

**YIELD, NUTRIENT UPTAKE, NITROGEN FIXATION AND RELEASE BY SOYBEAN,  
PEA, AND LENTIL AND IMPACT ON FOLLOWING CROPS IN ROTATION IN  
SASKATCHEWAN, CANADA**

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Saskatoon

By

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## ABSTRACT

As soybean acreage is expanding in western Canada, research is required to assess soybean production regarding the yield, nutrient uptake, N<sub>2</sub> fixation, and the effects on following crops under local soil-climatic conditions. Through a two-year rotation study conducted at four sites in the Black and Dark Brown soil zones in Saskatchewan, Canada, this research aimed to estimate the grain and straw yield, nutrient uptake, and N<sub>2</sub> fixation of three short-season soybean varieties in comparison to three pea and three lentil varieties, and the effects on the yield, nutrient uptake, and residue N recovery by following wheat and canola crops grown on the stubble of soybean, pea, or lentil. Soil nutrient supply rates and greenhouse gas emissions as influenced by soybean, pea, and lentil residues were also estimated in this research. Soybean produced similar or higher grain yield (2512 kg ha<sup>-1</sup>) and nutrient uptake (112 kg N ha<sup>-1</sup> and 14 kg P ha<sup>-1</sup>), and had similar effects on soil macro- and micronutrient availability to the following crops in comparison to pea and lentil. Lentil generally had lower grain yield yet similar rotational effects across the sites. Compared to pea and lentil, soybean had significantly higher K, Ca, Mg, and S concentrations in the grain across the sites, and similar or larger removal of these nutrient elements, suggesting potential for additional depletion of these elements from the soil over the long-term when soybean is grown. At maturity, soybean, pea, and lentil fixed similar amount of N in the above-ground plant components (grain + straw), with the majority (67-85%) of fixed N retained in the grain of soybean (119 kg N ha<sup>-1</sup>), pea (160 kg N ha<sup>-1</sup>), and lentil (89 kg N ha<sup>-1</sup>). Nitrogen derived from fixation comprised over 60% of the total above-ground N of the pulse crops. Soybean, pea, and lentil stubbles demonstrated similar effects on greenhouse gas emissions and soil N and P supplies measured under both field and controlled conditions. Overall, this research suggests promising prospects for soybean production under the soil-climatic conditions in the northern Great Plains, with considerations for soil P and K depletion in the long term when soybean is grown, although similar short-term effects on yield and nutrition of following crops were observed from soybean, pea, and lentil grown under similar conditions.

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## **DEDICATION**

This dissertation is dedicated to my parents who cultivated my love of life and nature. It is also written in appreciation for all those who dedicate their livelihoods to building humankind's knowledge of the world in which we live.

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## LIST OF ABBREVIATIONS

<b>BNF</b>	Biological N <sub>2</sub> fixation
<b>GHG</b>	Greenhouse gas
<b>cv</b>	Cultivar
<b>ANOVA</b>	Analysis of variance
<b>EC</b>	Electrical conductivity
<b>MDCD</b>	Minimum detectable concentration difference
<b>MIT</b>	Mineralization-immobilization turnover
<b>Ndfa</b>	Nitrogen derived from atmosphere
<b>Ndff</b>	Nitrogen derived from fertilizer
<b>Ndfs</b>	Nitrogen derived from soil
<b>Ndfr</b>	Nitrogen derived from residue
<b>NFRV</b>	Fertilizer N replacement value
<b>RCBD</b>	Randomized complete block design
<b>RDA</b>	Recommended daily allowance
<b>SCZ</b>	Soil-climatic zones
<b>%Ndfa</b>	Percentage nitrogen derived from atmosphere
<b>%Ndff</b>	Percentage nitrogen derived from fertilizer
<b>%Ndfr</b>	Percentage nitrogen derived from residue

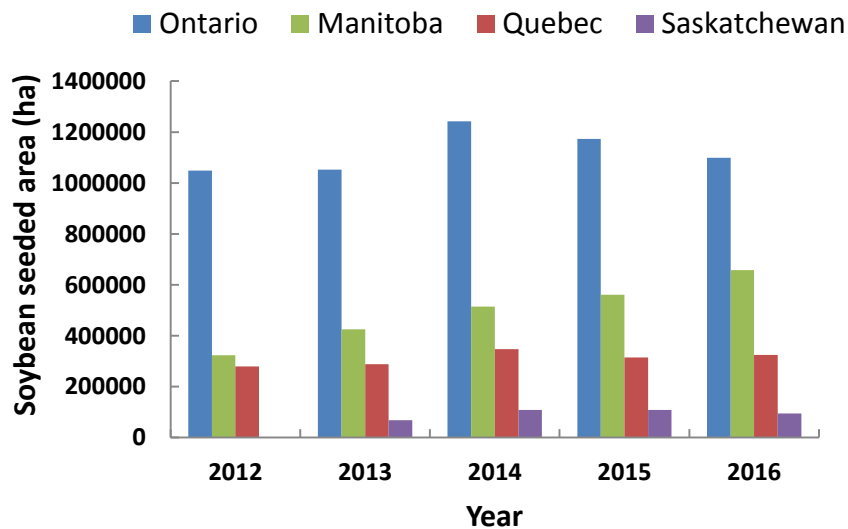
# 1. GENERAL INTRODUCTION

## 1.1 Introduction

Cropping systems in western Canada have been experiencing a shift from wheat-based monoculture to more diversified cropping sequences in the last few decades (Cowell et al., 1989; Lupwayi and Kennedy, 2007). Indeed, Saskatchewan has become a major producer of pulse crops (grain legumes), making Canada the world's largest exporter of peas (*Pisum sativum* L.) and lentils (*Lens culinaris* L.) (Statistics Canada, 2011). Accordingly, a variety of research projects have been conducted to assess factors affecting crop performance and N<sub>2</sub> fixation of grain legumes grown in western Canada (Cowell et al., 1989; McKenzie et al., 2001; Gan et al., 2010b), and the impact of legume production on soil fertility (Campbell et al., 1992; Soon and Arshad, 2004a), soil microbial processes (Lupwayi et al., 1998), yield and nutrient uptake of subsequent non-legume crops in rotation (Beckie and Brandt, 1997; Beckie et al., 1997), and environmental sustainability (Gan et al., 2009a; Zhong et al., 2009), under western Canadian soil-climatic conditions (Walley et al., 2007; Thiessen Martens et al., 2015).

Reported rotational benefits of grain legumes include both N benefits and non-N benefits. In particular, these rotational N benefits include N contribution to the soil N pool via root exudates and residue decomposition, improvement of yield and N uptake of subsequent crops in rotation; while non-N rotational benefits mainly include breaking pest cycles, increasing soil nutrient availability to following crops, and enhancing soil microbial structure and activity that contributes to crop N nutrition (Lupwayi and Kennedy, 2007; Peoples et al., 2009). As a rule of thumb, legumes with high N-fixing capability are more likely to have a positive contribution to the overall N budget in the long term, although it is also recognized by researchers that great variation and uncertainty exists in the estimates of N<sub>2</sub> fixation and N credits of grain legumes (Janzen et al., 2003; Walley et al., 2007).

Soybean [*Glycine max* (L.) Merr.] is a relatively new addition to the cropping rotation in western Canada, and soybean acreage is expanding in this region (Statistics Canada, 2016) (Fig. 1.1). However, there has been little research conducted to systematically assess the agronomic performance and environmental impact of soybean production under local soil-climatic conditions, including its grain and straw yield, N<sub>2</sub> fixation, nutrient uptake, and effects on the soil nutrient pool, greenhouse gas emissions, as well as the yield and nutrient uptake of following rotational crops. In contrast, pea and lentil are commonly grown grain legumes in this region, and have been relatively well researched regarding the agronomic and environmental implications (Cowell et al., 1989; Wright, 1990; Walley et al., 2007; Statistics Canada, 2011). In addition, although soybean is commonly classified as an oilseed, it is referred to as a pulse crop along with pea and lentil in this dissertation.



**Fig. 1.1.** Soybean acreage in Ontario, Manitoba, Quebec, and Saskatchewan from year 2012 to 2016. Data for Saskatchewan soybean acreage began to be collected and reported by Statistics Canada starting in 2012. Data resource: Statistics Canada (2016).

Therefore, the Ph.D. project described in this dissertation was conducted to systematically investigate the agronomic performance of soybean, including the uptake and release of nutrients and effects on greenhouse gas emissions, soil nutrient forms and availability, in comparison to pea and lentil under contrasting soil-climatic zones (SCZ). The project comprised a two-year field rotation experiment and a series of incubation experiments. In the field experiment, three modern varieties each of soybean, pea, and lentil, were grown in the first rotational year at four sites (two in Dark Brown SCZ, two in Black SCZ) in 2014, and in 2015 wheat or canola was grown on the stubble of the grain legumes. In the first rotational year, the grain and straw yield, macro- and micronutrient uptake, and N<sub>2</sub> fixation of soybean, pea, and lentil, were assessed, and in the second rotational year measurements made included the soil nutrient supply during the growing season, and the grain and straw yield and nutrient uptake of wheat and canola as influenced by different legume stubbles from the previous year. In addition, an 8-week incubation experiment was conducted to investigate the soil N and P supplies and greenhouse gas emissions from intact soil core samples taken in the fall of 2014 after soybean, pea, and lentil residue incorporation. Nitrogen-15 dilution technique was used to assess biological N<sub>2</sub> fixation (BNF) by the pulse crops, and to follow the recovery of above-ground residue N by following crops. Results of this Ph.D. project provide useful agronomic and environmental information for producers, policy makers, and scientists regarding fertility management in soybean production systems and their impact in western Canada.

## **1.2 Organization of the dissertation**

This dissertation is organized in manuscript format, with the general introduction (Chapter 1) followed by literature review (Chapter 2) and then four research chapters (Chapter 3 - Chapter 6) covering the field experiments and incubation experiment. Chapter 7 is the last chapter of the dissertation that synthesizes the major findings, conclusions, and needs for future research. The Appendix section includes ancillary results from both the field trials and greenhouse experiments that are not discussed in the research chapters of this dissertation.

The Literature review chapter, Chapter 2, reviews the current understanding of important topics related to my research, such as nutrient requirements, rotational benefits, and nitrogen fixation of grain legumes, as well as approaches for measuring N<sub>2</sub> fixation and our current knowledge of fate of N from crop residue in the soil-plant system. Each of the four following research chapters covers one main aspect of the research. Chapter 3 focuses on crop

yield, and the uptake of N and P in legumes and crops grown in the following year. This includes the yield and uptake of N and P of three varieties of soybean, pea, and lentil in the first rotational year, and of wheat and canola grown, depending on site, in the second year. In addition, this chapter also presents soil N and P supply rates measured in the field over the second growing season as influenced by different grain legume stubbles. Chapter 4 covers the uptake of mineral nutrients apart from N and P, including K, Ca, Mg, S, Cu, and Zn.

After considering crop yield and macro- and micro-nutrient uptake in Chapters 3 and 4, in Chapter 5 the dissertation turns to the important aspect of  $N_2$  fixation by the pulse crops and the fate of  $^{15}N$  in the rotation. Chapter 5 assesses the  $N_2$  fixation by soybean, pea, and lentil using  $^{15}N$  dilution techniques, and the enrichment of  $^{15}N$  in the surface soil after the growing season and in wheat in the second rotational year is covered in this chapter. After the field experiments covered in Chapters 3 to Chapter 5, Chapter 6 describes and interprets results of an incubation experiment conducted under controlled conditions over an 8-week period. This incubation used intact soil core samples with soybean, pea, or lentil residue taken in the fall of 2014 from the field sites. Soil supply rates of N and P were measured in the cores to indicate the effects of soybean, pea, and lentil residue on soil N and P supply under controlled conditions, along with  $CO_2$  and  $N_2O$  emissions, and  $^{15}N_2O$  enrichment in the gas fluxes measured through the incubation period. The final chapter, chapter 7, summaries and integrates the results of the different studies, with conclusions and recommendations made.



## 2. LITERATURE REVIEW

### 2.1 Nutrient requirements and rotational benefits of grain legumes

#### 2.1.1 Yield and nutrient uptake

Grain legumes play an important role in human nutrition due to their richness in protein, complex carbohydrates, vitamins and minerals, and their contribute to sustainable agriculture through N<sub>2</sub> fixation and other rotational and environmental benefits, such as breaking pest cycles, improving soil physical properties, enhancing nutrient uptake by subsequent non-legume crops in rotations, and mitigating greenhouse gas emissions (Crews and Peoples, 2004; Lupwayi et al., 2011). With the shift of cropping system rotational breaks on the Canadian Prairies from fallow-based to legume-based (Lupwayi and Kennedy, 2007), Canada has become a major contributor of pulses to the world. In 2011, Canada was the biggest exporter of dry peas (*Pisum sativum* L.) and lentils (*Lens culinaris* L.) in the global market, producing 21.7% and 34.8% of the world's total pea and lentil production, respectively, with most of them produced in Saskatchewan (Statistics Canada, 2011). Compared to pea and lentil, soybean [*Glycine max* (L.) Merr.] is relatively new to the Canadian Prairies, with the producing area increasing from 68,800 hectares in 2013 to 109,300 hectares in 2015 and 97,100 hectares in 2016 in Saskatchewan (Statistics Canada, 2016). Integrating grain legumes into the cropping systems has resulted in a variety of impact on agricultural production and environmental sustainability on the Canadian Prairies.

Sufficient nutrient supplies at early growth stages are important for attaining optimum seed yield of grain legumes, and different crop species vary in their nutrient requirements in different growth stages. For example, maximum N uptake rates take place at branching to early bud formation, and maximum biomass accumulation at early to late budding in pea and lentil (Malhi et al., 2007). Similarly, soybean yield is more directly related to mineral nitrogen

assimilation rather than  $N_2$  fixation in the early reproductive growth stages (Fabre and Planchon, 2000). According to Fabre and Planchon (2000), both soil N assimilation and biological  $N_2$  fixation were important pathways affecting the seed yield and protein content of soybean, with soil N assimilation more related to yield performance in early stages and  $N_2$  fixation involved in protein accumulation in the seed in later growth stages. Moreover, findings of Koutroubas et al. (1998) also implied the importance of biomass and N accumulation at early stages of soybean growth in achieving high seed yield, and soybean seed yield increased by inoculation but showed no responses to N fertilizer application as the soil N availability was sufficient.

Increasing the seed yield is generally the goal of agronomic management, genetic breeding and cultivar selection (Liu et al., 2005). Indeed, yield increase of soybean in North America ranged from  $22 \text{ kg ha}^{-1} \text{ yr}^{-1}$  to  $31 \text{ kg ha}^{-1} \text{ yr}^{-1}$  of different maturity groups over a 60-year period (Wilcox, 2001). Despite the difficulties in accurately quantifying the contribution of genetic improvement to yield increase, some regional studies have estimated the percentage of yield increase that could be attributed to genetic improvement through crop breeding. For example, contribution of crop breeding to the increase in soybean seed yield was 0.6% annually on average from 1950 to 2006 in Northeast China (Jin et al., 2010) and 0.5-0.7% per year in North America from 1945 to 2000 (Kumudini, 2002). In Canada, Cober and Voldeng (2012) suggested that on-farm soybean grain yield in Ontario increased at a rate of  $25 \text{ kg ha}^{-1} \text{ yr}^{-1}$  from 1971 to 2000, with 80% of the annual yield increase in soybean attributed to genetic improvement and 20% attributed to agronomic practices. Moreover, Yang et al. (2010) estimated that the average  $N_2$  fixation rate was  $95 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  of pulses, primarily including dry field pea, lentil, dry bean, fababean, and chickpea, and  $118 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  of soybean over 25 years across Canada, with great variation from one province to another for each crop. Together with breeding, soil-climatic conditions and agronomic management practices have contributed to large variation in legume yield and  $N_2$  fixation over time and from one region to another, making it rather difficult to accurately estimate the N contribution of legumes to soil N pools and subsequent crops in rotation (Unkovich and Pate, 2000; Walley et al., 2007).

On the western Canadian prairies, estimates of nutrient uptake and  $N_2$  fixation by legumes varied from one year to another and from one region to another as a result of different soil-climatic conditions and agronomic practices including tillage, fertilization, and crop

rotation. In a field study carried out in 1998-1999 at Melfort, Saskatchewan, nutrient uptake in the seed of pea (cv. Carneval) and lentil (cv. Laird) at harvest was estimated as 132 kg N ha<sup>-1</sup>, 14 kg P ha<sup>-1</sup>, and 40 kg K ha<sup>-1</sup> of pea, and 113 kg N ha<sup>-1</sup>, 11 kg P ha<sup>-1</sup>, and 30 kg K ha<sup>-1</sup> of lentil in 1998; while in 1999 the nutrient uptake was 79 kg N ha<sup>-1</sup>, 9 kg P ha<sup>-1</sup>, and 24 kg K ha<sup>-1</sup> of pea, and 19 kg N ha<sup>-1</sup>, 2 kg P ha<sup>-1</sup>, and 5 kg K ha<sup>-1</sup> of lentil, with this great temporal variation in nutrient uptake attributed to lower seed yield and harvest indexes in the second year due to hail damage (Malhi et al., 2007). A study in North Dakota reported the nutrient requirements of field pea as 0.034 kg N, 0.0041 kg P, and 0.0115 kg K per kilogram seed yield at harvest, and indicated that tillage practices, fertilization, and their interaction with climate influence biomass accumulation, seed yield, and nutrient uptake in the field pea production systems in the Northern Great Plains (Deibert and Utter, 2004). Soil water status in particular affects pulse crop yield and nutrient uptake, especially in drier regions such as the Brown soil-climatic zone. As Gan et al. (2010) reported, better soil water conditions increased N uptake in the seed but not straw, resulting in higher harvest N indexes (HNI) under better water conditions, with the HNI being 0.56 for dry pea and 0.55 for lentil under low water conditions, as opposed to 0.69 for dry pea and 0.75 for lentil under high soil water conditions.

Unlike pea or lentil, soybean grown in western Canada has received little research attention as to yield and nutrient uptake potential, while in Eastern Canada and other regions there have been studies investigating nutrient requirements of soybean as influenced by variable agronomic management and climatic conditions (Table 2.1). For example, in a tillage experiment conducted on a clay loam soil in Quebec, soybean and maize grain yield were 10-25% lower in plots with 11 years of no-till management as opposed to conventional tillage systems (Messiga et al., 2012). In the same study, grain yield did not respond to P fertilizer application, possibly due to high initial soil P levels. In northern soybean production regions of North America, such as Minnesota and Ontario, soybean yield performance was negatively impacted by the increase of corn residue in the no-till corn-soybean rotation systems, due to reduced soil available N levels and soybean nodulation as well as impaired soil physical properties such as reduce soil temperature and evaporative potential (Vanhie et al., 2015). In a three-year experiment conducted in northern Illinois, 275 kg N ha<sup>-1</sup>, 21 kg P ha<sup>-1</sup>, 175 kg K ha<sup>-1</sup>, and 113 kg Ca ha<sup>-1</sup> were required to produce 3500 kg ha<sup>-1</sup> grain yield of soybean, and P had the highest harvest index (81%) compared to other nutrients such as N (73%), Cu (62%), and S (61%),

indicating high P removal by soybean seed and the importance of monitoring soil P levels when soybean was used in the cropping systems (Bender et al., 2015).

In warmer areas of the world such as India, with different N, P, and K fertilizer additions, the total N uptake of soybean was estimated as 110-160 kg N ha<sup>-1</sup> under conventional tillage, in which crop residue was incorporated into the soil, and 112-164 kg N ha<sup>-1</sup> under conservation agriculture, in which crop residue was retained on the soil surface (Aulakh et al., 2012). Moreover, in northeast China, agronomic management practices were shown to be mainly responsible for soybean yield variability as opposed to soil properties, with P fertilizer application explaining about 61% of the yield variability as revealed by crop production prediction models (Zheng et al., 2009). Overall, previous studies have shown that both regional soil-climatic and agronomic management practices contributed to the yield and nutrient uptake of grain legumes in general, underlining the importance of multi-site assessment of legume performance, such as biomass accumulation and grain yield, nutrient uptake, N<sub>2</sub> fixation, and rotational impact on the soil nutrient pool and subsequent crops in rotation. Summary of yield and uptake of N and P in the grain of soybean grown under different soil-climatic conditions over the world is presented in Table 2.1.

**Table 2.1.** Yield and N uptake in soybean grain adapted from previous studies.

Region	Soil Type	Yield	N uptake	References
		kg ha <sup>-1</sup>		
Global	n/a	2690	155	(Salvagiotti et al., 2008)
Global	n/a	2300	86	(Herridge et al., 2008)
Northeast China	Mollisol (Black soil)	1450-2700	-- <sup>†</sup>	(Jin et al., 2010)
Gansu, China	Calcaric Cambisols	2200-3890	38-101	(Yang et al., 2006)
Bhopal, India	Vertisols	932-1715	--	(Ghosh et al., 2004)
Ludhiana, India	Typic Haplustept	1793-2514	37-55 <sup>‡</sup>	(Aulakh et al., 2012)
Paraná, Brazil	Rhodic Eutrudox	2057-3778	135-169	(Hungria et al., 2006)
	Typic Haplustox	1774-3454	154-189	
Thessaloniki, Greece	Typic Xerorthent	3400-4700	209-333	(Koutroubas et al., 1998)
Illinois, USA	Typic Endoaquolls-	3480±155	201±11	(Bender et al., 2015)
	Aquic Argiudolls			
Kentucky, USA	Cumulic Epiaquolls	2950-3860	175-244	(Egli and Bruening, 2007)
	Oxyaquic Argiudolls	2550-3050	147-194	
	Typic Paleudalfs	3200-4060	170-244	
Quebec, Canada	Dark Grey Gleysol	1010-4540	--	(Messiga et al., 2012)
Manitoba, Canada	Orthic Black	1098-3415	--	(Przednowek et al., 2004)
	Chernozem			

<sup>†</sup> Data not reported.

<sup>‡</sup> Calculated according to Herridge et al. (2008).

