular labeling intervals will increase labor costs as

well as the cost of isotope application. Many authors disregard the heterogeneity of ^{13}C label within the plant and continue to follow plant material through various soil C pools. Although we feel the method holds great promise we also believe caution should be applied to the interpretation of such data for the reasons outlined above and that it is absolutely necessary to assess the distribution of the label throughout the plant before embarking on future residue decomposition studies using the labeled plant material. Labeling should also be carefully planned in accordance to plant development, so it is necessary to have knowledge of plant metabolism and utilization of photosynthates. This will allow better pulse labeling methodology to be developed and hopefully improve the distribution of ^{13}C within the plant. In any study using this method to address research questions regarding crop influence on SOM pools, the first question should be how much heterogeneity in the labeling of the plant parts is acceptable so that labeled residues can be effectively used in future studies.

5. GENERAL DISCUSSION AND CONCLUSION

The recognition that pulse crops improve the sustainability of agricultural ecosystems has largely been attributed to biological N fixation and therefore a reduction in applied synthetic N during the pulse crop year. However, there has been recent speculation that including pulse crops in rotation may result in additional environmental and agronomic benefits, yet these anticipated benefits have not been adequately quantified for the Northern Great Plains region. Thus the primary goal of this study was to investigate further potential benefits of field pea in rotation with reference to carbon and nitrogen cycling.

A number of C and N parameters were monitored throughout the growing season during the field pea phase of rotation in addition to a growth chamber study designed to delineate differences in residue quantity and quality of field pea in comparison to canola. Although there are claims of increased mineral N available to crops in succession to pulse crops, these increases were not reflected in the field measurements of gross mineralization, gross nitrification, N₂O emissions or labile C pools. Because the field plots were established in 1997, we were able to measure the cumulative effect of field pea on C and N cycling processes. However, no effect of field pea in rotation was discernable within the properties we measured in this study. This indicated that the main influence on C and N cycling is likely attributed to the most recent crop residue addition which would originate from the previous crop in rotation. In our study, the previous crop in all rotations was wheat. Under this premise, it is therefore not surprising that differences were not detected. Field measurements of N and C soil processes and properties should continue into the following growing season in order to determine whether increases in gross mineralization and gross nitrification are realized in the year following field pea.

Because it is likely that recent residue additions are driving mineralization, residue quality and quantity may prove to better predict N credits for successive years. Results from the growth chamber study using ¹³C showed that field pea contributes less total carbon than canola but field pea residue had proportionately less ADF and ADL. Since ADF and ADL fractions are the least-degradable proportions of the plant, field pea

would have comparatively more degradable fractions, such as sugars, proteins and starches. This suggests it is likely that field pea residues will provide a more labile substrate for mineralization and potentially could release more N. In the field, during the growing season, we found no difference in labile C pools (MB-C and DOC), but differences may be more likely to appear in the next phase of the rotation when field pea residues will be the most recent residue addition.

Repeat-pulse labeling for crop residues shows promise as a useful tool in measuring total carbon additions from crop residue and may provide better estimates than root washing techniques. Our results were comparable to literature values of crop C additions within 100 cm of the soil surface which were derived from root washing. This suggests that these techniques may still be underestimating total C additions as our values were obtained within 15 cm of the soil surface.

In determining residue turnover and movement through C pools, it must be assumed that the label is evenly distributed throughout the plant. According to our data, it is likely that plant parts will have different levels of enrichment, especially those parts that develop in later growth stages, for instance, the pod. The contribution of these fractions to SOM via crop residues is significant but may be greatly underestimated if assumptions of homogeneity are not properly addressed. As well, because ADF fractions in this study were more enriched in ^{13}C than the ADL fractions, labile plant biochemical fraction contributions to SOM will likely be overestimated and contributions of more recalcitrant plant biochemical fractions will likely be underestimated. The consequences of accepting this assumption without testing the homogeneity of ^{13}C plant enrichment will lead to large biases in data interpretation.

This study comprised the first year of a multi year study. Future measurements of gross mineralization and gross nitrification may better explain literature observations of increased N availability to crops succeeding pulse crops. The trend of increasing mineralization rates in our study, although not statistically significant, should not be ignored. Nevertheless, this observation alone does not support our hypothesis that field pea in rotation will increase gross mineralization and nitrification rates. Our measurements of N_2O emissions can not conclusively support our hypothesis that field pea rotations would emit less N_2O not only because no significant differences among

rotations were found but because we had just three sampling events. More frequent measurements or continuous measurements of N₂O emissions would better determine the true affect of field pea.

Differences in crop residue quantity and quality should be explored further, particularly with respect to their effect on mineralization. Because we did find field pea contributed less quantity of a more labile residue in comparison to canola, it would be useful to investigate field pea and canola residue contributions to various SOM fractions. However, in order to do this, the repeat-pulse method proposed in this study needs to be modified. With more frequent labeling periods and increased labelling events during pod formation, it is likely that the distribution of ¹³C within the plant would be improved. Furthermore, the experimental design should be completely randomized. In order to facilitate this, chambers should be constructed on mobile bases so any affect of varying environmental conditions within the chamber would be minimized. As plants matured, they were constricted within the labeling chambers. This may have resulted in shading of various plant parts. The size and height of the labeling chamber should be carefully considered so overcrowding plants during the labeling events can be avoided.

Finally, with continued measurements of gross mineralization and nitrification at various sites within Saskatchewan, paired with a better understanding of the effect of pulse crops on carbon cycling facilitated by repeat-pulse labeling, predictions of the fate of N and C may be better defined. Ultimately a better definition of these processes could lead to better fertility and crop rotation recommendations that would improve both the economic and environmental status of Saskatchewan agroecosystems.

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APPENDIX A

Analysis of variance testing the influence of chamber and pot on ^{13}C labeling

Table A.1. Analysis of variance testing the influence of chamber and pot on ^{13}C labeling in pea using a nested design

Source	df	SS	F	Sig.
Stem				
Chamber	1	0.145	0.000	0.007
Chamber error	2	3706		
Pot (Chamber)	2	3706	6.648	0.011
MS (error)	12	3345		
Pod				
Chamber	1	2.065	0.001	0.974
Chamber error	2	3012		
Pot (Chamber)	2	3012	6.058	0.015
MS (error)	12	2983		
Leaf				
Chamber	1	172.8	0.101	0.780
Chamber error	2	3392		
Pot (Chamber)	2	3392	3.665	0.057
MS (error)	12	5553		
Root				
Chamber	1	9.101	0.015	0.912
Chamber error	2	1181		
Pot (Chamber)	2	1180	2.456	0.131
MS (error)	11	2643		

Table A.2. Analysis of variance testing the influence of chamber and pot on ^{13}C -labeling in canola using a nested design

Source	df	SS	F	Sig.
Stem				
Chamber	1	24.22	0.079	0.805
Chamber error	2	613.9		
Pot (Chamber)	2	613.9	1.865	0.197
MS (error)	12	1975		
Pod				
Chamber	1	57.27	0.451	0.571
Chamber error	2	254.1		
Pot (Chamber)	2	254.1	0.951	0.414
MS (error)	12	1603		
Leaf				
Chamber	1	5.597	0.029	0.881
Chamber error	2	387.2		
Pot (Chamber)	2	387.2	0.445	0.651
MS (error)	12	5222.8		
Root				
Chamber	1	336.8	0.332	0.623
Chamber error	2	2027		
Pot (Chamber)	2	2027	3.105	0.082
MS (error)	12	3917		