### 2.1.2 Rotational N benefits

Grain legumes are important to fertilization management and sustainable agricultural systems, mainly due to their ability to access atmospheric N ( $N_2$ ) via the root nodules formed following the infection by *Rhizobium* bacteria, which convert  $N_2$  to ammonia and supply ammonia to legume plants in exchange for carbon source and energy (Peoples et al., 2009). In this way, legumes are able to partially meet their N requirements, and thus reduce their N fertilizer demand and contribute to the soil N pool and following rotational crops with fixed atmospheric N through pathways such as rhizodeposition and residue decomposition (Brophy and Heichel, 1989; Peoples et al., 1995b; Arcand et al., 2014b). Compared to inorganic N fertilization,  $N_2$  fixation is more sustainable in that it mitigates the depletion of the soil N pool and replenishes the soil N pool without causing environmental issues related to excessive use of inorganic N fertilizers (Peoples et al., 1995b). Indeed, rotational benefits of grain legumes to soil nutrient store and succeeding crops that include both N and non-N benefits have been well documented in the last two decades under both field and laboratory conditions (Stevenson and van Kessel, 1996a; Przednowek et al., 2004; Salvagiotti et al., 2008; Peoples et al., 2009).

Salvagiotti et al. (2008) reviewed 108 studies on soybean  $N_2$  fixation and its response to N fertilization under field conditions, and found that  $N_2$  fixation met more than half (50-60%) of the soybean N demand across a wide range of yield levels and environments, with the proportion of plant N derived from air (Ndfa) through  $N_2$  fixation declining with increased N fertilizer inputs. Furthermore, the same review showed that the N balance was negative ( $-40 \text{ kg N ha}^{-1}$ ) in most studies when only above-ground plant N was considered, but the average N balance was almost neutral ( $-4 \text{ kg N ha}^{-1}$ ) if the below-ground parts were included as well. In addition to N fertilizer application, other factors affecting the  $N_2$  fixation and thus N contribution of legumes to succeeding cereal crops and soil N cycling include soil physical properties (Schipanski et al., 2010), soil water content and inoculation (Sadeghipour and Abbasi, 2012), and agronomic management practices (Rennie et al., 1988; Wheatley et al., 1995; Deibert and Utter, 2004). For example, soil water stress reduced  $N_2$  fixation, growth, and the grain yield of soybean, while applying N fertilizers increased the drought tolerance of soybean (Sadeghipour and Abbasi, 2012). Fertilizer N replacement value (NFRV) associated with legumes in rotation with a cereal is defined as the amount of fertilizer N required in a corn-corn sequence to produce yield equivalent to those in a legume-corn sequence without fertilizer N

(Shrestha et al., 1999). In central Ontario, NFRV was about 30 kg N ha<sup>-1</sup> for soybean-corn sequence compared to corn-corn sequence (Ding et al., 1998). Similar results were observed in the first year cereal crops following soybean in Lancaster, USA, but this N contribution decreased in the second year cereal crops (Vanotti and Bundy, 1995). Despite significant N contribution from soybean, non-N rotational benefits might even exceed the N benefits in crop rotations. Stevenson and van Kessel (1996) estimated that 92% of the yield advantage in a pea-wheat rotation was attributed to non-N rotation benefits in comparison to a wheat-wheat sequence in Saskatchewan. However, these non-N benefits may have decreased in recent years as acreages of pulse crops and other broad-leafed crops have increased while frequency of cereal in rotation has decreased, because using a cereal monoculture sequence as the reference usually caused overestimations of the non-N benefits of legumes in legume-cereal rotations, whereas using broadleaf crops as reference crops could avoid this overestimation (Beckie and Brandt, 1997; Beckie et al., 1997). These non-N effects were related to reducing root and leaf diseases and the increasing availability of P, K, S, and unidentified other growth substances released from legume residue (Peoples et al., 2009; Dayegamiye et al., 2012). Furthermore, the rotational benefits from grain legumes are influenced by agronomic practices to a significant degree through application of inoculants, methods of harvesting and residue management, and fertilizer application in following cereal crops. Inappropriate management practices could jeopardize the rotational benefits from pulse crops in rotation, and/or even cause negative effects to following cereal crops and to the environment (Ennin et al., 2004).

In comparison to soybean, field pea and lentil in Canadian agro-ecosystems were reported to have larger amount of dry matter allocated to agricultural harvest residue and higher N concentration in the residue on average (Janzen et al., 2003). Along with this, one study conducted in southern Manitoba reported the least N benefits from soybean to a following wheat crop while field pea had the highest (Przednowek et al., 2004). However, reported rotational benefits associated with grain legumes normally have great variation depending on the growth conditions for the legume and following crops. As a rule of thumb, field pea, lentil and fababean had positive N contribution to subsequent crops in the long term, while chickpea and common bean had less N contribution to a following crop or even caused soil N deficits in prairie soils in western Canada (Walley et al., 2007). However, comparisons

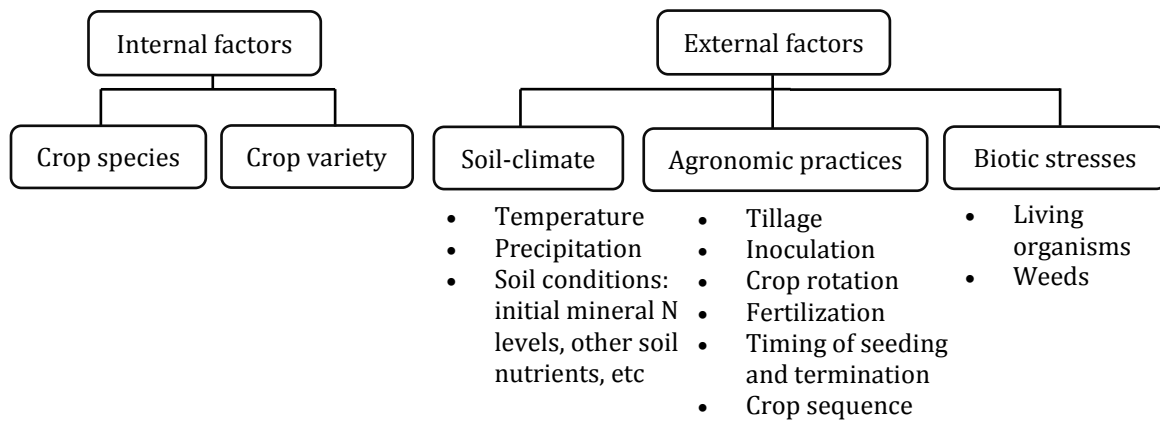
between soybean and other grain legumes regarding rotational benefits to following cereal crops under western Canadian conditions in a variety of soils have not been conducted to date.

## **2.2 Nitrogen fixation by grain legumes and approaches to measurement**

In agricultural systems, N inputs occur primarily through N fertilization, N<sub>2</sub> fixation, and atmospheric deposition, with N added to the soil via synthetic fertilizers, such as anhydrous ammonia, urea, ammonium nitrate, and ammonium sulfate, being the largest source of bioavailable N in many agricultural soils worldwide (Crews and Peoples, 2004). However, due to the growing desire to reduce reliance on commercial N fertilizers, N<sub>2</sub> fixation has received more attention than in the past (e.g. 1950's to 1990's), when fertilizer N was the main N source for agricultural systems and increasing yield rather than sustainability was the principal driving force for agricultural production (Peoples et al., 1995b). Grain legumes fix atmospheric N<sub>2</sub> via N<sub>2</sub> fixation by forming symbiotic associations with a variety of soil bacteria known as rhizobia, which, with the aid of nitrogenase enzymes, convert N<sub>2</sub> into ammonia (NH<sub>3</sub>) in root nodules, and the nodules are formed as a consequence of the *Rhizobium* infection in the plant roots (Zahran, 1999). Newly fixed NH<sub>3</sub> is provided for incorporation into metabolic N compounds for the legume plant, and in return, plants provide rhizobia with energy, amino acids, and sugars (Hardarson and Atkins, 2003). In this way, legumes are able to meet part or almost all of the N requirements for growth and reproduction and consequently, take up less N from the soil N pool. Reported amount of N derived from fixation by legumes show large variation (Unkovich and Pate, 2000). For example, Yang et al. (2010) reported a range in amount of N derived from fixation from 54-150 kg N ha<sup>-1</sup> from 1981 to 2006 in Canada, with a mean of 95 kg N ha<sup>-1</sup> of N<sub>2</sub> fixation by pulse crops mainly including dry field pea, lentil, dry bean, fababean, and chickpea.

The great variation in estimates of N<sub>2</sub> fixation by grain legumes can be attributed to both internal and external factors, such as crop factors, environmental factors, and agronomic practices (Peoples et al., 2012) (Fig. 2.1). Crop species and cultivars significantly affect the percentage of N derived from atmospheric N (%Ndfa) and the total amount of N fixed (Ndfa) by legumes. Unkovich and Pate (2000) reviewed legume N<sub>2</sub> fixation, including both shoot-N and root-N, from a variety of environmental conditions globally, and ranked soybean as the crop fixing the most N, followed by lupin, field pea, faba bean, common bean, lentil, and chickpea, although the authors also recognized that these estimates might have little relevance to

particular local growing conditions. For example, in one study conducted in southern Manitoba, soybean had the least apparent N benefits compared to pea, chickpea, and dry bean (Przednowek et al., 2004). In addition, N<sub>2</sub> fixation can also differ among cultivars of the same legume crop. For example, in Greece and Romania, soybean cultivars, including Chippewa, Williams, and Amsoy-71, had significant influence regarding the Ndfa levels that, in turn, were generally positively correlated with dry matter yield (Danso et al., 1987). In pea and lentil, similarly, the %Ndfa was significantly affected by cultivar and positively related to nodule numbers, as reported in a study in USA testing the N<sub>2</sub> fixation by five US cultivars of pea and of lentil, respectively, with wheat used as the reference crop (Abi-Ghanem et al., 2011). Moreover, environmental factors can greatly affect the N<sub>2</sub> fixation by legumes, including precipitation and temperature, soil water and mineral N conditions, other soil nutrient content such as soil P and K, soil temperature and pH, residual effects of sulfonylurea herbicides, pests, and soil salinity, as summarized in detail by Peoples et al. (2012) and Hardarson and Atkins (2003). Fig. 2.1 illustrates the main factors affecting the N<sub>2</sub> fixation by grain legumes, including internal factors (crop species and variety) and external factors (environmental and management factors).



**Fig. 2.1.** Factors affecting the N<sub>2</sub> fixation by grain legumes.



As a rule of thumb, N<sub>2</sub> fixation has a negative correlation to soil N availability (Herridge et al., 1984; Chalk, 2000; Schipanski et al., 2010), with the latter being closely related to other soil properties such as soil texture, soil water content, and soil organic matter content (Janzen, 2001). N<sub>2</sub> fixation was completely suppressed by applying urea at the rate of 100 kg N ha<sup>-1</sup> and significantly higher carbon isotope discrimination values were observed in plants with Ndfa than in plants obtaining N only from soil and fertilizer ((Knight et al., 1995), suggesting an impact of N source on water use efficiencies. Generally, environmental factors affect legume N<sub>2</sub> fixation mainly by regulating the soil physical, chemical, and biological conditions, which are crucial for dry matter accumulation, root nodulation, soil microbial activity, and accessibility of plant roots to soil water and nutrients (Bordeleau and Prévost, 1994). Agronomy management factors, similarly, indirectly affect the N<sub>2</sub> fixation of legumes through regulating growing conditions of the crops, such as inoculation (Sadeghipour and Abbasi, 2012), tillage (Wheatley et al., 1995), fertilizer application (Salvagiotti et al., 2008), timing of planting and termination (McCauley et al., 2012), cropping sequence (Peoples et al., 1995c; Matus et al., 1997), and frequency of legumes in rotation (Knight, 2012). These factors affect the N<sub>2</sub> fixation of legumes by regulating the conditions for symbiotic relationship forming, such as inoculating legume seeds with commercially available superior strains of rhizobia for attaining optimum nodulation (Hardarson and Atkins, 2003), and managing the cropping sequence (Peoples et al., 1995a), fertilizer applications, and legume frequency (Knight, 2012) to regulate soil starting N levels for legumes to meet the N requirements for early crop development without suppressing N<sub>2</sub> fixation in later stages. Therefore, N<sub>2</sub> fixation of legumes can be enhanced via such ways as breeding legume cultivars with higher dry matter yield and higher rhizobium infection rates supporting greater numbers of actively fixing nodules, and regulating the nodulation and growing conditions for legumes through above-mentioned agronomic practices. Large gains in N<sub>2</sub> fixation without associated yield penalty will require efforts to enhance biological efficiency in terms of energy consumed per unit of N fixed.

Walley et al. (2007) suggested that despite the great variation in the N benefits of legumes reported, common legume species on the northern Great Plains with higher levels of N<sub>2</sub> fixation, such as faba bean, field pea, and lentil, tended to have more N benefits in comparison to species with lower levels of N<sub>2</sub> fixation. Accurate quantification of N<sub>2</sub> fixation, therefore, is the key to assessing the N benefits of grain legumes in crop rotations and

understanding their roles in soil fertility and sustainable agriculture. To date, several approaches to measuring N<sub>2</sub> fixation have been reported, and commonly used ones include dry matter yield method, total N difference method, nodule observations, acetylene reduction assay, xylem-solute technique, and <sup>15</sup>N methodologies, including use of <sup>15</sup>N<sub>2</sub> gas, <sup>15</sup>N isotope dilution method, A-value method, and natural abundance method, as summarized by Hardarson and Danso (1993) and Unkovich and Pate (2000). Among these methods, <sup>15</sup>N isotope methods are generally more costly but considered more accurate if applied properly (Witty, 1983; Khan et al., 2002). This review will focus on the <sup>15</sup>N dilution method, which is the best way to quantify N<sub>2</sub> fixation due to its power to discriminate among Ndfa, N derived from fertilizer (Ndff), and N derived from soil (Ndfs) (Rennie, 1982).

The <sup>15</sup>N isotope dilution method was first proposed by McAuliffe et al. (1958), with the term “dilution” referring to the basis of this technique that atmospheric N fixed by plants dilutes the enriched N taken up from the soil and/or fertilizer by plants. Briefly, <sup>15</sup>N-enriched fertilizers are applied to soil for N-fixing crops to take up, with an appropriate non-fixing crop used as the reference crop, and the atom % <sup>15</sup>N of the legume crop and the reference crop is used to calculate the N<sub>2</sub> fixation by the legume crop (McAuliffe et al., 1958; Fried and Middelboe, 1977). Normally, <sup>15</sup>N-labeled fertilizer additions include inorganic salts [e.g. (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, NH<sub>4</sub>Cl, KNO<sub>3</sub>, NH<sub>4</sub>NO<sub>3</sub>] and <sup>15</sup>N-labelled urea fertilizer. In some other cases, <sup>15</sup>N-labelled organic matter such as enriched crop residue were also used, and this can mitigate the uneven distribution of the <sup>15</sup>N in space and time (Witty, 1983). Fertilizers can be added as a liquid by using a watering can, spraying or injecting into soil, or as a solid by broadcasting, banding, or mixing with soil (Chalk, 1985). Two assumptions that are important to the validity of this technique include: (1) the legume crop of interest and the reference crop take up the same proportion of fertilizer-derived enriched N and soil N through the growing season, and (2) the reference crop does not take up Ndfa released by the legume crop (Chalk, 1985; Hardarson and Danso, 1993). Therefore, choosing the appropriate reference crop is crucial to obtaining accurate estimates of N<sub>2</sub> fixation, and an ideal reference crop should have similar root patterns and N uptake profiles to the legume crop of interest through the growing season (Witty, 1983; Bremer et al., 1993).

Based on the assumption of this method, the differential dilution of <sup>15</sup>N in the legume crop and the reference crop can be determined, and the percentage of N derived from the

atmosphere (%Ndfa) by the legume crop can be calculated according to McAuliffe et al. (1958) and Fried and Middelboe (1977):

$$\%Ndfa = \left(1 - \frac{\text{atom}\% \text{ }^{15}\text{N excess legume}}{\text{atom}\% \text{ }^{15}\text{N excess reference plant}}\right) \times 100 \quad (\text{Eq. 2.1})$$

$$Ndfa \text{ (kg)} = \%Ndfa \times \text{totalN (plant)} \quad (\text{Eq. 2.2})$$

with the term 'atom% <sup>15</sup>N excess' referring to the <sup>15</sup>N enrichment above background. In addition, N derived from soil (Ndfs) and fertilizer (Ndff) can be calculated as follows:

$$\%Ndff = \frac{\text{atom}\% \text{ }^{15}\text{N excess (plant)}}{\text{atom}\% \text{ }^{15}\text{N excess (fertilizer)}} \quad (\text{Eq. 2.3})$$

$$Ndff \text{ (kg)} = \%Ndff \times \text{totalN(plant)} \quad (\text{Eq. 2.4})$$

$$Ndfs \text{ (kg)} = \text{total plant N} - Ndfa - Ndff \quad (\text{Eq. 2.5})$$

Since its first description, the <sup>15</sup>N isotope dilution technique has received relatively thorough evaluation thereafter, with both demerits (Witty, 1983; Chalk, 1985) and merits (Danso, 1986) of this method being elaborated on in the last century, such as difficulties in validation of the assumptions, dilution of applied enriched N by soil N, uneven distribution of applied enriched N with soil depth and growing period, interactions between the legume and the reference crop, and difficulties in choosing an appropriate reference plant, as opposed to the method's advantages of independence of crop yield, time-integrated estimates of N<sub>2</sub> fixation, and ability to discriminate among different N sources, namely, Ndfa, Ndff, and Ndfs. Overall, this method has been applied in a variety of environments worldwide, ranging from controlled environmental conditions to field conditions with various soil-climatic conditions, in order to study the rotational benefits of legumes in cropping systems and to trace transformations of N<sub>2</sub> fixation derived N in the plant-soil system (Rennie et al., 1988; Watanabe et al., 1990; Carranca et al., 1999; Issah et al., 2014).

### **2.3 Following the fate of crop nitrogen in the soil-plant system**

Unlike other soil nutrient elements, which can be released from weathering rocks, N in most agricultural soils is exclusively derived from external sources via pathways including fertilizer application, N<sub>2</sub> fixation, organic amendments, and atmospheric deposition (Robertson and Vitousek, 2009). Through these pathways, N is introduced to soil and normally

immediately starts to go through a sequence of mineralization-immobilization processes and in this way, added N is transformed among different soil organic and inorganic N pools (Mary et al., 1996). These transformations regulate soil N availability and N losses, affecting crop yield and nutrient uptake, greenhouse gas emissions, and groundwater contamination (Mary et al., 1996; Schoenau and Campbell, 1996; Janzen et al., 2003).

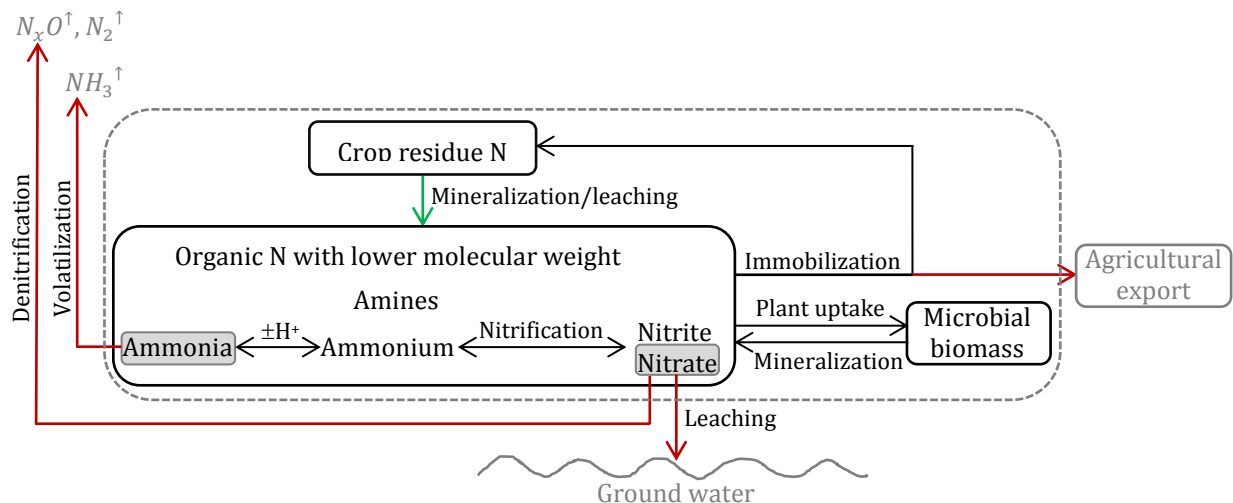
The N added to soil via crop residue differs from inorganic fertilizer N in that prior to being available for plant uptake, it generally needs to go through a sequence of enzymatic reactions and processes, which are considered to follow a sequence moving from higher molecular weight (e.g. proteins) to lower molecular weight organic N (e.g. peptides), amines, and ultimately to ammonium. The entire process is collectively referred to as mineralization. Some studies also reported direct uptake of organic N by soil microorganisms in addition to inorganic N assimilation as significant in some environments such as arctic soils (Robertson and Vitousek, 2009; Geisseler et al., 2010). One important biological pathway that transforms N among different inorganic forms is nitrification, which refers to the biological oxidation of ammonia ( $\text{NH}_3$ ) and ammonium ( $\text{NH}_4^+$ ) to nitrite ( $\text{NO}_2^-$ ), followed by the oxidation of nitrite ( $\text{NO}_2^-$ ) to nitrate ( $\text{NO}_3^-$ ). Nitrification is carried out by certain soil microorganisms under aerobic conditions, including ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) in the conversion of ammonium ( $\text{NH}_4^+$ ) to nitrite ( $\text{NO}_2^-$ ), and other bacterial species (*Nitrobacter*) in the second step to form nitrate (Taylor et al., 2012).

Soil N can enter the soil microbial biomass pool via immobilization when the soil microbial community experiences N-limited conditions (McSwiney et al., 2013). Therefore, the amount of mineral N present in soil at any time is the net effect of the magnitude of the two concurrent opposing processes of mineralization and immobilization. This internal N cycling within the soil featured by the biological mineralization-immobilization turnover (MIT) is driven by soil microorganisms and affected by various factors (Mary et al., 1996). The rate and degree of the MIT of nutrients released from crop residue in soil is affected by a variety of biotic and abiotic factors, including the biochemical composition of added residue such as the C:N ratio and lignin concentration (Kumar and Goh, 2003), soil microbial community composition and activity (Baumann et al., 2009), agronomic practices such as residue placement (Aulakh et al., 1991) and tillage (Lupwayi et al., 2004), and soil decomposing conditions such as soil water content and temperature (Mary et al., 1996). Incorporating crop

residues with very high C:N ratios (>30), such as cereal straw, generally results in net immobilization, because soil microorganisms have to take up mineral N from the soil inorganic N pool in order to break down and metabolize added residue (Schoenau and Campbell, 1996). When residues with low C:N ratios (<20) are added to soil, mineral N will be released through microbial decomposition, resulting in net mineralization (Lupwayi and Soon, 2009).

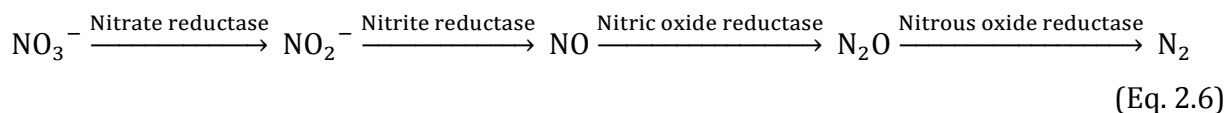
By incubating an agricultural soil from Iowa, USA, amended with wheat, corn, soybean, or vetch residue under controlled conditions, Aulakh et al. (1991) found that initial decomposing rates were negatively related to the C:N ratio of added residue, and residue decomposition, nitrification, and denitrification were mostly affected by residue placement and soil water content within the first 10 days following residue additions. Marschner et al. (2011) also reported that during a 30-day incubation of wheat residue inoculated with a microbial suspension, microbial biomass reached peaks within the first 4 days, and microbial community composition changed most drastically in the first 1-2 weeks of incubation and rather slowly thereafter, presumably due to the depletion of readily available and decomposable organic compounds in the added residue. It is recognized that the combination of the bioavailability of substrate and soil conditions regulates the microbial community population and structure, which in turn, carry out the decomposition of the substrate and transformations of nutrients released from the substrate, driving the MIT in soil (Mary et al., 1996).

In addition to the biochemical MIT, physical leaching of soluble nutrients from crop residue is another way for nutrients to become bioavailable for microbial assimilation or plant uptake (Cermak et al., 2004). Through MIT or physical leaching, nutrients contained in crop residue become part of the inorganic N pool, and can be either taken up by plants, or immobilized by soil microbial organisms, or lost from the soil-plant system through pathways such as leaching and gaseous losses such as denitrification to N<sub>2</sub>, NH<sub>3</sub> volatilization, and N<sub>2</sub>O and NO<sub>x</sub> fluxes (Janzen et al., 2003; Robertson and Vitousek, 2009). Due to these possible pathways of N losses from the root zone or the soil-plant system, it is not always desirable to have large amount of N in inorganic forms in the soil (Lupwayi et al., 2004). An illustration of the main pathways of N inputs and losses of in agro-soil system is presented in Fig. 2.2.



**Fig. 2.2.** Fate of N released from crop residue in the agro-soil system. The boundary of the system is the N that is available for plant use and the soil microbial community, so processes within the grey dashed box do not cause N losses from the agro-soil system. Green arrows denote N inputs into the plant available N pool, and red arrows denote N exports from the plant available and total N pools.

In agro-ecosystems, N is lost from the soil-plant system primarily via denitrification, ammonia volatilization, and leaching (Janzen et al., 2003). In addition, in regions with large amount of precipitation, N losses due to erosion and runoff can also occur to a considerable extent (Mosier et al., 1998). Denitrification refers to a sequence of microbial reductions of nitrate to N gases carried out by a group of heterotrophic anaerobic bacteria as illustrated in eq. 2.6, or the chemical reactions of nitrite with soil components, known as chemodenitrification (Cayuela et al., 2013).



Main regulators of the production of N gases via denitrification include the availability of nitrate and soil organic C, soil aeration, pH, and temperature (Aulakh et al., 2001; Khalil et al., 2004). Due to the production of gaseous N, especially  $N_2O$ , a greenhouse gas with large global warming potential, denitrification can cause adverse environmental impact, in addition to reduction of soil fertility. Indeed, studies have shown that a significant proportion ( $\geq 30\%$ ) of applied N via either inorganic fertilizer or crop residue amendments is lost from the soil-plant

system through denitrification, while reducing this loss can be achieved by regulating soil aeration status through management of tillage and irrigation/drainage, timing of fertilizer application, fertilizer placement, and crop rotation (Aulakh et al., 1991, 2001; Gregorich et al., 2008; Muhammad et al., 2011; van Kessel et al., 2013). For example, as a meta-analysis showed, compared to conventional tillage, no-tillage or reduced tillage significantly reduced soil N<sub>2</sub>O emissions in studies longer than 10 years, especially in drier regions (van Kessel et al., 2013). On the other hand, Jahangir et al. (2012) also pointed out that by providing enough C resource in subsoils, excess nitrate in subsoils can be converted to N<sub>2</sub> through complete denitrification without producing large amount of intermediate gaseous N, reducing the chance of nitrate leaching into groundwater and potential groundwater pollution.

Leaching is the loss pathway in which N is leached from the root zone with the downward percolating water. Nitrate is the primary N form leached due to its high solubility and therefore mobility (Dinnes et al., 2002). Movement below the root zone renders the N unavailable for plant uptake and causes potential nitrate pollution of groundwater and surface water systems. Despite the difficulty in accurately quantifying the magnitude of N lost through leaching, especially in dryland agriculture, it was estimated that almost 0.43 Tg N was lost from Canadian agroecosystems per year through leaching, although the authors also recognized that large variation existed among regions with different precipitation (Janzen et al., 2003). For example, leaching is relatively negligible on the prairies in western Canada, where the precipitation is limited and excess standing water and rapid deep percolation are infrequent occurrences compared to southern British Columbia (Janzen et al., 2003). In addition, rates of N fertilizer can also affect the magnitude of leaching, and higher rates applied in the previous year unused by the crop can cause more N leaching in the following spring (Zhang et al., 2004). Therefore, practices for reducing residual inorganic N in the soil can be employed to control N leaching, such as adding crop residues with high C:N ratios to immobilize soil inorganic N (Muhammad et al., 2011), using cover crops, applying nitrification inhibitors, and adjusting the timing, form, method, and rate of N fertilization according to soil tests (Dinnes et al., 2002).

Ammonia volatilization is another pathway via which N can be lost from soil (eq. 2.7), and generally this loss occurs from surface placement of NH<sub>3</sub> and NH<sub>4</sub><sup>+</sup>-containing or producing fertilizers, or decomposition of N-rich organic matter which releases ammonia through enzymatic ammonification (Harper et al., 1983; Schoenau and Campbell, 1996). The magnitude

of N lost via ammonia volatilization varies greatly, ranging from  $\approx 0$  to  $\geq 50\%$  of applied N fertilizer depending on different factors, and generally ammonia volatilization is most significant under such conditions as alkaline soils, surface application of  $\text{NH}_4^+$ -containing fertilizers, high wind speed, and drying soils with warm temperatures (Sommer et al., 2004). Therefore, management of ammonia volatilization can be achieved by controlling these factors.



Many researchers, such as Janzen et al. (2003), Dinnes et al. (2002), and Bouwman et al. (2013), recognized the difficulties in accurately quantifying and eliminating N losses through such pathways as denitrification, leaching, and ammonia volatilization, due to the fact that there is always inorganic N in soil and it is technically difficult to precisely match plant demand and N release in the soil. Only a certain portion of added N is consumed by crops in the first growing season, with a significant amount of added N retaining in soil after the first growing season or lost from the soil-plant system during the first growing season under the prairie conditions (Schoenau and Campbell, 1996).

The residual N can be available to crops in the following seasons or lost from the soil-plant system via previously discussed pathways, including denitrification, leaching, and volatilization. It is generally accepted that increasing crop recovery is paramount to reducing N losses from the soil-plant system (Janzen, 2001). Management approaches to increasing N fertilizer use efficiency proposed and in use generally aim to match the supply of plant available N in soil with plant demand. This includes consideration of the timing and rate of fertilization with commercial inorganic N sources, fertilizer placement, tillage, irrigation and drainage, regulating soil pH, the use of controlled-release N fertilizers and inhibitors, and crop rotation. Crop rotations including N-fixing grain legumes can offer ability to reduce fertilizer use and increase efficiency, due to the fact that legume crops can meet a substantial portion of their N requirements through fixation and also release N and other nutrients to subsequent crops from their residue (Janzen, 2001; Janzen et al., 2003; Sommer et al., 2004; Gardner and Drinkwater, 2009). To compensate and adjust for this properly, the nutrient requirements and release characteristics of different grain legume crops need to be evaluated and understood. The work in this thesis aims to address this gap.



### **3. YIELD AND UPTAKE OF NITROGEN AND PHOSPHORUS IN SOYBEAN, PEA, AND LENTIL, AND EFFECTS ON SOIL NUTRIENT SUPPLY AND CROP YIELD IN THE SUCCEEDING YEAR IN SASKATCHEWAN, CANADA**

#### **3.1 Preface**

Increasing crop yield is the primary goal of agricultural production, with N and P uptake in the plant as important considerations regarding nutrient cycling. In addition, the partitioning of crop biomass, N, and P in different plant components is important for assessing crop performance and nutrient budget of the plant-soil system, influencing soil nutrient dynamics and following crops in rotation. As soybean is a relatively new legume crop in Saskatchewan, this information is essential for assessing the agronomic prospects for soybean expanding on the prairies. Therefore, this chapter investigates the yield and uptake of N and P in the grain and the straw of three short-season varieties of soybean in comparison to three pea varieties and three lentil varieties grown in 2014 at four sites in the Dark Brown soil zone (Saskatoon and Scott) and the Black soil zone (Rosthern and Yorkton) in Saskatchewan, Canada. In the subsequent year (2015), the grain and straw yield and N and P uptake in following wheat and canola crops as influenced by different previous legume crops, namely, soybean, pea, and lentil at the four sites were assessed. In addition, the soil N and P supply rate, an important indicator of the effects of previous crop residue, was measured through the subsequent growing season (2015) at the Rosthern and Saskatoon sites. This chapter provides basic information on the yield, N, and P for soybean production and the effects on following crops in the subsequent year.

#### **3.2 Abstract**

Soybean [*Glycine max* (L.) Merr.] production is expanding in western Canada, but there is limited information on the yield and associated nutrient removal of soybean grown under

western Canadian field conditions. Also of interest is the impact of soybean in comparison to other pulse crops on the availability of nutrients to subsequent crops in rotation. Through a two-year field experiment conducted at four sites in Saskatchewan, this study quantified grain and straw yield and uptake of N and P in three short-season varieties of soybean in comparison to three varieties of pea (*Pisum sativum* L.) and three varieties of lentil (*Lens culinaris* L.), and in the following year assessed the soil supplies of available N and P and the yield and uptake of N and P in the grain and the straw of wheat (*Triticum aestivum* L.) and canola (*Brassica napus*, L.). Soybean grain yield (929-3534 kg ha<sup>-1</sup>) was similar to results from other studies in western Canada, but about 20% lower when compared to results from eastern Canada or mid-western USA. Selected soybean varieties had similar or higher grain yield compared to pea (1565-6644 kg ha<sup>-1</sup>) and lentil (664-2755 kg ha<sup>-1</sup>), while lentil had relatively lower grain yield and lower harvest indexes among the three grain legumes. Compared to pea and lentil, soybean had relatively high N (39-48 g kg<sup>-1</sup>) and P (5.1-6.8 g kg<sup>-1</sup>) concentration and uptake (40-168 kg N ha<sup>-1</sup> and 5.8-21.2 kg P ha<sup>-1</sup>) in the grain, and relatively lower N and P concentration and uptake in the straw. As a result, the amount of N and P returned to the field via residue was lower with soybean than with pea or lentil. In the following year, soil supply of available N was lower in soybean stubble plots than in lentil stubble plots in the first two months following seeding, and soil supply of available P was higher in soybean stubble plots than in lentil stubble plots throughout most of the growing season at one site. Wheat and canola showed no large consistent response to soybean, pea, and lentil stubbles regarding yield and N and P uptake in the next year, implying comparable rotational benefits of soybean to pea and lentil under Saskatchewan conditions. High grain P removal of soybean needs to be considered in long-term fertility maintenance planning for rotations, and studies examining rotational effects over several cycles of the rotation would be desirable under western Canadian soil-climatic conditions.

### **3.3 Introduction**

Including grain legumes in crop rotations provides both economic and environmental benefits, such as mitigating inorganic N fertilizer costs and greenhouse gas emissions related to excess use of inorganic N fertilizers, due to the N fixing ability of grain legumes (Knight, 2012). Through N<sub>2</sub> fixation, grain legumes are able to partially meet their N demands and thus lessen soil nutrient depletion (Ha et al., 2008). Furthermore, benefits of grain legumes include the

exudation of N and other nutrients to the soil via the root system, enhancement of the soil microbial activity, and break of insect pest cycles and disease infestation of subsequent crops (Lupwayi et al., 2011; Arcand et al., 2014b). Therefore, rotational grain legumes benefit not only the soil nutrient stores in the present year but also succeeding crops in rotation. A two-year rotation study in central Ontario showed that the N contribution by soybean to succeeding cereal crops was about 30 kg N ha<sup>-1</sup> for soybean-corn sequence compared to corn-corn sequence (Ding et al., 1998). Similar results were observed in first year cereal crops following soybean in Lancaster, PA, USA, but this N contribution decreased in the second year of cereal crops following soybean (Vanotti and Bundy, 1995). Non-N rotational benefits from grain legumes are sometimes reported to exceed the N benefits in crop rotations. Stevenson and van Kessel (1996a) estimated that 92% of the yield advantage in a pea-wheat rotation was attributed to non-N rotation benefits in comparison to a wheat-wheat sequence in Saskatchewan, Canada. These non-N effects included reducing root and leaf diseases and increasing availability of P, K, S, and growth substances released from legume residues (Stevenson and van Kessel, 1996b).

The N and non-N rotational benefits of grain legumes vary with different soil-climatic conditions, crop species and cultivars, and agronomic practice (Malhi et al., 2008). For instance, agronomic practices affect the production and contribution of grain legumes through such factors as application of inoculants, methods of harvesting, and fertilizer application (Ennin et al., 2004). Primarily, these factors affect the biomass accumulation, N<sub>2</sub> fixation, and nutrient partitioning in grain legumes, which then determine the amount of nutrients returned to soil via residue for recharging the soil nutrient pool and for being passed on to following crops in rotation, provided that the crop residue is not removed from the field at harvest (van Kessel, 1994; Hungria et al., 2006; Zakeri et al., 2012). Nutrient partitioning between the grain and the straw of grain legumes is significant in evaluating nutrient removal and rotational benefits of legumes, because it determines the amount of nutrients that stays in the field after harvest. Furthermore, the availability of nutrients in legume residues to the soil nutrient pool and following crops is largely related to the biochemical characteristics of the residue such as the C:N ratio and the amount of lignin, and environmental factors influencing decomposing processes in the soil system, such as soil temperature, soil water content, and the composition of the soil biota (Kumar and Goh, 2003). As a result, the impact of previous legume crops on the

yield and nutrition of succeeding crops in rotation commonly varies with crop species in the previous year and soil-climatic conditions (Przednowek et al., 2004; Jani et al., 2015).

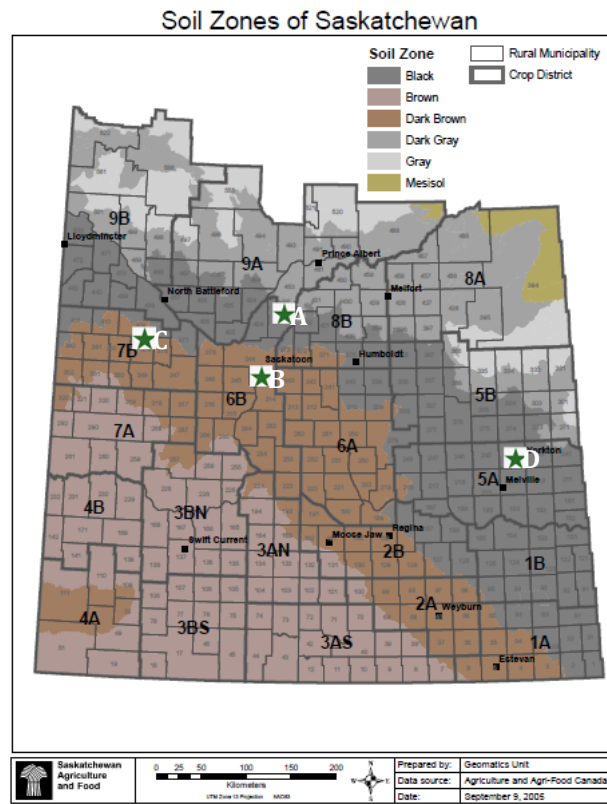
Production of soybean [*Glycine max* (L.) Merr.] is expanding in western Canada due to development of short-season herbicide tolerant varieties. For instance, the soybean acreage in Saskatchewan has increased more than ten-fold from 2011 to 2013. Unlike soybean, field pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.) are well-established grain legume crops grown by producers for many years in Saskatchewan. For pea and lentil, the rotational benefits and environmental impact were mainly attributed to low C:N ratios in the residue, and their N benefits including N<sub>2</sub> fixation and contribution to the availability of soil N (Stevenson and van Kessel, 1996a; Adderley et al., 2006). Despite the expanding acreage of soybean in this region, there is little information on the yield and nutrient assimilation in different plant components (grain vs. straw) of new short-season soybean varieties grown under western Canadian soil-climatic conditions. Nutrient uptake can be related to immediate crop requirements for added fertilizers as well as impact on soil fertility in future years when the amount of nutrient in harvested grain is determined along with the amount contained in above-ground straw that is returned to the soil. This information is needed for producers to make short and longer-term fertilization decisions.

Nitrogen and phosphorus are essential elements for plant growth due to their important roles in photosynthesis, the development of amino acids, enzymes, and proteins, and energy storage and transformations. Therefore, it is essential to quantify the amount of N and P that crops remove from the soil and the impact on yield and N and P supply to following crops. Through a two-year field trial at four sites encompassing two Dark Brown and two Black Chernozemic soils (Typic Borolls, USDA taxonomy system) in south-central Saskatchewan in 2014 and 2015, this chapter aimed to assess several aspects of soybean production in Saskatchewan, including (1) yield, N and P uptake in the grain and the straw of three modern varieties each of soybean, pea, and lentil in the 2014 field trials; (2) soil supply of available N and P in soybean, pea, and lentil stubble plots during the growing season in the following year; and (3) yield, N and P uptake in the grain and straw of wheat (*Triticum aestivum* L.) and canola (*Brassica napus*, L.) in the subsequent year (2015).

### 3.4 Materials and Methods

#### 3.4.1 Site description and experimental design

The experiment was conducted in 2014 and 2015 at four sites in south-central Saskatchewan, Canada, near Rosthern, Saskatoon, Scott, and Yorkton (Fig. 3.1). The soil at the Rosthern site (legal location: NE-09-43-02-W3) belongs to the Hamlin-Blaine Lake Association and is an Orthic Black Chernozemic soil formed in a mixture of loamy and silty lacustrine materials (Saskatchewan Soil Survey, 1989a). The soil at the Saskatoon site (legal location: SW-31-35-03-W3) is of the Elstow Association, an Orthic Dark Brown Chernozemic soil from a medium to moderately fine textured, moderately calcareous, silty glacio-lacustrine deposits (Saskatchewan Soil Survey, 1989b). At the Scott site (legal location: SW-19-39-20-W3), the soil is the Scott-Elstow Association, an Orthic Dark Brown Chernozem formed from silty lacustrine deposits (Saskatchewan Soil Survey, 1989c). The soil at the Yorkton site (legal location: NE-06-26-04-W2) is of the Oxbow-Hamlin Association, an Orthic Black soil formed in a mixture of loamy glacial till and loamy lacustrine materials (Saskatchewan Soil Survey, 1989d).



**Fig. 3.1.** Locations of the four sites in Saskatchewan: (A) Rosthern site, (B) Saskatoon site, (C) Scott site, and (D) Yorkton site.

To assess baseline soil nutrients before the experiment, pre-seeding soil composite samples from the 0-15cm depth of the soil profile were obtained from each site in May, 2014, by taking eight surface soil cores across the site area with a hand auger and combining the cores to produce a composite sample (Table 3.1). Soil samples collected were air-dried, sieved and the <2 mm fraction was retained and analyzed for various extractable nutrients and chemical properties.

The experimental design was a randomized complete block design (RCBD) with four replicates at each site. In 2014, three modern varieties of soybean, pea, and lentil, respectively, were selected to represent varieties and classes commonly grown by producers at the time of the study (Table 3.2). Before seeding, soybean seed was pretreated with ApronMaxx® RTA® fungicide at the rate of 142mL per 45kg seed. Granular TagTeam™ inoculant (Novozymes BioAg) for soybean (*Bradyrhizobium japonicum*) and Nodulator® XL inoculant (BASF Canada, Inc., 2013) for pea/lentil (*Rhizobium leguminosarum*) were mixed with the legume seed on site immediately before seeding, at double the normal rate for soybean inoculant. Doubling the rate of soybean inoculant is a recommended practice for inoculating soybean in soils where soybean has not been grown before, as the abundance of *Bradyrhizobium japonicum* populations in these soils is typically very low. Hard red spring wheat (cv. CDC Abound) was planted beside the legume crops for assessing N fixation as discussed in Chapter 5 at the seeding rate of 248 seeds m<sup>-2</sup>. Seeding was carried out at the end of May to the first week of June 2014 by the Crop Development Centre, University of Saskatchewan, and at each site all crops were seeded on the same day (Table 3.3). Plot size for each crop and site was 3.66 m × 1.4 m, with 3 rows per plot. The inter-row spacing was 35 cm, and row length was 3.66 m. Seeding rates were 65 seeds m<sup>-2</sup> for soybean, 86 seeds m<sup>-2</sup> for pea, and 129 seeds m<sup>-2</sup> for lentil (Saskatchewan Ministry of Agriculture, 2014). No fertilizers were applied for any of the crops, as significant deficiencies were not identified by pre-seeding soil analysis, as shown in Table 3.1. Odyssey™ (imazamox-imazethapyr) was sprayed across the plots at each site in the last week of June for weed control.

In 2015, hard red spring wheat (cv. CDC Abound) was seeded in the second week of May on the stubble of soybean, pea, and lentil at the Rosthern, Saskatoon, and Scott sites by the Crop Development Centre, University of Saskatchewan. Seeding rate was 248 seeds m<sup>-2</sup>, and no fertilizers were applied at the three sites. At the Yorkton site, canola (*Brassica napus* L. cv. Nexera 1016RR) was seeded on the stubble of soybean, pea, or lentil, as this site was managed

by a producer who normally follows a grain legume–canola rotation. At the Yorkton site, 55 kg ha<sup>-1</sup> of 11-52-0 fertilizer was applied in the seed-row at the time of seeding. Seeding dates are presented in Table 3.3. Odyssey™ (imazamox-imazethapyr) was sprayed across the plots at each site in the last week of May for weed control in wheat and glyphosate for canola.

**Table 3.1.** Pre-seeding soil properties in the 0-15cm soil profile at the four sites in May, 2014.

Site	Texture	pH	EC	NO <sub>3</sub> <sup>-</sup> -N	P	K	SO <sub>4</sub> <sup>2-</sup> -S	Cu	Mn	Zn	B	Fe
			kg ha <sup>-1</sup>									
			dS m <sup>-1</sup>									
Rosthern	Loam	6.2	0.1	6	28	322	5	1.1	37.1	3.3	1.7	174
Saskatoon	Loam	5.9	0.6	19	33	>600	>48	1.3	24.1	6.0	2.4	320
Scott	Loam	6.6	0.1	25	26	>545	9	1.5	27.9	2.0	2.4	118
Yorkton	Loam	7.9	0.1	8	47	463	5	0.9	12.9	5.5	2.4	29

**Table 3.2.** Crop varieties used in the study.

Crop	Variety	Market class	Breeder	Herbicide resistance
Soybean	P001T34R (S1)	Oilseed	Pioneer Dupont	Group 2
Soybean	TH3303R2Y (S2)	Oilseed	Thunder Seeds	Group 2
Soybean	NSC Moosomin (S3)	Oilseed	Northstar Genetics	Group 2
Pea	CDC Meadow (P1)	Yellow	CDC	Group 2
Pea	CDC Amarillo (P2)	Yellow	CDC	Group 2
Pea	CDC Limerick (P3)	Green	CDC	Group 2
Lentil	CDC Impower (L1)	Large green	CDC	Group 2
Lentil	CDC Invincible (L2)	Small green	CDC	Group 2
Lentil	CDC Maxim (L3)	Small red	CDC	Group 2
Wheat	CDC Abound (W)	Hard red	CDC	Group 2
Canola	Nexera 1016RR (C)	Argentine hybrid	Dow AgroSciences	Glyphosate

**Table 3.3.** Dates of seeding, harvesting, and residue return, and days to maturity at the four sites in 2014 and 2015.

Year	Site	Seeding	Harvesting		Days to maturity		Residue returned
			Pea/Lentil	Soybean	Pea/Lentil	Soybean	
2014†	Rosthern	May 31	Sept. 9	Sept. 17	101	109	Oct. 16
	Saskatoon	May 22	Sept. 2	Sept. 23	103	124	Oct. 16
	Scott	June 1	Sept. 4	Sept. 17	95	108	Oct. 14
	Yorkton	May 23	Sept. 5	Sept. 22	105	122	Oct. 15
<b>Wheat/canola</b>							
2015	Rosthern	May 13	Aug. 19		98		Sept. 30
	Saskatoon	May 12	Aug. 19		99		Sept. 30
	Scott	May 6	Aug. 20		106		Sept. 29
	Yorkton	May 10	Aug. 18		100		Oct. 15

† In 2014, soybean was harvested later than pea and lentil due to its later maturity.

### 3.4.2 Plant and soil sampling and analyses

Soil composite samples were air-dried and sieved, and the <2 mm fraction was retained and analyzed for various extractable nutrient levels and chemical properties. Soil pH and electrical conductivity (EC) were measured in a 1:2 soil:water suspension (Nelson and Sommers, 1982). Soil nitrate ( $\text{NO}_3^-$ ) and sulphate ( $\text{SO}_4^{2-}$ ) were extracted with the 0.01M  $\text{CaCl}_2$  (Houba et al., 2000). Automated colorimetry was used to analyze the extracts for concentrations of  $\text{NO}_3^-$ -N and  $\text{SO}_4^{2-}$ -S. Available phosphorus (P) and potassium (K) were measured on the soil depth sample of 0-15cm using a modified Kelowna extraction procedure (Qian et al., 1994). Extracts were then colorimetrically analyzed for P using a Technicon Auto-analyzer II segmented flow automated system (Technicon Industrial Systems, Tarrytown, NY, USA). Potassium concentration in the extracts was analyzed using flame atomic absorption (Varian Spectra 220 Atomic Absorption Spectrometer; Varian Inc., Palo Alto, CA, USA). Plant available Cu and Zn was extracted from samples using a 0.005M diethylene-triamine-penta acetic acid (DTPA) solution (Lindsay and Norvell, 1978).

In fall 2014 and 2015, crops were harvested at physiological maturity by hand cutting and completely removing above-ground crop materials, including grain, straw, and leaves from the plot area. Harvesting dates for each site are shown in Table 3.3. In 2014, soybean was harvested later than pea and lentil due to its later maturity. Three square meters of above-ground crop samples were taken within each plot, including all 3 three rows in the plot, to estimate crop yield and for further plant nutrient analyses.

Plant samples were dried at 30°C, and weighed. The crop samples were threshed using a stationary mechanical thresher. When threshing, grain and straw sub-samples ( $\approx 10$  g) were collected from each sample, and the rest of the sample was used to determine grain yield and total biomass yield. After threshing, the residue component (straw, pods, and leaves) was collected from each sample and bagged, and returned and spread evenly across each plot from which it was harvested, followed by a light rotary tilling in order to anchor the residue in place. The residue was spread evenly over the plot area using a rake, and a plastic frame was used to constrain the residue within the plot area during residue incorporation when the weather was windy (Fig. 3.2). Collected sub-samples were ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) before being analyzed. Grain and straw N and P concentration was



determined by using a hydrogen peroxide-sulphuric acid digestion at 360°C as described by Thomas et al. (1967), followed by automated colorimetric measurements of N and P concentration in the digests (Technicon AutoAnalyzer; Technicon Industrial Systems, Tarrytown, NY, USA). Carbon content in the straw samples was measured by using a Leco TruMac CNS combustion analyzer (LECO Corporation, St. Joseph, MI, USA).

In September of 2014, post-harvest soil samples were collected from the 0-15cm, 15-30cm and 30-60cm depths of each plot. Three soil cores were taken per plot and the sample depths from each core combined to provide a composite sample. Soil extractable nitrate was determined by extraction with 0.01M CaCl<sub>2</sub> and available P by modified Kelowna method as described previously.



**Fig. 3.2.** Residue return and incorporation in the field. Photo A depicts spreading residue across the plot area evenly by using a rake; photo B shows incorporating residue into the soil by light rotary tilling.

### 3.4.3 Soil nutrient supply rates

In May 2015 before seeding, soil samples were collected from the 0-15cm depth of the soil profile in plots of one variety of each previous crop, including TH3303R2Y soybean, CDC Meadow pea, and CDC Maxim lentil at the Rosthern and Saskatoon sites, for determining pre-seeding available N and P. Initial available N and P were used as the baseline soil nutrient supply for the 2015 growing season. Soil samples were air dried and ground before the analysis. The “Sandwich” technique was used to measure soil supplies of available N and P in the soil samples, following the protocol described by Qian et al. (2008). Briefly, anion-exchange and cation-exchange membranes were soaked in a 0.5M NaHCO<sub>3</sub> solution for 2h and the membrane was then placed between two vial lids filled with soil subsamples, of which the water content was brought up to field capacity using deionized water beforehand. Prepared “sandwiches” were then stored at 20°C for 24h. After 24h, membranes were rinsed with deionized water until they were free of residual soil and eluted with a 0.5M HCl solution. The eluate from anion-exchange membranes was analyzed for NO<sub>3</sub><sup>-</sup>-N and PO<sub>4</sub><sup>3-</sup>-P, and the eluate from cation-exchange membranes was analyzed for NH<sub>4</sub><sup>+</sup>-N colorimetrically (Technicon AutoAnalyzer; Technicon Industrial Systems, Tarrytown, NY, USA).

During the growing season of 2015, wheat plots seeded on the stubble of one variety of each previous legume crop (TH3303R2Y soybean, CDC Meadow pea, and CDC Maxim lentil) were measured for soil available N and P supply rates using Plant Root Simulator (PRST<sup>TM</sup>) ion exchange resin membrane probes (Western Ag Innovations Inc., Saskatoon, SK, Canada) at the Rosthern and Saskatoon sites. Two anion-exchange and two cation-exchange PRST<sup>TM</sup> probes were buried in each plot (3 crop stubbles × 4 reps × 2 PRST<sup>TM</sup>-probes of each exchange type). The first burial took place one week after seeding by inserting PRST<sup>TM</sup>-probes vertically into the 0-15cm soil profile, with PVC cylinders surrounding the probes to prevent root competition. The probes were replaced with a new set of probes biweekly throughout the entire growing season and the area within the PVC cylinders where the probes were located was kept free of plant growth. Probes were replaced by inserting a set of newly-regenerated probes in the same soil slots as the previous probes. The last sets of probes were removed at harvest at the two sites. PRST<sup>TM</sup>-probes were brought back to the laboratory shortly after each removal.

The analysis and regeneration of PRST<sup>TM</sup>-probes followed the protocol of Hangs et al. (2013). Briefly, probes were first washed using deionized water until they were free of soil and

then eluted with a 0.5M HCl solution. The eluate was analyzed for  $\text{NO}_3^-$ -N,  $\text{PO}_4^{3-}$ -P, and  $\text{NH}_4^+$ -N colorimetrically (Technicon AutoAnalyzer; Technicon Industrial Systems, Tarrytown, NY, USA), which represented the biweekly supply rates of soil  $\text{NO}_3^-$ -N,  $\text{PO}_4^{3-}$ -P, and  $\text{NH}_4^+$ -N. PRS<sup>TM</sup>-probes were regenerated by shaking three times in a 0.5M  $\text{NaHCO}_3$  solution for 4h, and between each shaking deionized water was used to thoroughly rinse the probes. Cumulative supply rates of these nutrients were calculated according to the PRS handbook manual (Western Ag Inc., 2006).

#### 3.4.4 Statistical analysis

Data were analyzed using SAS 9.4 for Windows, and a significance level of 0.05 was used unless specified. Data distribution normality was tested using the Kolmogorov-Smirnov test by the PROC UNIVARIATE procedure, and homogeneity of variance was tested using the Levene's test. Initially, the effects of crop species, crop variety, site, and their interaction were analyzed through a three-way ANOVA. Comparison of means at each site was conducted using the PROC Mixed procedure, with crop variety as the fixed effect and block as the random effect (Yang, 2010). The Tukey HSD method was used to conduct multiple comparisons among group means.

### 3.5 Results

#### 3.5.1 Yield and uptake of nitrogen and phosphorus in soybean, pea, and lentil

At the four sites, the previous crop prior to the experiment was spring wheat (*Triticum aestivum* L.) and the tillage management was no-till. Monthly cumulative precipitation and mean temperatures in the growing season of 2014 were close to or above the average level of the last 30 years, whereas the growing season of 2015 was drier in spring, with the monthly cumulative precipitation lower than the historical mean at the four sites, according to data from Environmental Canada weather stations near the sites (Table 3.4).

Analysis of variance (Table 3.5) showed that site, crop, and crop variety all had significant impact on the yield and uptake of N and P by soybean, pea, and lentil in 2014. In addition, the interaction between site  $\times$  crop and site  $\times$  variety also had significant effects in this study. Overall, results indicated that regional variation was important factor affecting the yield and nutrient uptake of grain legumes, making multisite trials necessary when new crops are tested. Selecting proper varieties also plays important roles in increasing crop yield and nutrient uptake, give a certain crop to include in rotations in this region.

**Table 3.4.** Monthly precipitation and mean monthly temperature during the growing season (May-August) in 2014 and 2015, as compared to the historical (1983-2013) data at the four sites.

Site	Month	Precipitation (mm)			Temperature (°C)		
		2014	2015	HM†	2014	2015	HM†
Rosthern	May	61	0	53	10.1	11.0	10.6
	June	95	14	107	14.1	17.7	15.4
	July	45	84	57	18.3	19.5	17.9
	August	19	45	41	17.9	17.7	17.1
	Sum/Mean‡	220	144	258	15.1	16.4	15.3
Saskatoon	May	73	10	18	10.1	10.7	10.9
	June	102	21	82	14.4	17.6	15.5
	July	66	94	61	18.2	19.3	18.0
	August	17	81	41	18.0	17.4	17.4
	Sum/Mean	258	206	203	15.2	16.3	15.5
Scott	May	60	4	36	12.3	9.3	11.2
	June	95	19	62	17.8	16.0	16.6
	July	143	46	72	19.9	18.1	19.4
	August	82	75	46	18.9	16.8	18.3
	Sum/Mean	379	144	216	17.2	15.1	16.4
Yorkton	May	46	8	55	10.5	10.5	10.4
	June	235	28	82	15.4	16.7	15.7
	July	22	123	78	18.2	19.3	18.8
	August	87	46	51	17.6	17.5	17.2
	Sum/Mean	389	205	266	15.4	16.0	15.5

† Historical mean data (1983-2013) from the nearest Environment Canada Meteorological Station to each of the four research sites.

‡ Precipitation data denotes cumulative precipitation from May to August and temperature data denotes mean monthly temperature during this period.

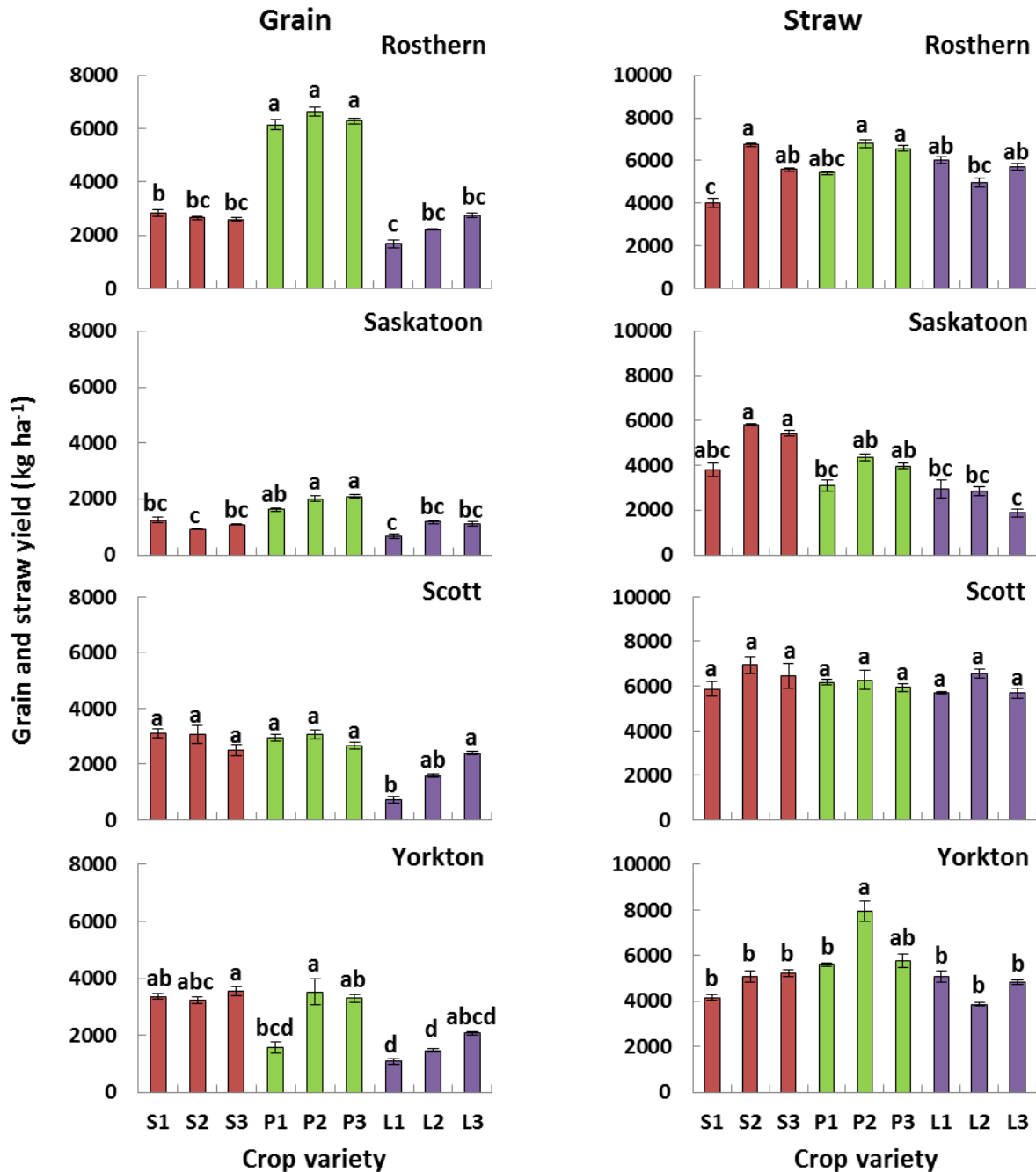
**Table 3.5.** Summary of ANOVA and significance of variables of yield and uptake of N and P in the grain and the straw of soybean, pea, and lentil in 2014.

Source	df	Grain (kg ha <sup>-1</sup> )			Straw (kg ha <sup>-1</sup> )		
		Yield	N	P	Yield	N	P
Site	3	***	***	***	***	***	***
Crop	2	***	***	***	***	***	***
Variety	8	***	***	***	***	***	***
Site × Crop	6	***	***	***	***	***	***
Site × Variety	24	***	***	***	***	***	***

\*, \*\*, and \*\*\* Significant at  $p < 0.05$ ,  $P < 0.01$ , and  $p < 0.001$ , respectively, according to a Tukey's HSD test.

In general, grain yield of soybean, pea, and lentil at the four sites (Fig. 3.3) was at or above the provincial average yield levels for each crop in Saskatchewan in 2014, which were 1278 kg ha<sup>-1</sup>, 2287 kg ha<sup>-1</sup>, and 1537 kg ha<sup>-1</sup> for soybean, pea, and lentil, respectively (Government of Saskatchewan, 2014). The three short-season soybean varieties yielded similarly to selected pea and lentil varieties, with pea having superior yield at the Rosthern site. Overall, CDC Impower (large green lentil market class) had low grain yield but high straw yield due to its relatively tall plants compared to the other crops, while crops generally showed an inconsistent trends regarding straw yield across the sites. Among the four sites, Saskatoon generally had the lowest crop yield and Rosthern had the highest pea yield.

Grain yield was generally lower than straw yield across the sites for all three crops, and crop yield varied from site to site. Soybean grain yield ranged from 929 kg ha<sup>-1</sup> to 3534 kg ha<sup>-1</sup>, and soybean straw yield from 3799 kg ha<sup>-1</sup> to 6956 kg ha<sup>-1</sup>. Pea yield ranged from 1565 kg ha<sup>-1</sup> to 6644 kg ha<sup>-1</sup> of grain, and from 3101 kg ha<sup>-1</sup> to 7946 kg ha<sup>-1</sup> of straw. Lentil grain yield was 664 kg ha<sup>-1</sup> to 2755 kg ha<sup>-1</sup> and straw yield was 1870.5 kg ha<sup>-1</sup> to 6551 kg ha<sup>-1</sup>. Overall, crop harvest indexes were intermediate in soybean compared to pea and lentil, ranging from 13% to 46% (95<sup>th</sup> percentile: 45%) across the sites, as opposed to 9% to 55% (95<sup>th</sup> percentile: 52%) in pea, and 5% to 43% (95<sup>th</sup> percentile: 36%) in lentil. Carbon concentration ranged from 41% to 45 % in the straw of soybean, pea, and lentil across the sites, and straw C:N ratio in soybean straw (26 to 131) was significantly higher than in pea (20 to 51) and lentil (15 to 38) at the Scott and Yorkton sites, while at the Rosthern and Saskatoon sites, the three crops had similar straw C:N ratios of approximately 36.



**Fig. 3.3.** Grain and straw yield ( $n=4$ ) of soybean (red), pea (green), and lentil (purple) at four sites in 2014. S1, S2, and S3 denote P001T34R, TH3303R2Y, and NSC Moosomin soybean varieties; P1, P2, and P3 denote CDC Meadow, CDC Amarillo, and CDC Limerick pea varieties; L1, L2, and L3 denote CDC Impower, CDC Invincible, and CDC Maxim lentil varieties. Error bars represent one standard error. Within a site, bars with the same letters are not significantly different ( $p \geq 0.05$ ) according to a Tukey's HSD test.

As expected, N and P concentration in the grain was significantly higher than that in the straw for the three crops. In general, soybean varieties had higher grain N and P concentration, while only low to similar levels of straw N and P concentration as compared to pea and lentil (Table 3.6). Compared to soybean and pea, lentil had relatively high N and P concentration in the straw. Soybean and pea had higher N and P uptake in the grain than in the straw, whereas lentil had similar or lower N and P uptake in the grain than in the straw. In addition, soybean and pea had similar amount of grain N and P uptake at the Saskatoon, Scott, and Yorkton sites, whereas pea had superior grain N and P uptake at the Rosthern site (Table 3.7). Lentil had relatively lower grain N and P uptake but similar or higher straw N and P uptake compared to soybean and pea. At the Yorkton site, all three varieties of soybean had significantly lower straw N yield than pea and lentil, and lower straw P yield in soybean than CDC Amarillo pea and all three lentil varieties. Harvest N indexes (HNI) and harvest P indexes (HPI) appeared to be higher for soybean and pea, while lentil had relatively lower HNI and HPI across the sites except at the Saskatoon site, where the three crops had similar HNI and HPI (Table 3.7).

**Table 3.6.** N and P concentration (n=4) in the grain and the straw of soybean, pea, and lentil in 2014.

Site	Crop	Variety	N concentration		P concentration	
			Grain	Straw	Grain	Straw
			g N kg <sup>-1</sup>		g P kg <sup>-1</sup>	
Rosthern	Soybean	P001T34R	43.1 a <sup>†</sup>	8.6 a	5.5 a	0.8 c
		TH3303R2Y	43.0 a	7.4 a	5.7 a	1.0 bc
		NSC Moosomin	44.5 a	8.2 a	5.7 a	0.9 bc
	Pea	CDC Meadow	26.7 d	8.7 a	3.2 c	0.6 c
		CDC Amarillo	26.7 d	9.1 a	3.3 c	0.6 c
		CDC Limerick	29.7 cd	8.9 a	3.7 bc	0.6 c
	Lentil	CDC Impower	30.4 c	12.9 a	4.1 b	1.5 ab
		CDC Invincible	34.4 b	14.1 a	4.1 b	1.8 a
		CDC Maxim	31.4 bc	9.1 a	3.9 b	1.1 abc
Saskatoon	Soybean	P001T34R	41.2 a	7.5 b	5.1 abc	1.2 a
		TH3303R2Y	43.5 a	8.4 b	6.3 ab	1.5 a
		NSC Moosomin	45.9 a	10.1 b	6.8 a	1.6 a
	Pea	CDC Meadow	19.9 c	13.8 ab	3.7 c	1.1 a
		CDC Amarillo	26.8 bc	14.6 ab	4.2 bc	1.1 a
		CDC Limerick	28.9 bc	18.0 a	4.4 bc	1.5 a
	Lentil	CDC Impower	28.8 bc	13.0 ab	4.2 bc	1.3 a
		CDC Invincible	30.3 b	10.8 b	3.9 bc	1.2 a
		CDC Maxim	28.8 bc	10.7 b	3.7 c	1.0 a
Scott	Soybean	P001T34R	47.3 a	9.5 abc	5.6 a	0.8 bc
		TH3303R2Y	39.3 a	3.4 c	5.5 a	0.6 c
		NSC Moosomin	45.7 a	6.5 bc	5.4 ab	0.7 bc
	Pea	CDC Meadow	37.4 a	14.9 a	4.0 c	1.2 abc
		CDC Amarillo	36.9 a	12.8 ab	4.0 c	0.9 bc
		CDC Limerick	36.7 a	16.0 a	4.3 bc	1.1 abc
	Lentil	CDC Impower	37.6 a	16.9 a	3.7 c	1.9 a
		CDC Invincible	35.7 a	14.9 a	3.7 c	1.7 ab
		CDC Maxim	37.2 a	14.6 ab	3.3 c	1.4 abc
Yorkton	Soybean	P001T34R	44.5 b	4.2 d	5.9 a	0.8 d
		TH3303R2Y	43.6 b	3.8 d	5.6 a	1.0 cd
		NSC Moosomin	47.6 a	4.4 d	6.0 a	0.9 cd
	Pea	CDC Meadow	31.1 d	12.2 c	4.3 b	1.4 bcd
		CDC Amarillo	30.6 d	13.2 bc	4.3 b	1.3 bcd
		CDC Limerick	33.2 cd	14.7 bc	4.5 b	1.5 bc
	Lentil	CDC Impower	32.6 cd	14.3 bc	4.3 b	1.9 ab
		CDC Invincible	35.0 c	16.4 ab	4.4 b	2.3 a
		CDC Maxim	35.5 c	19.3 a	4.3 b	2.4 a

<sup>†</sup> Within a column, means within a site followed by the same letter are not significantly different from each other (p≥0.05) according to a Tukey's HSD test.



**Table 3.7.** N and P uptake (n=4) in the grain and the straw of soybean, pea, and lentil in 2014.

Site	Crop	Variety	N yield		P uptake		HNI <sup>†</sup>	HPI <sup>†</sup>
			Grain	Straw	Grain	Straw	%	
			kg N ha <sup>-1</sup>		kg P ha <sup>-1</sup>			
Rosthern	Soybean	P001T34R	121.4 b <sup>‡</sup>	34.0 a	15.5 bc	3.1 b	78 a	84 ab
		TH3303R2Y	113.9 bc	50.1 a	15.1 bc	6.8 ab	69 ab	69 abc
		NSC Moosomin	116.0 bc	45.5 a	14.8 bc	5.3 ab	72 ab	74 ab
	Pea	CDC Meadow	163.4 a	47.6 a	19.8 ab	3.1 b	77 a	86 a
		CDC Amarillo	177.6 a	61.1 a	21.8 a	4.1 b	74 ab	84 ab
		CDC Limerick	186.9 a	58.4 a	23.1 a	4.0 b	76 a	85 a
	Lentil	CDC Impower	51.2 e	78.3 a	6.8 d	9.3 a	40 c	42 d
		CDC Imvincible	76.6 de	72.0 a	9.1 d	9.1 a	52 bc	50 cd
		CDC Maxim	86.4 dc	54.1 a	10.8 cd	6.6 ab	61 ab	62 bc
Saskatoon	Soybean	P001T34R	51.3 ab	25.8 b	7.0 abc	4.0 c	67 a	64 a
		TH3303R2Y	40.4 abc	48.6 ab	5.8 abc	8.5 ab	45 abc	41 a
		NSC Moosomin	50.1 ab	55.3 ab	7.4 ab	8.8 a	48 ac	46 a
	Pea	CDC Meadow	33.7 bc	43.0 ab	6.1 abc	3.4 c	44 bc	64 a
		CDC Amarillo	54.0 ab	63.9 ab	8.4 ab	4.6 bc	46 abc	64 a
		CDC Limerick	60.8 a	71.9 a	9.2 a	5.8 abc	46 abc	61 a
	Lentil	CDC Impower	19.1 c	38.6 ab	2.8 c	4.0 c	33 c	41 a
		CDC Imvincible	35.6 abc	31.7 ab	4.6 bc	3.5 c	53 abc	57 a
		CDC Maxim	31.6 bc	20.5 b	4.1 bc	2.0 c	61 ab	67 a
Scott	Soybean	P001T34R	150.1 a	57.9 abc	17.0 a	4.9 ab	72 ab	77 a
		TH3303R2Y	124.3 ab	24.8 c	16.4 a	4.1 b	83 a	80 a
		NSC Moosomin	117.2 ab	39.6 bc	13.5 ab	4.8 ab	75 ab	74 ab
	Pea	CDC Meadow	109.5 abc	91.8 ab	11.8 abc	7.1 ab	54 bc	62 abc
		CDC Amarillo	112.3 abc	77.8 abc	12.1 abc	5.3 ab	59 abc	70 ab
		CDC Limerick	98.9 abc	94.8 ab	11.3 abc	6.8 ab	51 bcd	62 abc
	Lentil	CDC Impower	26.8 c	97.0 ab	2.5 d	10.8 a	22 d	19 d
		CDC Imvincible	57.0 bc	98.1 a	5.9 cd	11.0 a	37 cd	35 cd
		CDC Maxim	90.1 abc	83.6 ab	7.8 bcd	8.1 ab	52 bc	49 bcd
Yorkton	Soybean	P001T34R	149.9 ab	18.0 c	19.9 a	3.2 e	89 a	86 a
		TH3303R2Y	141.6 ab	19.5 c	18.1 a	4.6 de	88 a	80 ab
		NSC Moosomin	168.0 a	22.6 c	21.2 a	4.7 cde	88 a	82 a
	Pea	CDC Meadow	48.4 de	68.4 b	6.6 c	7.9 bcd	41 bc	46 cd
		CDC Amarillo	106.1 bcd	104.1 a	14.9 ab	10.2 ab	50 bc	59 bcd
		CDC Limerick	109.1 abc	85.0 ab	14.5 ab	8.4 abc	56 b	63 abc
	Lentil	CDC Impower	35.2 e	73.2 ab	4.5 c	9.6 ab	32 c	32 d
		CDC Imvincible	50.4 cde	63.1 b	6.4 c	9.0 ab	44 bc	42 cd
		CDC Maxim	76.3 cde	93.0 ab	9.2 bc	11.8 a	45 bc	44 cd

<sup>†</sup> HNI= harvest nitrogen index, HPI= harvest phosphorus index.

<sup>‡</sup> Within a column, means within a site followed by the same letter are not significantly different from each other (p≥0.05) according to a Tukey's HSD test.

### 3.5.2 Fall 2014 available nitrogen and phosphorus concentration and nitrogen and phosphorus supply rates in the 2015 season

In 2014 fall after harvest of the pulse crops, soil  $\text{NO}_3^-$ -N concentration in the surface soil (0-15cm) was lower in soybean stubble plots than in pea or lentil stubble plots (Table 3.8). This effect was significant at Rosthern, Saskatoon and Yorkton. Soil modified Kelowna extractable P concentration was similar in soybean, pea, and lentil stubble plots across the sites, except for the Yorkton site where the soil available P concentration in soybean stubble plots was 27% and 40% lower than that in pea and lentil stubble plots, respectively.

The two sites (Rosthern and Saskatoon) selected to follow soil available N and P supply rates over the 2015 growing season also had different initial soil supply rates of  $\text{NO}_3^-$ -N,  $\text{PO}_4^{3-}$ -P, and  $\text{NH}_4^+$ -N before seeding in May 2015 (Table 3.9). At the Rosthern site, plots with lentil grown in the previous year (2014) had higher supply rates of  $\text{NO}_3^-$ -N than plots with soybean grown in 2014, with pea stubble plots having intermediate soil  $\text{NO}_3^-$ -N supply rates. At the Saskatoon site, plots with different previous crops grown had similar  $\text{NO}_3^-$ -N supply rates. Trends of the  $\text{NO}_3^-$ -N supply rate in the spring of 2015 followed those of soil extractable  $\text{NO}_3^-$ -N in the fall of 2014, with lowest values in the soybean stubble plots. On the other hand, soybean, pea, and lentil plots had similar pre-seeding soil  $\text{NH}_4^+$ -N and  $\text{PO}_4^{3-}$ -P supply rates at both sites, consistent with the similar soil  $\text{PO}_4^{3-}$ -P concentration measured in 2014 fall.

During the 2015 growing season, soil available ( $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N) N supply showed different trends at the two sites (Fig. 3.4). At the Rosthern site, soybean and pea stubbles had similar N supplies at the beginning of the growing season, while the lentil stubble had a higher initial soil N supply, as also revealed by the initial soil N supply via 24 h PRS<sup>TM</sup> measurement (Table 3.9). In soybean and pea stubble plots, the cumulative soil N supply was lower than in lentil stubble plots in the first 5 weeks following seeding, but appeared to increase more in soybean stubble plots and pea stubble plots later in the growing season. Overall, by harvest, there were no significant differences among legume stubbles regarding the total amount of N supplied through the growing season at the Rosthern site. However, these results indicate a difference in available N release patterns among the different crop stubbles at the Rosthern site, with a greater early release of available N from the lentil stubble but a greater late season contribution from the pea stubble. At the Saskatoon site, on the other hand, plots had similar soil N supply patterns with the three pulse stubbles through the entire growing season.

Moreover, compared to the Rosthern site, the steeper slopes of the cumulative soil N supply rate curves at the Saskatoon site indicated that at the Saskatoon site, N mineralization preceded at a steadier rate through the season compared to the Rosthern site.

Similarly, soil P ( $\text{PO}_4^{3-}\text{-P}$ ) supplies showed different trends at the Rosthern site compared to the Saskatoon site during the growing season (Fig. 3.5). At the Rosthern site, soybean stubble plots had higher soil P supplies than lentil stubble plots, agreeing with the initial 24 hour soil supply rates and also the extractable soil P in the previous fall which was highest in soybean stubble at this site. At the Saskatoon site, on the other hand, there was a trend for soybean stubble having lower soil P supplies, but differences were not statistically significant. Furthermore, as for soil N supply rates, the Saskatoon site also had steeper cumulative soil P supply curves during the growing season, which even became steeper towards the end of the growing season at this site. Cumulative soil P supplies in soybean stubble plots did not increase to as great an extent later in the season compared to that in pea or lentil stubble plots.

Overall, by the end of the growing season, soybean, pea, and lentil stubbles did not produce significantly different cumulative soil N and P supplies at either of the two sites. However, at the Rosthern site, soybean stubble plots had lower soil N supply rates in the first 5 weeks of the growing season, but higher soil P supply rates through almost the entire growing season compared to lentil stubble plots. Despite superior pea yield at the Rosthern site in the previous year, cumulative soil N and P supplies in pea stubble plots were similar to that in soybean or lentil stubble plots, although the release rate of available N from the pea stubble at Rosthern was increasing later in the season compared to other stubble types. In contrast, at the Saskatoon site, plots with different grain legume stubbles had similar cumulative soil N and P supply rates through the entire growing season.

**Table 3.8.** Post-harvest soil properties (0-15cm) (n=4) at the four sites in fall 2014.

Site	Crop <sup>†</sup>	NO <sub>3</sub> <sup>-</sup> -N	MK-P <sup>‡</sup>	Moisture	EC	pH
		mg kg <sup>-1</sup>			%	
Rosthern	Soybean	7.2 b <sup>§</sup>	13.4 a	17.1 a	0.11 a	6.8 a
	Pea	10.5 a	11.9 a	21.2 a	0.15 a	6.9 a
	Lentil	10.7 a	9.9 a	19.7 a	0.16 a	6.9 a
Saskatoon	Soybean	6.2 b	17.8 a	29.7 a	2.11 a	6.3 a
	Pea	11.2 a	17.7 a	30.3 a	2.73 a	6.4 a
	Lentil	9.1 ab	18.0 a	31.2 a	2.39 a	6.2 a
Scott	Soybean	5.3 a	8.6 a	16.5 a	0.23 a	7.2 a
	Pea	13.9 a	8.7 a	15.6 a	0.30 a	7.2 a
	Lentil	17.9 a	9.1 a	16.0 a	0.28 a	6.9 a
Yorkton	Soybean	7.7 b	7.5 b	18.9 a	0.33 a	7.9 a
	Pea	16.4 a	10.3 ab	18.4 a	0.39 a	7.8 a
	Lentil	12.8 a	12.5 a	19.0 a	0.34 a	7.8 a

<sup>†</sup> The crop that was grown in the plot during the 2014 growing season.

<sup>‡</sup> Modified Kelowna extractable available P.

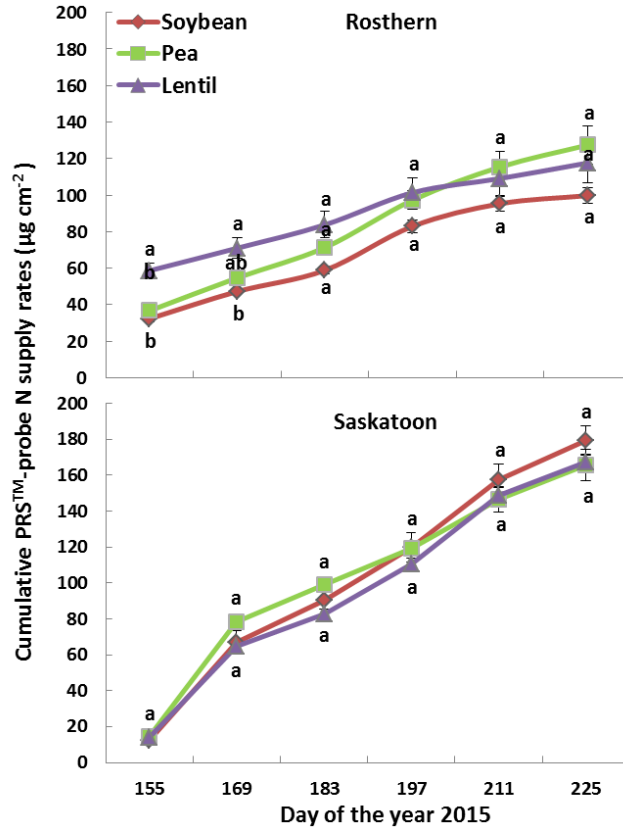
<sup>§</sup> Within a column, means within a site followed by the same letter are not significantly different from each other ( $p \geq 0.10$ ) according to a Tukey's HSD test.

**Table 3.9.** Pre-seeding soil N and P supply (n=4) at the Rosthern and Saskatoon sites in May, 2015.

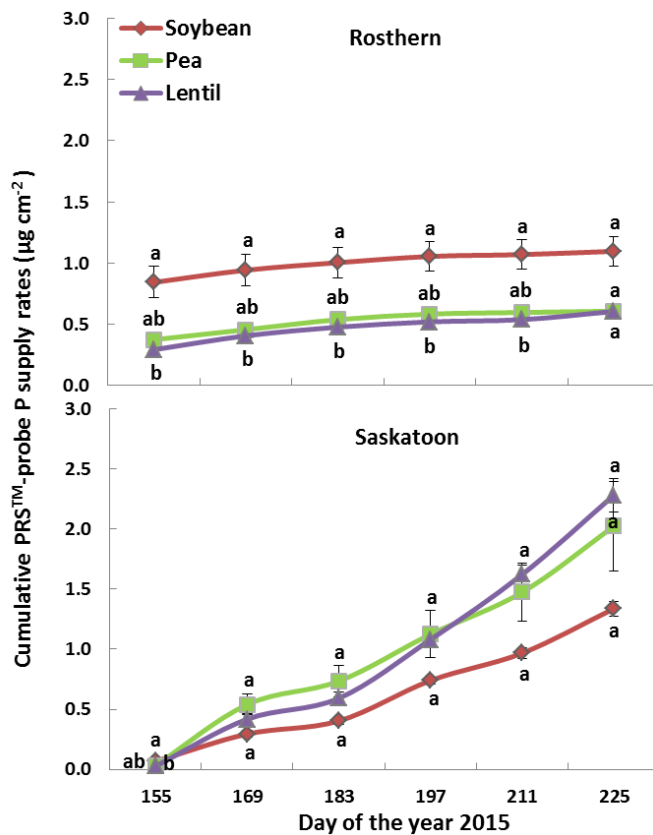
Site	Previous crop <sup>†</sup>	NO <sub>3</sub> <sup>-</sup> -N	PO <sub>4</sub> <sup>3-</sup> -P	NH <sub>4</sub> <sup>+</sup> -N
		µg cm <sup>-2</sup> (24 h) <sup>-1</sup>		
Rosthern	Soybean	11.4 b <sup>‡</sup>	1.5 a	0.1 a
	Pea	16.6 ab	1.1 a	0.1 a
	Lentil	19.6 a	1.0 a	0.1 a
Saskatoon	Soybean	6.0 a	0.3 a	0.2 a
	Pea	6.5 a	0.0 a	0.1 a
	Lentil	9.2 a	0.1 a	0.2 a

<sup>†</sup> The crop that was grown in the same plot in 2014.

<sup>‡</sup> Within a column, means within a site followed by the same letter are not significantly different from each other ( $p \geq 0.10$ ) according to a Tukey's HSD test.



**Fig. 3.4.** Cumulative soil N ( $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N) supply ( $n=4$ ) at the Rosthern and Saskatoon sites during the 2015 growing season. Values are the mean cumulative soil N supply rates through the 2015 growing season, with soybean, pea, or lentil grown in the previous crop year (2014). Error bars represent one standard error. For each sampling date, cumulative soil N supplies with the same letter are not significantly different ( $p \geq 0.10$ ) according to a Tukey's HSD test.



**Fig. 3.5.** Cumulative soil available  $\text{PO}_4^{3-}\text{-P}$  supply ( $n=4$ ) at the Rosthern and Saskatoon sites during the 2015 growing season. Values are the mean cumulative soil P supplies through the 2015 growing season, with soybean, pea, or lentil grown in the previous crop year. Error bars represent one standard error. For each sampling date, cumulative soil N supplies with the same letter are not significantly different ( $p \geq 0.10$ ) according to a Tukey's HSD test.

### 3.5.3 Yield and uptake of nitrogen and phosphorus in crops grown the following year

For spring wheat grown on the stubble of soybean, pea, or lentil at the Rosthern, Saskatoon, and Scott sites, the ANOVA revealed that soybean, pea, and lentil stubbles had significant impact on the grain yield and grain N uptake, as well as the straw P uptake in wheat (Table 3.10). Site had significant effects on the yield and N and P uptake in both the grain and the straw of spring wheat in 2015. Different stubble varieties significantly impacted the grain yield and N uptake, and straw P uptake in wheat. In addition, the interaction between previous site × stubble had significant effects on the grain and straw P uptake, and straw N and P uptake, while the interaction between site × stubble variety only had significant impact on the straw P uptake of spring wheat grown in the 2015 season.

In 2014 all sites received near normal amount of precipitation during the growing season, whereas in 2015 the growing season precipitation at the four sites was below normal, and for May and June the precipitation was well below normal (Table 3.4). The average grain yield of wheat was reported as 2488 kg ha<sup>-1</sup>, and for canola 2287 kg ha<sup>-1</sup> in Saskatchewan in 2015 (Government of Saskatchewan, 2015). In the present study, spring wheat grown in 2015 on the soybean, pea, or lentil stubble had similar grain yield at the Saskatoon and Scott sites (Table 3.11). At the Rosthern site, wheat grown on the stubble of P001T34R soybean, TH3303R2Y soybean, and CDC Amarillo pea had slightly lower grain yield than on other legume stubbles. Wheat straw yield was similar in plots with different crops grown in the previous year at each of the three sites. Overall, wheat in 2015 had similar grain and straw yield on the soybean, pea, and lentil stubbles, while wheat grain yield was generally lower on the wheat stubble than on legume stubbles at the Rosthern and Saskatoon sites.

**Table 3.10.** Summary of ANOVA and significance of variables of yield and uptake of N and P in the grain and the straw of wheat in 2015.

Source	df	Grain (kg ha <sup>-1</sup> )			Straw (kg ha <sup>-1</sup> )		
		Yield	N	P	Yield	N	P
Site	2	***	***	**	***	***	***
Stubble	2	**	***	ns	ns	ns	**
Stubble variety	8	*	**	ns	ns	ns	*
Site × Stubble	4	ns <sup>†</sup>	ns	*	ns	*	*
Site × Stubble variety	16	ns	ns	ns	ns	ns	*

<sup>†</sup> Not significant ( $p \geq 0.05$ ) according to Tukey's HSD test.

\*, \*\*, and \*\*\* Significant at  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively, according to a Tukey's HSD test.

Compared to the grain and straw yield, N uptake in wheat (Table 3.11) had more variation among different grain legume stubbles in general. At the Rosthern site, the grain N uptake of wheat was lower on the stubble of P001T34R soybean, TH3303R2Y soybean, NSC Moosomin soybean, and CDC Amarillo pea than on the stubble of CDC Invincible lentil and CDC Maxim lentil. Wheat grown on different grain legume stubbles had similar straw N uptake at the Rosthern site. At the Saskatoon site, wheat had higher grain N uptake on CDC Limerick pea and CDC Invincible lentil stubbles than on the P001T34R soybean stubble, while wheat had higher straw N uptake on CDC Meadow pea, CDC Amarillo pea, CDC Limerick pea, and CDC Invincible lentil stubble than on the P001T34R soybean stubble. Wheat grown on wheat stubble had the lowest grain N uptake. At the Scott site, the 2015 wheat grain and straw N uptake was similar on different grain legume stubbles.

At the Rosthern site, wheat had higher grain P uptake on the CDC Maxim lentil stubble than on the CDC Amarillo pea stubble, while wheat had higher straw P uptake on the CDC Amarillo pea than that on the CDC Impower lentil stubble. At the Saskatoon site, the grain P uptake of wheat was lower on the P001T34R soybean stubble than on CDC Meadow pea, CDC Limerick pea, and CDC Invincible lentil stubbles, whereas straw P uptake did not differ with different grain legume stubbles from the previous year. At the Scott site, previous grain legume species did not result in different P uptake in the grain or straw of wheat grown in the following year. Overall, wheat grown on soybean stubble had similar P uptake compared to that on pea and lentil stubbles, while wheat grain P uptake was lower on the wheat stubble from 2014 than on grain legume stubbles. At the Yorkton site where canola was grown rather than wheat, the yield, N and P uptake in the grain and the straw of canola did not vary with different grain legume stubbles from the previous year.



**Table 3.11.** Yield, N and P uptake (n=4) in the grain and the straw of wheat at the Rosthern, Saskatoon, and Scott sites, and of canola at the Yorkton site in 2015.

Site	Stubble	Variety	Yield		N uptake		P uptake	
			Grain	Straw	Grain	Straw	Grain	Straw
			kg ha <sup>-1</sup>		kg N ha <sup>-1</sup>		kg P ha <sup>-1</sup>	
Rosthern	Soybean	P001T34R	3052 bc <sup>†</sup>	3639 a	65.9 bc	16.8 a	13.8 abc	1.3 ab
		TH3303R2Y	2905 c	3396 a	64.2 bc	18.0 a	13.3 abc	1.7 ab
		NSC Moosomin	3093 abc	3749 a	67.9 bc	18.2 a	13.8 abc	1.4 ab
	Pea	CDC Meadow	3183 abc	3744 a	72.2 ab	19.4 a	14.4 abc	1.0 ab
		CDC Amarillo	2742 c	4236 a	60.4 bc	25.0 a	12.5 bc	2.3 a
		CDC Limerick	3193 abc	3846 a	73.3 ab	22.3 a	14.0 abc	1.8 ab
	Lentil	CDC Impower	3191 abc	3924 a	72.1 ab	18.3 a	13.6 abc	0.6 b
		CDC Imvincible	3642 ab	4056 a	83.4 a	21.0 a	15.2 ab	0.9 ab
		CDC Maxim	3746 a	4016 a	85.7 a	20.1 a	16.2 a	1.0 ab
	Wheat	CDC Abound	2649 c	3108 a	57.8 c	17.2 a	11.6 c	1.2 ab
Saskatoon	Soybean	P001T34R	2097 bc	2208 ab	45.1 bc	7.3 c	9.4 bc	1.9 a
		TH3303R2Y	2629 ab	2705 ab	59.9 ab	13.4 abc	13.0 ab	2.9 a
		NSC Moosomin	2706 ab	2907 ab	64.0 ab	13.3 abc	13.5 ab	2.8 a
	Pea	CDC Meadow	2925 ab	2972 ab	66.4 ab	15.4 ab	14.2 a	2.5 a
		CDC Amarillo	2850 ab	3236 a	64.4 ab	14.7 ab	13.9 ab	2.1 a
		CDC Limerick	3044 ab	3323 a	71.5 a	16.5 a	14.8 a	2.5 a
	Lentil	CDC Impower	2886 ab	3016 a	65.8 ab	13.4 abc	13.7 ab	2.3 a
		CDC Imvincible	3098 a	3184 a	73.7 a	15.3 ab	14.8 a	2.2 a
		CDC Maxim	2889 ab	2880 ab	66.6 ab	13.9 abc	14.0 a	2.3 a
	Wheat	CDC Abound	1484 c	1710 b	34.2 c	9.1 bc	7.5 c	2.2 a
Scott	Soybean	P001T34R	4775 a	3703 a	111.8 a	19.9 a	16.3 a	1.3 a
		TH3303R2Y	3830 a	3009 a	86.3 a	17.5 a	14.3 a	1.3 a
		NSC Moosomin	4636 a	3574 a	107.4 a	18.0 a	16.3 a	1.1 a
	Pea	CDC Meadow	4331 a	3277 a	103.3 a	17.2 a	14.9 a	0.9 a
		CDC Amarillo	3998 a	2992 a	93.7 a	16.3 a	13.8 a	0.9 a
		CDC Limerick	3909 a	2870 a	93.9 a	15.8 a	14.1 a	0.9 a
	Lentil	CDC Impower	4354 a	3257 a	105.0 a	15.9 a	14.4 a	0.8 a
		CDC Imvincible	4944 a	3582 a	118.1 a	18.2 a	16.5 a	0.9 a
		CDC Maxim	4465 a	3418 a	105.5 a	18.3 a	14.9 a	1.0 a
	Yorkton	Soybean	P001T34R	1851 a	5306 a	50.5 a	23.7 a	13.0 a
TH3303R2Y			1750 a	5219 a	50.6 a	21.7 a	13.3 a	2.8 a
NSC Moosomin			1911 a	5167 a	53.7 a	18.2 a	13.6 a	2.1 a
Pea		CDC Meadow	1786 a	5012 a	50.9 a	23.0 a	12.9 a	1.7 a
		CDC Amarillo	1983 a	6010 a	55.8 a	20.5 a	14.8 a	1.6 a
		CDC Limerick	2278 a	5952 a	68.2 a	24.3 a	16.3 a	2.0 a
Lentil		CDC Impower	2054 a	5861 a	62.9 a	24.6 a	15.9 a	2.3 a
		CDC Imvincible	2017 a	5214 a	55.8 a	24.1 a	14.5 a	2.7 a
		CDC Maxim	2349 a	6338 a	62.1 a	19.7 a	16.1 a	1.8 a

<sup>†</sup> Within a column, means within a site followed by the same letter are not significantly different from each other (p≥0.05) according to a Tukey's HSD test.

### 3.6 Discussion

As Bullock and Nadler (2013) reported, although a lack of heat units and insufficient soil moisture may be considered major limits for soybean production in western Canada, the future of soybean production was noted as positive due to the increasing average air temperature across southern Canada over the last century, with both maximum and minimum air temperature increasing in western Canada in the last decade. Indeed, in the present study, the grain yield of selected modern short-season varieties of soybean (929-3534 kg ha<sup>-1</sup>, HI of 14-45%), pea (1565-6644 kg ha<sup>-1</sup>, HI of 22-53%), and lentil (664-2755 kg ha<sup>-1</sup>, HI of 11-37%) observed at the four sites is comparable to previous studies conducted in western Canada. Przednowek et al. (2004) reported the grain yield of soybean as 1098-3415 kg ha<sup>-1</sup> and of pea as 1194-3563 kg ha<sup>-1</sup> in a three-year (1998-2000) field trial carried out at four locations in southern Manitoba. Moreover, they found that compared to soybean, pea yielded better and provided more N benefits to wheat grown in the subsequent year in rotation. In another rotation experiment conducted nearly two decades ago at three locations in Saskatchewan, the grain yield of pea were 1656-2227 kg ha<sup>-1</sup>, and the grain yield of wheat was 43% higher on the pea stubble than on the wheat stubble (Stevenson and van Kessel, 1996a), indicating the rotational benefits of legumes to succeeding crops as opposed to non-legumes. Moreover, lentil grain yield was within 1720-2640 kg ha<sup>-1</sup> (HI of 38-45%) at two adjacent sites in southeast Saskatchewan (Zakeri et al., 2012).

When compared to results from eastern Canada and northern USA, though, soybean grain yield in the present study is generally on the lower range. For instance, in a long-term tillage fertilization field trial conducted in eastern Canada, soybean grain yield was 3360-3730 kg ha<sup>-1</sup> (Messiga et al., 2012). In Ontario, the average grain yield of soybean was 2250-4000 kg ha<sup>-1</sup> over 30 years (1970-2000), with an annual increasing rate of 20 kg ha<sup>-1</sup>, of which 80% was attributed to genetic improvement and the rest to improvement of agronomic practices (Cober and Voldeng, 2012). In northern USA, the grain yield of soybean was also reported to be increasing at a rate of about 1% yr<sup>-1</sup> over 60 years (1940-2000), and the yield of tested soybean cultivars was around 3500 kg ha<sup>-1</sup> by the year 2000 and demonstrated a continued increasing trend (Wilcox, 2001). In a cover-crop experiment conducted in eastern South Dakota, USA, the grain yield of soybean ranged within 2500-3000 kg ha<sup>-1</sup> under different cover-crop treatments, with harvest indexes of 46-54% (Dagel et al., 2014). Despite the differences among particular

varieties, differences in the soybean grain yield between Saskatchewan and eastern Canada and USA can be mainly attributed to the relatively drier and colder environmental conditions in Saskatchewan.

There is little recent information on nutrient uptake by soybeans grown in Canada reported in peer-reviewed papers. Parsons (2005) reported N uptake of soybean as 62-136 kg ha<sup>-1</sup> and P uptake as 6-13 kg ha<sup>-1</sup> from a two-year rotation experiment with different manure treatments conducted in Truro, Nova Scotia. In the current study, the above-ground N uptake (grain N + straw N) in soybean was around 100-150 kg ha<sup>-1</sup>, comparable with the Nova Scotia study, while soybean P uptake in the present study was higher than that reported by Parsons (2005). In other regions, there has been significant amount of research on nutrient uptake conducted, but with variable amounts reported. For instance, in a meta-analysis, data from 480 experiments suggested the mean N uptake of soybean was 219 kg ha<sup>-1</sup>, ranging within 44-480 kg ha<sup>-1</sup>, with 50% of the data falling between 154-280 kg ha<sup>-1</sup> (Salvagiotti et al., 2008). Variation in yield and nutrient uptake of soybean is attributed to different growing conditions, fertility treatments applied in each experiment, and differences in plots harvest and plant sample processing. Overall, results of N and P uptake from the present study are similar to results of other studies in Canada. As a significant portion of N in the soybean ( $\approx$ 60-70%) is derived from atmospheric N<sub>2</sub> fixation according to our <sup>15</sup>N study (see chapter 5), the contribution of short season soybean to N fertility from fixation is significant, and nearly as high or equivalent to pea.

2014 received near normal precipitation and temperatures during the growing season compared to historical weather data at the research sites, and this was considered to be beneficial for the production of soybeans in this region. Indeed, in the present study, selected modern short-season varieties of soybean yielded as much as or higher than pea and lentil at three of the four sites, with pea having superior yield at the Rosthern site. However, rotational benefits of grain legumes to soil nutrient supply rates and succeeding crops are determined by the amount of nutrients retained in the soil system after the removal of grain from the field at harvest. In addition, other factors, such as biochemical composition of the above- and below-ground residue, environmental conditions affecting the decomposition processes, and agronomic management, also affect the nutrient benefits of grain legumes to the soil nutrient pool and following crops in rotation (Kumar and Goh, 2003; Campbell et al., 2011). In fact, soybean generally had similar or greater grain N and P uptake and smaller straw uptake,

implying its potentially negative impact on the availability of nutrients to following crops as the grain is harvested, although factors such as C:N and C:P ratios also affect nutrient release from the residue. Moreover, rotational benefits of grain legumes include not only nutrient benefits such as rhizosphere deposits, less soil N consumption, and returning of nutrients to the soil system via residue, but also the enhancement of other soil properties and soil microbial aspects that influence the yield and nutrient uptake of following crops (Przednowek et al., 2004; Adderley et al., 2006; Arcand et al., 2014b). For instance, Dayegamiye et al. (2012) reported that the rotational benefits of soybean to following corn crops lasted up to two years following the last introduction of soybean in the cropping system versus corn crops grown on the corn stubble, due to the relatively slow decomposition in the cold and humid environmental conditions in eastern Canada.

Although the absolute amount of residue biomass and residue N and P returned to the field after harvest is not a precise indicator for the amount of immediately bioavailable C, N, and P, the returned amount is meaningful for indicating the potential contribution of preceding crops to soil nutrient stores. It represents nutrients of which a large portion will ultimately become available to following crops. With comparable biomass yield and grain yield of selected short-season soybean varieties, the amount of N and P returned to the field via soybean straw was relatively low compared to that via pea or lentil residue as a consequence of high uptake in grain of N and P. However, in the subsequent year, wheat and canola crops did not show large or consistent differences in uptake among different previous crop stubbles, in spite of the lower straw N and P returned via soybean straw, especially at the Yorkton site. This lack of significant response in the following crops was possibly a consequence of relatively high soil available N levels and mineralization input in the Black soil at the Yorkton site, which muted the nutrient input differences from the different legume stubbles from 2014. In addition, N benefits from previous crops are further complicated by other factors such as decomposing conditions and mineralization rates of added residues (Walley et al., 2007), making it difficult to accurately estimate short-term N contribution from pulse residues. Overall, soybean, pea, and lentil showed similar impact on crops in the subsequent year, but high levels of grain N and P with relatively low straw N and P in soybean indicate that under Saskatchewan growing conditions, when selected soybean varieties are included in crop rotation for a long term, fertilization of N and P for following crops may need to be elevated due to the relatively low amount of N and P

returned by soybean residue and higher amount exported in soybean grain compared to conventional grain legumes in Saskatchewan such as pea and lentil. Although a significant portion of the N in the harvested grain is derived from atmospheric N<sub>2</sub> fixation in the pulse crops, the rest is derived from soil N reserves and greater total N contained in seed indicates greater soil N removal from soil at harvest, agreeing to the amount of grain N derived from soil as shown in Chapter 5.

Soil nutrient supply rates during the growing season of 2015 showed different patterns with time at the two sites probably due to different decomposition conditions at the two sites. The Saskatoon site had salinity conditions during the 2015 growing season and the soil microbial activity was likely suppressed. However, overall, the cumulative soil supply of available N by the end of the growing season at the Rosthern and Saskatoon sites were similar to results reported by Adderley et al. (2006) at sites in southern Saskatchewan, whereas the cumulative soil supply of available P was only about 20-35% of their results, likely due to the limited precipitation at the sites in the early spring of 2015. Given the dry conditions in 2015 spring, it is possible that mineralization was not reaching the maximum, muting the effects of previous crops on the soil nutrient supply, yield and nutrient uptake of following crops. This was in line with the observation that crops in 2015 showed no large consistent response to different previous legume crops at any of the sites, despite soybean had lower N and P returned to the field via residue in the fall of 2014 compared to pea and lentil. However, when soybean is used in crop rotation for a long term, soil N and P levels need to be monitored due to the high export in soybean grain and relatively low return via straw compared to pea and lentil. Overall, the yield and nutrient uptake of wheat and canola grown on the different grain legume stubbles across sites were not greatly influenced by grain legume crop stubble types in this study. Our findings therefore imply that under the growing conditions experienced at the four sites in 2014 and 2015, soybean, pea, and lentil were able to yield comparably and benefit the crop grown in the following year at a similar level in a normal year in western Canada, without experiencing extreme climatic conditions or disease damage.

### **3.7 Conclusion**

Selected modern short-season soybean varieties had similar or higher grain yield compared to pea and lentil under Saskatchewan growing conditions, although site had a significant influence on the yield and nutrient uptake by the crops related to the growing

season conditions. The N and P concentration was higher in the grain than in the straw of all three crops, and soybean had higher grain N and P concentration but relatively lower straw N and P concentration compared to pea and lentil. In addition, compared to pea and lentil, soybean had similar to high levels of N and P uptake in the grain, but relatively lower N and P uptake in the straw, and this effect was the most evident at the Yorkton site. As a result, soybean had high harvest N index (HNI) and harvest P index (HPI), whereas lentil generally had lower HNI and HPI across the sites, suggesting a higher percentage of N and P in soybean plants removed from the field in grain at harvest. Grain and straw yield results implied promising agronomic potential of soybean production in Saskatchewan, with consideration of potential needs for higher fertilization of N and P for crops following soybean in rotation in a long term due to the large removal of N and P in the grain of soybean.

In 2015, at the Rosthern site, soil supplies of available N were lower in soybean stubble plots than in lentil stubble plots in the first one and half months following seeding, but by the end of the season, the cumulative supplies were similar among different stubbles. Soil available P supplies were higher in soybean stubble plots than in lentil stubble plots at the Rosthern site through most of the growing season, despite higher grain P removal and relatively lower P straw return with soybean. At the Saskatoon site, soil supplies of available N and P during the growing season was similar among soybean, pea, and lentil stubbles throughout the entire growing season. Soybean, pea, and lentil as previous rotational crops did not produce large or consistent effects on the grain and straw yield, N and P uptake in wheat or canola crops grown the year after the grain legumes. Overall, in the present study, compared to pea and lentil, selected soybean varieties showed similar production potential and rotational contribution to crops grown in the following year.

## **4. UPTAKE AND PARTITIONING OF POTASSIUM, CALCIUM, MAGNESIUM, SULFUR, COPPER, AND ZINC IN SOYBEAN, PEA, AND LENTIL, AND IMPACT ON NUTRITION OF SUCCEEDING CROPS IN SASKATCHEWAN, CANADA**

### **4.1 Preface**

Following chapter 3, which covered the yield, N, and P aspects of soybean production in Saskatchewan, this chapter turns to the crop nutrition aspect apart from N and P of soybean grown under Saskatchewan conditions. Due to the nutritional value of grain legumes with respect to the generally high levels of mineral elements contained in grain legumes as opposed to cereals, it is important to quantify the mineral nutrients in the grain and the straw of soybean in comparison to pea and lentil, which are commonly grown in Saskatchewan. This chapter, therefore, assesses several important mineral nutrients, including potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), copper (Cu), and zinc (Zn) in the grain and the straw of soybean in comparison to pea and lentil at the four sites near Rosthern, Saskatoon, Scott, and Yorkton in Saskatchewan. In addition, these mineral elements in following wheat and canola grown in the second year as influenced by soybean, pea, and lentil as previous rotational crops are also covered in this chapter.

### **4.2 Abstract**

Mineral nutrition of crops in rotation is important for yield and nutritional quality. As grain legume crop acreage increases and new crops like soybean [*Glycine max* (L.) Merr.] expand into the western Prairies, there is need to assess the mineral nutrient uptake by these crops and crops that commonly follow in rotation. The aim of this component of the thesis research was to assess the concentration and uptake of secondary nutrients and micronutrients including K, Ca, Mg, S, Cu, and Zn, in the grain and the straw of soybean in comparison to pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.), and in the wheat (*Triticum*

*aestivum* L.) and canola (*Brassica napus* L.) grown on the stubble of each of the three crops in the following year. The research was conducted at four sites, including two sites in the Dark Brown (Saskatoon, Scott) and two sites in Black (Rosthern, Yorkton) soil climatic zones over two years in Saskatchewan, Canada. Soybean grain had highest K (16.8-20.3 g kg<sup>-1</sup>), Ca (2.1-2.7 g kg<sup>-1</sup>), Mg (2.6-2.7 g kg<sup>-1</sup>), and S (3.2-3.5 g kg<sup>-1</sup>) concentration, and intermediate to high concentration of Cu (10.2-14.8 mg u kg<sup>-1</sup>) and Zn (30.7-44.4 mg kg<sup>-1</sup>) compared to pea and lentil. Straw concentration was more variable and showed different patterns, with soybean having intermediate straw nutrient concentration (11.3-16.8 g K kg<sup>-1</sup>, 7.2-13.0 g Ca kg<sup>-1</sup>, 4.5-6.2 g Mg kg<sup>-1</sup>, 0.2-1.7 g S kg<sup>-1</sup>, 2.6-4.2 mg Cu kg<sup>-1</sup>, and 5.5-53.3 mg Zn kg<sup>-1</sup>) compared to pea and lentil. Soybean had consistently higher Ca removal in grain (2.2-8.1 kg ha<sup>-1</sup>) than pea (1.1-5.1 kg ha<sup>-1</sup>) and lentil (1.0-2.4 kg ha<sup>-1</sup>) across the sites, while the three crops did not show consistent differences in the grain and straw uptake of the other mineral nutrients evaluated. In the subsequent year, grain and straw mineral concentration and removal of wheat or canola did not differ significantly in soybean, pea, and lentil stubble plots, implying comparable impact from soybean, pea, and lentil on succeeding crops in the following rotational year.

### 4.3 Introduction

While nitrogen (N) and phosphorus (P) receive the greatest attention as primary nutrients most likely to limit crop growth on the Canadian Prairies (see Chapter 3), mineral elements including potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) along with copper (Cu) and zinc (Zn) are also important considerations in nutrient management for crop yield and quality. From a human nutritional standpoint, although some mineral elements are only required in very small amount in human diets for metabolic purposes, inadequate or excessive intake of these mineral nutrients could cause health risks in animals and humans. For instance, Zn deficiencies in humans can result in delayed growth and an impaired immune system (Fischer Walker et al., 2009), and Cu deficiencies have been related to cardiovascular risks (Mertz, 1981). Various mineral nutrient deficiencies occur worldwide with wide geographical variation. For example, the rates of age-adjusted hip fracture, which is a result of lack of Ca, ranged from 52.9 per 100 000 in Chile, 94.0 in Venezuela, to 247 in Hong Kong according to a joint report from FAO and WHO (FAO/WHO, 2001). The accumulation of mineral nutrients in crops, therefore, is an important consideration in crop nutritional value.



Compared to cereals, grain legumes typically contain higher contents of mineral nutrients (Ray et al., 2014), making grain legumes valuable sources of nutritional minerals for human diets and animal feeds. For example, in a study conducted in Canada, pulse consumers (13% of the population) had reduced levels of Mg, P, and Zn deficiency compared to non-consumers of pulse crops (Mudryj et al., 2012). Both genetics and environmental conditions can influence the content of mineral nutrients in crops. For example, mineral nutrients in the seed of six genotypes of field pea (*Pisum sativum* L.) grown at six locations in North Dakota, USA, were reported in the range of 39-63 mg Zn kg<sup>-1</sup>, 1350-1427 mg Mg kg<sup>-1</sup>, and 46-54 mg Fe kg<sup>-1</sup> (Amarakoon et al., 2012). In Saskatchewan, the seed Zn concentration ranged within 44-55 mg Zn kg<sup>-1</sup> in 19 lentil (*Lens culinaris* L.) genotypes grown at eight locations in a two-year field study (Thavarajah et al., 2009). In a two-year study conducted in southern Saskatchewan, Ray et al. (2014) found that location had substantial effects on the mineral nutrient concentration of grain legumes including common bean (*Phaseolus vulgaris* L.), field pea, lentil, and chickpea (*Cicer arietinum* L.), reflecting the significant role of site-specific soil-climatic properties in the mineral nutrient concentration in grain legumes.

Unlike mineral nutrient content in the seed of grain legumes, which have been reported for grain legumes commonly grown in western Canada (Bueckert et al., 2011; Garrett et al., 2013; Ray et al., 2014), the mineral nutrient content of in straw of pulse crops is less frequently reported in western Canada. Knowledge of the nutrient content of the straw is important in assessing the total nutrient requirement of a crop and in predicting the impact of straw removal versus retention in a cropping system. Therefore, information on the concentration and uptake of mineral nutrients, including K, Ca, Mg, S, Cu, and Zn, in both the grain and the straw is crucial to understanding the cycling of these nutrients in the plant-soil system. In addition, knowledge on these nutrients in the straw of grain legumes can help in predicting the impact of including different grain legumes in rotation on nutrient fertility, and thus help improve fertilization strategies.

While pea and lentil have been grown for decades on the Canadian prairies, soybean [*Glycine max* (L.) Merr.] production is more recent, with significant production occurring in Saskatchewan only in the last few years with the development of short-season varieties (Saskatchewan Ministry of Agriculture, 2014). The effects of pulse crop type, variety, and site on nitrogen and phosphorus content, soil supply and uptake by the succeeding crop were

covered in Chapter 3 of this thesis. While the soybean acreage is expanding in western Canada, there is little information on the content and removal of mineral nutrients including K, Ca, Mg, S, Cu, and Zn by the grain and the straw of soybean grown under western Canadian conditions. In addition, there is no recent research on Ca and Mg uptake by grain legumes in western Canada. Therefore, there is a need to evaluate the concentration, uptake, and removal of these nutrients in soybean, and the impact on the concentration and uptake by following crops commonly grown on the soybean stubble in rotation. Through a two-year rotation experiment conducted at four sites in Saskatchewan, Canada, this chapter assesses the concentration and uptake of mineral nutrients, including K, Ca, Mg, S, Cu, and Zn, in the grain and the straw of soybean, pea, and lentil grown in the first study year (2014), and in wheat and canola grown on the stubble of these grain legumes in the second rotational year (2015). The objectives were (1) to evaluate the concentration and uptake of K, Ca, Mg, S, Cu, and Zn in the grain and the straw of soybean in comparison to pea and lentil, which are well-established grain legumes in western Canada, under Saskatchewan field conditions, and (2) to evaluate the concentration and uptake of these mineral elements in the grain and the straw of wheat and canola crops as affected by soybean, pea, and lentil stubbles from the previous year. Results of this study can provide information on relative soybean, pea, and lentil mineral nutrient (K, Ca, Mg, S, Cu, and Zn) content, requirements and removal under Saskatchewan soil-climatic conditions, and insight into requirements for fertilization in rotations with grain legumes followed by cereals and oilseeds.

#### **4.4 Materials and Methods**

##### **4.4.1 Site description and experimental design**

The experiment was conducted in 2014 and 2015 at four sites in south-central Saskatchewan, Canada, including sites located near Saskatoon, Scott, Rosthern, and Yorkton (as described in Chapter 3). Monthly cumulative precipitation and mean temperatures in the growing season of 2014 were close to or above the average level of the last 30 years, whereas the growing season of 2015 was drier, with the monthly cumulative precipitation lower than the historical mean in spring at the four sites (Table 3.4). To assess baseline soil nutrients before the experiment, pre-seeding composite samples from the 0-15cm depth of the soil profile were obtained from each site in May, 2014, by taking eight surface soil cores across the site area with a hand auger and combining the cores to produce a composite sample. Soil

samples were air-dried, sieved and the <2 mm fraction was retained and analyzed for various extractable nutrient levels and chemical properties, as described in Chapter 3 (Table 3.1).

The experimental design was a randomized complete block design (RCBD) with four replicates at each site. In 2014, three modern varieties of soybean, pea, and lentil were selected to represent varieties and classes commonly grown by producers at the time of the study. Grain and straw yield, N and P uptake by the three crops were quantified (see Chapter 3). One variety of each crop (TH3303R2Y soybean, CDC Meadow pea, and CDC Maxim lentil) was randomly selected to assess K, Ca, Mg, S, Cu, and Zn concentration and uptake in the grain and the straw as covered in this chapter. Information on selected varieties is provided in Table 4.1. Detailed information on seed treatment and seeding procedure was presented Chapter 3. Seeding at the four sites was carried out from the last week of May to the first week of June 2014 by the Crop Development Center, University of Saskatchewan according to site preparation and soil and weather conditions at each site. At each site, all crops were seeded on the same day (Table 3.3). Plot size for each crop and location was 3.66m × 1.4m with 3 rows per plot. Seeding rates were 65 seeds m<sup>-2</sup> for soybean, 86 seeds m<sup>-2</sup> for pea, and 129 seeds m<sup>-2</sup> for lentil. No fertilizers were applied for any of the crops, as significant deficiencies were not identified by pre-seeding soil analysis (Table 3.1). Odyssey™ (imazamox-imazethapyr) was sprayed in the last week of June at each site for weed control.

In 2015, hard red spring wheat (*Triticum aestivum* L. cv. CDC Abound) was seeded in the second week of May on the stubble of soybean, pea, or lentil at the Rosthern, Saskatoon, and Scott sites by the Crop Development Centre, University of Saskatchewan. Seeding rate was 248 seeds m<sup>-2</sup>, and no fertilizers were applied at the three sites. At the Yorkton site, canola (*Brassica napus* L. cv. Nexera 1016RR) was seeded on the stubble of soybean, pea, or lentil, as this site was managed by a producer who normally follows a grain legume–canola rotation. At the Yorkton site, 55 kg ha<sup>-1</sup> of 11-52-0 fertilizer was applied in the seed-row at the time of seeding. Seeding dates were shown in Chapter 3 (Table 3.3). Imazamox-imazethapyr was sprayed across the plots at each site in the last week of May for weed control in wheat and glyphosate was used for weed control in canola.

**Table 4.1.** Crop varieties assessed for K, Ca, Mg, S, Cu, and Zn in 2014-2015.

<b>Crop</b>	<b>Variety</b>	<b>Market class</b>	<b>Breeder</b>	<b>Herbicide resistance</b>
Soybean	TH3303R2Y	Oilseed	Thunder Seeds	Group 2
Pea	CDC Meadow	Yellow	CDC	Group 2
Lentil	CDC Maxim	Small red	CDC	Group 2
Wheat	CDC Abound	Hard red	CDC	Group 2
Canola	Nexera 1016RR	Argentine hybrid	Dow AgroSciences	Glyphosate

#### 4.4.2 Plant and soil sampling

In fall 2014 and 2015, crops were harvested at physiological maturity by hand cutting and completely removing above-ground crop materials, including grain, straw, and leaves from the plot area. Harvesting dates for each site are shown in Table 3.3. Soybean was harvested later than pea and lentil due to its later maturity. Three square meters of above-ground crop samples were taken from each plot to estimate crop yield and for further plant nutrient analyses. Post-harvest soil samples were collected from the 0-15cm, 15-30cm and 30-60cm depths of each plot, with three soil cores taken per plot and the sample depths from each core combined to provide a composite sample (Table 3.8).

Plant samples were brought back to the field lab at the University of Saskatchewan, SK, Canada. All samples were dried at 30°C, and weighed. The crop samples were threshed using a stationary mechanical thresher. When threshing, grain and straw sub-samples were collected from each sample and the rest of the sample was used to determine grain yield and total biomass yield. After threshing, the residue component (stems and leaves) was collected from each sample and bagged, and returned and spread evenly across each plot from which it was harvested, followed by a light rotary tilling in order to anchor the residue in place. Residue was spread evenly over the plot area using a rake, and a plastic frame was used to constrain the residue within the plot area when conducting residue incorporation (Fig. 3.1).

Analysis of nutrient elements was conducted at the Soil Science Department, University of Saskatchewan, Canada. Collected sub-samples were ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) to pass a 2mm sieve before being analyzed. Grain and straw mineral nutrient content was determined by using a nitric acid digestion as described in the United States Environmental Protection Agency method 3050A (USEPA, 1992), and concentration of K, Ca, Mg, Cu, and Zn in the digests was analyzed using flame atomic absorption spectrometry (Varian Spectra 220 Atomic Absorption Spectrometer; Varian Inc.,

Palo Alto, CA, USA). Grain and straw S content was determined using a LECO S-144DR Sulfur Analyzer (LECO Corporation, St. Joseph, MI, USA). Quality assurance was completed by the inclusion of a National Institute of Standards and Technology (NIST) certified plant material standard of known concentration every 40 samples, and ensuring that the sample value for mineral element concentration obtained was within 5% of the NIST certified value.

Soil composite samples collected were air-dried, sieved and the <2 mm fraction was analyzed for various extractable nutrient levels and chemical properties. Soil pH and electrical conductivity (EC) were measured in a 1:2 soil:water suspension (Nelson and Sommers, 1982). Soil nitrate ( $\text{NO}_3^-$ ) and sulphate ( $\text{SO}_4^{2-}$ ) were extracted using 0.01M  $\text{CaCl}_2$  extraction methodology described by Houba et al. (2000). Automated colorimetry was used to analyze the extracts for  $\text{NO}_3\text{-N}$  and microwave induced plasma spectroscopy (Agilent Technologies MP-AES 4100) for  $\text{SO}_4\text{-S}$ . Available P and K were measured on the soil depth sample of 0-15cm using a modified Kelowna extraction procedure (Qian et al., 1994). Extracts were colorimetrically analyzed for P using a Technicon Auto-analyzer II segmented flow automated system (Technicon Industrial Systems, Tarrytown, NY, USA). Concentration of K, Ca, and Mg in the extracts was analyzed using flame atomic absorption (Varian Spectra 220 Atomic Absorption Spectrometer; Varian Inc., Palo Alto, CA, USA). Plant available Cu and Zn were extracted from samples using a 0.005M diethylene-triamine-penta acetic acid (DTPA) solution (Lindsay and Norvell, 1978).

#### 4.4.3 Statistical analysis

Grain and straw uptake of mineral nutrients was calculated by multiplying nutrient concentration by corresponding grain and straw yield (see Chapter 3). Data were analyzed using SAS 9.4 for Windows, and a significance level of 0.05 was used. Distribution normality of the data was tested using the Kolmogorov-Smirnov test by the PROC UNIVARIATE procedure, and homogeneity of variance was tested using the Levene's test. Initially, effects of crop species, site, and their interaction on selected crop parameters were analyzed in a two-way ANOVA. Comparison of means at each sites was conducted using the PROC GLM procedure. The Tukey HSD method was used to conduct multiple comparisons among group means.

## 4.5 Results

### 4.5.1 Mineral nutrient concentration in the grain and straw of soybean, pea, and lentil

The analysis of variance (Table 4.2) showed that the effect of crop species was significant in affecting the concentration of grain and straw K, Ca, Mg, S, Cu, and Zn except straw Zn. Site also affected grain and straw nutrient concentration significantly except grain Mg concentration. The interaction between crop species × site was less important for mineral nutrient concentration in the grain than in the straw. The crop × site interaction was significant for grain Ca concentration, and straw K, Ca, Mg, and S concentration. Overall, crop species and site were significant factors affecting grain and straw mineral nutrients, whereas the interaction between these two factors was more significant in affecting evaluated nutrient content in the straw than in the grain.

**Table 4.2.** Summary of ANOVA and significance of variables of K, Ca, Mg, S, Cu, and Zn in the grain and the straw of soybean, pea, and lentil in 2014.

Source	df	g kg <sup>-1</sup>				mg kg <sup>-1</sup>	
		K	Ca	Mg	S	Cu	Zn
		----- <b>Grain</b> -----					
Crop	2	***	***	***	***	***	**
Site	3	***	***	ns	***	***	***
Crop × site	6	ns <sup>†</sup>	***	ns	ns	ns	ns
		----- <b>Straw</b> -----					
Crop	2	***	***	***	***	***	ns
Site	3	***	**	**	***	***	***
Crop × site	6	***	*	ns	***	*	ns

\*, \*\*, and \*\*\* Significant at  $p < 0.05$ ,  $P < 0.01$ , and  $p < 0.001$ , respectively, according to a Tukey's HSD test.

<sup>†</sup> Not significant ( $p \geq 0.05$ ) according to a Tukey's HSD test.

Soybean had significantly higher grain concentration of K, Ca, Mg, and S than pea and lentil across the sites, with the only exception being that lentil had similar grain S content (3.2 g kg<sup>-1</sup>) to soybean (3.5 g kg<sup>-1</sup>) at the Saskatoon site (Table 4.3). This crop difference in grain mineral nutrient content was most evident in Ca, for which soybean grain Ca concentration was about 3 times higher than that in pea or lentil. Pea and lentil generally had similar grain K, Ca, and S concentration, except that pea had higher grain K concentration at the Saskatoon site. Pea had higher grain Mg concentration than lentil at three of the four sites, whereas the two crops had similar grain Mg concentration at the Rosthern site. For grain Cu and Zn, soybean, pea, and lentil did not show consistent differences across the sites. Crops at the Saskatoon site had high grain S, consistent with high pre-seeding soil available sulfate at this site (Table 3.1). In addition, pea and lentil at the Saskatoon site had almost twice the grain Zn concentration (61.1 mg kg<sup>-1</sup> in pea and 58.9 mg kg<sup>-1</sup> in lentil) as crops at other sites. This site had the highest soil extractable available Zn in pre-seeding surface (0 - 15cm) soil (Table 3.1).

Nutrient concentration in the straw of soybean, pea, and lentil (Table 4.3) had larger variation (wider ranges for each crop) and showed different patterns compared to grain mineral nutrients, with soybean having similar to high straw mineral nutrient content compared to pea and lentil. Differences in the straw nutrient concentration of soybean, pea, and lentil were not consistent across the sites, and at the Saskatoon site all crops had much higher straw S and Zn concentration than at other sites. Overall, soybean generally had the highest K, Ca, Mg, S, Cu, and Zn concentration in the straw at the Rosthern and Saskatoon sites, except S (1.7 g kg<sup>-1</sup>) at the Saskatoon site, where pea had the highest straw S concentration (10.4 g kg<sup>-1</sup>). At the Scott and Yorkton sites, straw nutrient differences were rather inconsistent among crops.

Compared to the grain, the straw generally had similar K concentration, high Ca and Mg concentration, and lower S, Cu, and Zn concentration. In addition, partitioning of mineral nutrients between the grain and the straw also differed with different nutrients and crop species. At the Saskatoon site, the S concentration was higher in soybean grain than in pea grain, while in the straw soybean had only about 16% of the S concentration found in pea. At the Scott site, similarly, compared to pea and lentil, the S concentration was the highest in soybean grain.

**Table 4.3.** K, Ca, Mg, S, Cu, and Zn concentration (n=4) in the grain and the straw of soybean, pea, and lentil in 2014.

Site	Crop	K	Ca	Mg	S	Cu	Zn
		g kg <sup>-1</sup>				mg kg <sup>-1</sup>	
		<b>Grain</b>					
Rosthern	Soybean	16.8 a <sup>†</sup>	2.7 a	2.6 a	3.2 a	14.8 a	30.7 a
	Pea	10.3 b	0.8 b	1.2 b	1.8 b	9.2 b	22.6 b
	Lentil	9.5 b	0.9 b	1.1 b	1.9 b	11.0 b	31.7 a
Saskatoon	Soybean	19.6 a	2.3 a	2.6 a	3.5 a	10.2 a	44.4 b
	Pea	14.0 b	0.7 b	1.5 b	2.5 b	5.9 a	58.9 a
	Lentil	12.1 c	0.9 b	1.1 c	3.2 ab	7.3 a	61.1 a
Scott	Soybean	19.5 a	2.1 a	2.7 a	3.2 a	14.0 a	33.9 ab
	Pea	13.8 b	0.7 b	1.4 b	1.9 b	10.2 a	38.0 a
	Lentil	11.2 b	0.8 b	1.0 c	2.3 b	9.7 a	30.3 b
Yorkton	Soybean	20.3 a	2.5 a	2.7 a	3.3 a	11.2 ab	31.8 ab
	Pea	11.7 b	0.8 b	1.4 b	1.7 b	8.2 b	29.0 b
	Lentil	11.7 b	1.0 b	1.2 c	2.0 b	12.1 a	35.8 a
		<b>Straw</b>					
Rosthern	Soybean	11.3 a	13.0 a	6.1 a	0.4 a	2.6 ab	9.4 a
	Pea	10.7 a	15.7 a	3.1 b	0.6 a	1.1 b	5.3 a
	Lentil	13.0 a	5.9 b	1.8 b	0.7 a	4.5 a	13.0 a
Saskatoon	Soybean	16.8 a	12.8 a	6.2 a	1.7 b	4.2 ab	53.3 a
	Pea	5.8 b	14.9 a	4.5 a	10.4 a	3.3 b	71.0 a
	Lentil	12.8 a	7.7 b	3.5 a	6.8 ab	5.1 a	47.2 a
Scott	Soybean	11.6 c	7.2 b	4.8 a	0.2 b	3.3 c	5.5 b
	Pea	18.1 a	14.4 a	3.9 a	1.8 ab	6.7 b	13.1 a
	Lentil	15.3 b	8.3 b	2.4 b	1.9 a	8.7 a	15.5 a
Yorkton	Soybean	11.3 ab	12.1 b	4.5 a	0.2 c	2.9 a	9.0 b
	Pea	6.4 b	18.9 a	2.1 b	0.6 b	2.7 a	18.7 ab
	Lentil	14.4 a	11.1 b	1.9 b	0.9 a	6.2 a	22.1 a

<sup>†</sup> Within a column, means within a site for the same component followed by the same letter are not significantly different from each other (p≥0.05) according to a Tukey's HSD test.



#### 4.5.2 Uptake of mineral nutrients by soybean, pea, and lentil

Soybean, pea, and lentil had similar grain K uptake at the Scott and Yorkton sites, respectively, while at other sites, pea had the greatest grain K removal and lentil had the least (Table 4.4). At the Rosthern site, pea had the most grain K removal, followed by soybean, and lentil had the least grain K removal. At the Saskatoon site, soybean and pea had similar grain K removal, while lentil had less grain K removal than soybean and pea. Uptake of K was similar in soybean, pea, and lentil straw at the Rosthern site. At the Saskatoon site, K uptake in was more than fivefold in soybean straw as in pea straw and in lentil straw. At the Scott site, soybean straw N uptake was less than pea but similar to lentil, with the latter two crops having similar K uptake in the straw. At the Yorkton site, soybean and lentil had similar straw K uptake, whereas pea had significantly less K uptake in the straw than soybean and lentil.

Lack of soil Ca has not been identified as a concern in pulse production in western Canada, but no information has been collected on plant uptake and partitioning between the grain and the straw. In the present study, grain Ca removal showed consistent differences among the pulse crops across the four sites (Table 4.4). Soybean grain Ca uptake was significantly higher than pea and lentil, with pea and lentil having similar grain Ca removal at all sites. Straw Ca uptake in the three grain legumes was about tenfold higher than grain Ca across the sites, such that harvesting and removal of straw would have a much greater effect on soil Ca fertility than grain harvest, while on the Prairies soil Ca depletion is not a concern. At the Rosthern site, soybean and pea had similar straw Ca uptake that was more than twice as much as that of lentil. At the Saskatoon site, straw Ca uptake by soybean was 1.5 times greater than by pea, and more than 5 times that by lentil. At the Scott and Yorkton sites, soybean and lentil had similar straw Ca uptake, and pea had significantly more straw Ca uptake than soybean and lentil. Overall, most of the Ca taken up by the three crops during the growing season is retained in the straw and would be returned to the soil via residue, provided crop residue was not taken away from the field after harvest.

**Table 4.4.** K, Ca, Mg, S, Cu, and Zn uptake (n=4) in soybean, pea, and lentil in 2014.

Site	Crop	kg ha <sup>-1</sup>					
		K	Ca	Mg	S	Cu	Zn
		----- Grain -----					
Rosthern	Soybean	44.6 b <sup>†</sup>	7.2 a	6.8 a	8.5 ab	0.04 b	0.08 b
	Pea	62.9 a	5.1 b	7.1 a	10.6 a	0.06 a	0.14 a
	Lentil	26.2 c	2.4 c	3.1 b	5.3 b	0.03 b	0.09 b
Saskatoon	Soybean	18.2 ab	2.2 a	2.4 a	3.2 a	0.01 a	0.04 b
	Pea	22.7 a	1.2 b	2.5 a	4.0 a	0.01 a	0.10 a
	Lentil	13.3 b	1.0 b	1.2 b	3.4 a	0.01 a	0.07 ab
Scott	Soybean	61.5 a	6.4 a	8.0 a	9.9 a	0.04 a	0.11 a
	Pea	41.0 a	2.1 b	4.3 b	5.6 a	0.03 ab	0.11 a
	Lentil	26.6 a	2.0 b	2.4 b	5.4 a	0.02 b	0.07 a
Yorkton	Soybean	65.3 a	8.1 a	8.6 a	10.7 a	0.04 a	0.10 a
	Pea	18.5 a	1.1 b	2.3 b	2.6 b	0.01 b	0.05 b
	Lentil	25.0 a	2.3 b	2.6 b	4.2 b	0.03 a	0.08 ab
		----- Straw -----					
Rosthern	Soybean	76.2 a	86.8 a	40.8 a	2.4 a	0.02 a	0.06 a
	Pea	58.2 a	85.5 a	16.9 b	3.4 a	0.01 a	0.03 a
	Lentil	75.0 a	34.3 b	10.5 b	3.9 a	0.03 a	0.08 a
Saskatoon	Soybean	97.7 a	74.3 a	36.1 a	10.0 b	0.02 a	0.31 a
	Pea	18.1 b	45.1 b	13.7 b	31.0 a	0.01 b	0.23 ab
	Lentil	25.0 b	14.5 c	6.0 b	11.2 b	0.01 b	0.09 b
Scott	Soybean	81.1 b	51.1 b	33.5 a	1.4 a	0.02 b	0.04 b
	Pea	111.9 a	88.7 a	24.0 ab	11.0 a	0.04 a	0.08 a
	Lentil	86.6 ab	47.7 b	14.1 b	10.7 a	0.05 a	0.09 a
Yorkton	Soybean	56.8 ab	60.7 b	22.7 a	1.2 b	0.02 a	0.05 b
	Pea	36.3 b	105.4 a	11.8 b	3.6 a	0.01 a	0.10 ab
	Lentil	69.3 a	53.7 b	9.3 b	4.6 a	0.03 a	0.11 a

<sup>†</sup> Within a column, means within a site for the same component followed by the same letter are not significantly different from each other (p≥0.05) according to a Tukey's HSD test.

**Table 4.5.** Harvest index of K, Ca, Mg, S, Cu, and Zn <sup>†</sup> (n=4) of soybean, pea, and lentil in 2014.

Site	Crop	K	Ca	Mg	S	Cu	Zn
Rosthern	Soybean	0.37 b <sup>‡</sup>	0.08 a	0.14 c	0.79 a	0.71 ab	0.58 b
	Pea	0.53 a	0.05 a	0.29 a	0.76 a	0.91 a	0.83 a
	Lentil	0.26 b	0.06 a	0.22 b	0.58 a	0.54 b	0.55 b
Saskatoon	Soybean	0.15 c	0.03 b	0.06 b	0.29 a	0.28 a	0.12 b
	Pea	0.57 a	0.03 b	0.18 a	0.13 a	0.46 a	0.33 a
	Lentil	0.37 b	0.06 a	0.16 a	0.23 a	0.45 a	0.44 a
Scott	Soybean	0.41 a	0.11 a	0.19 a	0.89 a	0.64 a	0.72 a
	Pea	0.26 b	0.02 b	0.15 a	0.37 b	0.41 b	0.57 ab
	Lentil	0.23 b	0.04 b	0.15 a	0.36 b	0.32 b	0.45 b
Yorkton	Soybean	0.53 a	0.12 a	0.28 a	0.90 a	0.71 a	0.69 a
	Pea	0.34 ab	0.01 b	0.15 a	0.41 b	0.48 a	0.32 b
	Lentil	0.26 b	0.04 b	0.22 a	0.48 b	0.48 a	0.41 b

<sup>†</sup> The ratio of grain uptake:total above-ground uptake.

<sup>‡</sup> Within a column, means within a site for the same component followed by the same letter are not significantly different from each other (p≥0.05) according to a Tukey's HSD test.

Similar to Ca, data on Mg uptake by grain legumes is not available for western Canada. At the Rosthern and Saskatoon sites, soybean and pea had similar grain Mg uptake, which was about twice as much as that of lentil (Table 4.4). At the Scott and Yorkton sites, grain Mg uptake by soybean was about twice that by pea and lentil, consistent with grain Mg concentration patterns of the three crops. For the straw, similarly, soybean had the most straw Mg uptake compared to pea and lentil across the sites, with the only exception being that at the Scott site pea had similar straw Mg uptake to soybean. Overall, straw Mg uptake was about two to five times the grain Mg uptake for the three crops evaluated, and total above-ground Mg uptake was the most in soybean and least in lentil across the sites.

Soybean, pea, and lentil had similar S uptake in the grain at the Saskatoon and Scott sites, respectively (Table 4.4). At the Rosthern site, soybean and pea had similar grain S removal, while the grain S removal by lentil was the lowest. At the Yorkton site, soybean had significantly higher grain S removal than pea and lentil. Compared to grain S uptake, straw S uptake in the crops had more variation. At the Rosthern and Scott sites, the straw S uptake was not significantly different among the three pulse crops, while at the Saskatoon site, pea had about three times the straw S uptake than soybean and lentil did. At the Yorkton site, soybean had significantly less straw S uptake than pea and lentil was observed. Crops at the Saskatoon site generally had rather high straw S uptake but low grain S uptake compared to crops at other sites, possibly due to the high soil S concentration associated with sulfate salts but poorer grain yield at the Saskatoon site (see Chapter 3), which was attributed to the salinity occurring within the root zone due to a high water table in the 2014 growing season at this site.

Soybean and lentil had similar grain Cu removal at the Rosthern site, while had significantly higher grain Cu uptake than soybean and lentil, reflecting the superior grain yield of pea at the site. At the Saskatoon site, soybean, pea, and lentil had similar Cu in the grain. At the Scott and Yorkton sites, however, soybean had the most grain Cu uptake, while lentil had the least at the Scott site and pea had the least at the Yorkton site. Unlike K, Ca, Mg, or S, partitioning of Cu in the grain and straw was relatively evenly, and the three crops had similar straw uptake at the Rosthern and Yorkton sites, ranging from 0.01 kg ha<sup>-1</sup> to 0.03 kg ha<sup>-1</sup>. At the Saskatoon site, soybean had higher straw Cu uptake than pea and lentil, while at the Scott site soybean had lower straw uptake than pea and lentil. This pattern was consistent with the Cu

concentration in the straw of the three crops. Overall, Cu uptake was generally similar among soybean, pea, and lentil at the study sites at 2014 harvest.

Patterns of Zn uptake in the grain of soybean, pea, and lentil were inconsistent across the sites (Table 4.4). At the Rosthern site, pea had higher grain Zn uptake compared to soybean and lentil, consistent with the high grain yield of pea at this site. At the Saskatoon site, soybean had a lower grain Zn removal than pea, and lentil had similar grain Zn uptake to soybean and pea. At the Scott site, the three crops had similar grain Zn removal, ranging from 0.07-0.11 kg ha<sup>-1</sup>. At the Yorkton site, soybean and lentil had similar grain Zn removal, whereas pea had significant less grain Zn. Similar to Cu uptake, Zn partitioning in the three crops was relatively even, except at the Saskatoon site, where the Zn in soybean straw was almost 8 times of that in soybean grain, and the Zn in pea straw was more than twice as much as that in pea grain. At the Rosthern site, soybean, pea, lentil had similar straw Zn uptake, ranging within 0.03-0.08 kg ha<sup>-1</sup>, while at the Scott and Yorkton sites soybean had the least straw Zn compared to pea and lentil.

Partitioning of nutrients is indicated in the harvest index, which is the proportion of grain uptake to total above-ground uptake in crops (Table 4.5). In general, soybean had higher harvest K, Ca, Mg, S, Cu, and Zn indexes at the Scott and Yorkton sites, and lentil had significantly lower harvest nutrient indexes, except the harvest Mg index, of which soybean, pea, and lentil had similar levels at the two sites (0.15-0.19 at the Scott site and 0.15-0.28 at the Yorkton site). Harvest S indexes of soybean, pea, and lentil were similar at two sites, ranging within 0.58-0.79 at the Rosthern site and 0.13-0.29 at the Saskatoon site, while at the Scott and Yorkton sites, harvest S indexes of soybean were about twice of that of pea or lentil. Overall, harvest nutrient indexes in soybean, pea, and lentil were highest for S and Cu, intermediate for K and Zn, low for Mg, and extremely low for Ca across the sites.

#### 4.5.3 Mineral nutrients in crops grown on soybean, pea, and lentil stubble

The analysis of variance showed that site had substantial effects on nutrient concentration in the grain and the straw of wheat except straw K and straw Cu in 2015, while crop stubbles from the previous year only significantly affected grain K and straw Zn concentration in wheat grown on the stubbles (Table 4.6). The interaction between stubble × site did not significantly affect the grain or straw nutrient concentration in wheat.

Wheat grown on the soybean, pea, or lentil stubble generally had similar grain K, Ca, Mg, S, Cu, and Zn concentration at each of the Rosthern, Saskatoon, and Scott sites in 2015 (Table 4.7), with the only two exceptions that at the Saskatoon and Scott sites, wheat on the soybean stubble had significantly higher grain K concentration (6.0 g kg<sup>-1</sup> at the Saskatoon site and 5.3 g kg<sup>-1</sup> at the Scott site) than wheat on the pea (5.5 g kg<sup>-1</sup> at the Saskatoon site and 4.9 g kg<sup>-1</sup> at the Scott site) and lentil (5.5 g kg<sup>-1</sup> at the Saskatoon site and 4.9 g kg<sup>-1</sup> at the Scott site) stubbles. Other than grain K concentration at these two sites, concentration of evaluated elements was rather similar among different legume stubble treatments at each site. For canola grown at the Yorkton site, grain K, Ca, Mg, S, Cu, and Zn concentration was similar among different legume stubble treatments. Similarly, the straw of wheat and canola did not show response to different legume stubbles in this study at any of the sites.

**Table 4.6.** Summary of ANOVA and significance of variables of K, Ca, Mg, S, Cu, and Zn in the grain and the straw of wheat in 2015.

Source	df	g kg <sup>-1</sup>				mg kg <sup>-1</sup>			
		K	Ca	Mg	S	Cu	Zn		
		-----				<b>Grain</b>		-----	
Crop	2	***	ns	ns	ns	ns	ns	ns	
Site <sup>‡</sup>	2	***	*	***	***	**	***	***	
Crop × site	4	ns	ns	ns	ns	ns	ns	ns	
		-----				<b>Straw</b>		-----	
Crop	2	ns	ns	ns	ns	ns	ns	*	
Site	2	ns	**	***	**	ns	ns	***	
Crop × site	4	ns	ns	ns	ns	ns	ns	ns	

\*, \*\*, and \*\*\* Significant at  $p < 0.05$ ,  $P < 0.01$ , and  $p < 0.001$ , respectively, according to a Tukey's HSD test.

<sup>†</sup> Not significant ( $p \geq 0.05$ ) according to a Tukey's HSD test.

<sup>‡</sup> Includes the Rosthern, Saskatoon, and Scott sites.

**Table 4.7.** K, Ca, Mg, S, Cu, and Zn concentration (n=4) in the grain and the straw of wheat at the Rosthern, Saskatoon, and Scott sites, and of canola at the Yorkton site in 2015.

Site	Stubble	g kg <sup>-1</sup>				mg kg <sup>-1</sup>			
		K	Ca	Mg	S	Cu	Zn		
		-----				<b>Grain</b>		-----	
Rosthern	Soybean	6.3 a <sup>†</sup>	0.2 a	2.4 a	2.0 a	5.9 a	37.4 a		
	Pea	6.2 a	0.2 a	2.3 a	2.0 a	6.3 a	35.7 a		
	Lentil	5.7 a	0.2 a	2.3 a	2.0 a	6.8 a	36.3 a		
Saskatoon	Soybean	6.0 a	0.3 a	2.2 a	2.3 a	6.1 a	59.8 a		
	Pea	5.5 b	0.2 a	2.1 a	2.3 a	6.7 a	55.9 a		
	Lentil	5.5 b	0.2 a	2.1 a	2.3 a	6.2 a	56.7 a		
Scott	Soybean	5.3 a	0.2 a	2.2 a	2.4 a	8.2 a	39.4 a		
	Pea	4.9 b	0.2 a	2.1 a	2.4 a	8.3 a	42.7 a		
	Lentil	4.9 b	0.2 a	2.1 a	2.2 a	8.6 a	40.1 a		
Yorkton	Soybean	10.4 a	4.4 a	4.1 a	4.8 a	8.3 a	30.9 a		
	Pea	10.0 a	4.2 a	4.0 a	4.9 a	7.2 a	30.4 a		
	Lentil	9.4 a	4.1 a	3.9 a	4.7 a	7.6 a	30.2 a		
		-----				<b>Straw</b>		-----	
Rosthern	Soybean	21.8 a	1.6 a	1.3 a	1.5 a	24.5 a	17.8 a		
	Pea	22.5 a	1.6 a	1.3 a	1.1 a	25.2 a	17.7 a		
	Lentil	20.4 a	1.6 a	1.4 a	0.7 a	9.0 a	10.8 a		
Saskatoon	Soybean	23.5 a	1.5 a	1.2 a	1.8 a	22.6 a	36.9 a		
	Pea	22.4 a	1.6 a	1.7 a	2.6 a	25.9 a	33.2 a		
	Lentil	23.6 a	1.6 a	1.5 a	2.1 a	12.5 a	27.4 a		
Scott	Soybean	26.0 a	1.7 a	2.0 a	1.7 a	21.1 a	16.4 a		
	Pea	26.6 a	1.8 a	2.0 a	1.9 a	28.6 a	20.5 a		
	Lentil	23.2 a	2.0 a	2.0 a	1.9 a	14.3 a	13.1 a		
Yorkton	Soybean	19.8 a	6.5 a	1.0 a	2.5 a	2.1 a	4.6 a		
	Pea	19.3 a	6.9 a	1.2 a	2.5 a	4.3 a	5.8 a		
	Lentil	18.7 a	6.1 a	0.9 a	1.7 a	3.4 a	4.2 a		

<sup>†</sup> Within a column, means within a site for the same component followed by the same letter are not significantly different from each other ( $p \geq 0.05$ ) according to a Tukey's HSD test.

#### 4.5.4 Uptake of mineral nutrients by wheat and canola in the second year

Grain uptake of nutrients, including K, Ca, Mg, S, Cu, and Zn, of wheat in 2015 was basically similar on different legumes stubbles at the Rosthern, Saskatoon, and Scott sites (Table 4.8). Exceptions included that at the Rosthern site, wheat on the soybean stubble had significantly lower grain S removal than wheat on the lentil stubble, with wheat on the pea stubble having an intermediate grain S removal. At the Yorkton site, canola grown on the soybean, pea, or lentil stubble had similar grain Ca uptake, Mg, S, and Cu, whereas for K and Zn, canola on the soybean and pea stubbles had significantly lower grain K removal than canola grown on the lentil stubble. Similar to grain nutrient uptake, straw uptake of K, Ca, Mg, S, Cu, and Zn on the soybean stubble was similar to that on pea and lentil stubbles for both wheat and canola at the four sites.

At the Rosthern, Saskatoon, and Scott sites, wheat straw K uptake was about four times of grain K, and straw Ca was about nine times of grain Ca. Similar, wheat straw Cu uptake was 2-3 times that of grain Cu across the sites. On the other hand, for Mg, S, and Zn, wheat had relatively even partitioning between the grain and straw. Canola at the Yorkton site had different partitioning patterns from wheat, with straw K being more than five times grain K uptake, straw Ca about 4 times of grain Ca uptake, and straw Zn being less than half of grain. The grain and straw of canola had relatively similar of Mg, S, and Cu uptake.

Overall, harvest nutrient indexes of wheat and canola in 2015 did not show responses to different legume stubbles from the previous year (Table 4.9). Wheat had relatively low harvest indexes of K, Ca, and Cu, reflecting that relatively small portions of plant K, Ca, and Cu are exported out of the plant-soil system at grain harvest. Harvest Cu indexes of canola were relatively high compared to that of wheat, implying the impact of crop species on Cu partitioning between different plant components.

**Table 4.8.** K, Ca, Mg, S, Cu, and Zn uptake (n=4) in wheat at the Rosthern, Saskatoon, and Scott sites, and of canola at the Yorkton site in 2015.

Site	Stubble	K	Ca	Mg	S	Cu	Zn
		kg ha <sup>-1</sup>					
		----- <b>Grain</b> -----					
Rosthern	Soybean	18.1 a <sup>†</sup>	0.6 a	7.0 a	5.8 b	0.02 a	0.11 a
	Pea	19.6 a	0.7 a	7.4 a	6.4 ab	0.02 a	0.11 a
	Lentil	21.2 a	0.8 a	8.5 a	7.6 a	0.03 a	0.14 a
Saskatoon	Soybean	15.8 a	0.7 a	5.7 a	5.9 a	0.02 a	0.16 a
	Pea	16.1 a	0.6 a	6.2 a	6.7 a	0.02 a	0.16 a
	Lentil	16.0 a	0.7 a	6.0 a	6.5 a	0.02 a	0.16 a
Scott	Soybean	20.0 a	0.7 a	8.4 a	9.1 a	0.03 a	0.15 a
	Pea	21.2 a	0.8 a	9.0 a	10.2 a	0.04 a	0.19 a
	Lentil	21.9 a	0.9 a	9.4 a	10.0 a	0.04 a	0.18 a
Yorkton	Soybean	18.0 b	7.6 a	7.2 a	8.5 a	0.02 a	0.05 b
	Pea	17.8 b	7.5 a	7.2 a	8.8 a	0.01 a	0.05 b
	Lentil	22.1 a	9.5 a	9.2 a	11.1 a	0.02 a	0.07 a
		----- <b>Straw</b> -----					
Rosthern	Soybean	74.0 a	5.6 a	4.0 a	5.0 a	0.08 a	0.06 a
	Pea	84.5 a	5.9 a	4.7 a	4.0 a	0.10 a	0.07 a
	Lentil	82.5 a	6.6 a	5.7 a	2.6 a	0.04 a	0.04 a
Saskatoon	Soybean	64.1 a	3.9 a	3.3 a	5.0 a	0.06 a	0.10 a
	Pea	67.1 a	4.7 a	5.1 a	7.9 a	0.08 a	0.10 a
	Lentil	68.1 a	4.5 a	4.3 a	6.0 a	0.04 a	0.08 a
Scott	Soybean	77.5 a	5.2 a	5.7 a	5.0 a	0.07 a	0.05 a
	Pea	92.4 a	5.8 a	6.8 a	6.4 a	0.09 a	0.07 a
	Lentil	79.6 a	6.7 a	6.8 a	6.2 a	0.05 a	0.04 a
Yorkton	Soybean	104.3 a	33.7 a	5.4 a	12.9 a	0.01 a	0.02 a
	Pea	97.5 a	34.7 a	5.9 a	12.7 a	0.02 a	0.03 a
	Lentil	119.5 a	38.7 a	5.8 a	10.9 a	0.02 a	0.03 a

<sup>†</sup> Within a column, means within a site for the same component followed by the same letter are not significantly different from each other (p≥0.05) according to a Tukey's HSD test.



**Table 4.9.** Harvest index<sup>†</sup> for K, Ca, Mg, S, Cu, and Zn (n=4) of wheat at the Rosthern, Saskatoon, and Scott sites, and of canola at the Yorkton site in 2015.

Site	Stubble	K	Ca	Mg	S	Cu	Zn
Rosthern	Soybean	0.20 a <sup>‡</sup>	0.10 a	0.62 a	0.57 a	0.23 a	0.65 a
	Pea	0.19 a	0.10 a	0.61 a	0.62 a	0.20 a	0.63 a
	Lentil	0.21 a	0.11 a	0.60 a	0.75 a	0.44 a	0.76 a
Saskatoon	Soybean	0.20 a	0.16 a	0.63 a	0.56 a	0.28 a	0.61 a
	Pea	0.20 a	0.11 a	0.56 a	0.48 a	0.26 a	0.63 a
	Lentil	0.19 a	0.13 a	0.59 a	0.54 a	0.36 a	0.68 a
Scott	Soybean	0.21 a	0.13 a	0.59 a	0.64 a	0.37 a	0.76 a
	Pea	0.21 a	0.13 a	0.58 a	0.63 a	0.36 a	0.75 a
	Lentil	0.22 a	0.12 a	0.58 a	0.61 a	0.48 a	0.80 a
Yorkton	Soybean	0.15 a	0.18 a	0.58 a	0.41 a	0.57 a	0.70 a
	Pea	0.16 a	0.18 a	0.56 a	0.41 a	0.40 b	0.66 a
	Lentil	0.16 a	0.20 a	0.62 a	0.54 a	0.47 ab	0.73 a

<sup>†</sup> The ratio of grain uptake:total above-ground uptake.

<sup>‡</sup> Within a column, means within a site for the same component followed by the same letter are not significantly different from each other ( $p \geq 0.05$ ) according to a Tukey's HSD test.

#### 4.6 Discussion

Grain mineral content of pea in the present study falls within the range of results reported by Wang and Daun (2004), who examined mineral composition in four varieties of pea selected from western Canada, and showed that both variety and environmental conditions significantly affected grain K content, but only variety significantly affected grain Ca and Cu, and only environmental conditions had significant impact on grain Mg and Zn. Thavarajah et al. (2010) showed a positive effect of temperature on the grain Zn and Fe content of lentil, with 69 mg Zn kg<sup>-1</sup> and 116 mg Fe kg<sup>-1</sup> in the lentil seed in a higher temperature regime (Lucknow, India) as opposed to 61 mg Zn kg<sup>-1</sup> and 113 mg Fe kg<sup>-1</sup> in a lower temperature regime (Saskatoon, Canada), although in fact the difference seemed rather small. In addition, the present study showed a lower grain Zn concentration than 69 mg kg<sup>-1</sup>, with only lentil at the Saskatoon site having similar grain Zn, indicating that even under generally similar climatic-conditions (Saskatchewan), grain Zn concentration of lentil can still vary substantially. Indeed, the present study showed significant effects of site on all evaluated elements in the grain except for Mg, which is in line with findings of Ray et al. (2014), who suggested that site had substantial effects on grain minerals nutrients in Saskatchewan, making multi-locational tests necessary to accurately estimate mineral content in the grain legumes.

In the present study, the concentration of evaluated elements in the grain of soybean, pea, and lentil suggested that these legumes could be good sources of micronutrients for humans according to the recommended daily allowance (RDA) suggested by Health Canada and United States Department of Agriculture (USDA) (United States Department of Agriculture, 2016). For example, compared to the soybean nutrition reported in the USDA national nutrient database, soybean grown in Saskatchewan in the present study had slightly higher K content, similar Ca and Mg content, and slightly less Cu and Zn, indicating promising nutritional quality of soybean grown under Saskatchewan soil-climatic conditions. Deibert and Utter (1989) assessed two soybean varieties in North Dakota, and showed similar K concentration and uptake to in the present study. Therefore, significant removal of these elements from the soil system by soybean harvest can also be anticipated when soybean grain yield is high. In addition, soybean and pea generally had higher grain Ca uptake than lentil across the sites. For other mineral elements evaluated, pea and lentil generally showed similar levels in the grain across the sites. Overall, soybean, pea, and lentil had about twice the K and Cu concentration, and four

times the Ca concentration in the grain compared to wheat grain grown in the second year, reflecting the benefits of grain legumes in respect to evaluated nutritional minerals. In addition, the canola grown at the Yorkton site in the second rotational year generally showed higher levels of K, Ca, Mg, and S in the grain than wheat, with similar grain K, Cu, and Zn to the legumes, and higher levels of grain Ca, Mg, and S than the legumes grown in 2014.

Compared to the grain, the straw of soybean, pea, and lentil generally had higher levels of Ca and Mg, similar levels of K, and lower levels of S, Cu, and Zn. At the Saskatoon site, the straw of pea and lentil had unexpectedly high levels of S, and all three legumes had high straw Zn, possibly related to the poor drainage, a high water table, and sulfate salts that accumulated in the soil profile during the growing season at this site. This was consistent with the pre-seeding soil properties as shown in Table 3.1, which showed high levels of EC,  $\text{SO}_4^{2-}$ -S, and Zn. Patterns in partitioning between the grain and the straw varied from one mineral element to another. This, in turn, affected not only the nutritional quality of the crops, as the grain is used for human consumption, but also the amount of minerals returned to the soil via straw residue after harvest, provided that the straw residue is not exported from the field at harvest. The present study showed significant grain uptake and removal of S, Cu, and Zn as opposed to the straw uptake across the sites, suggesting larger removal of S, Cu, and Zn from the field. Indeed, harvest indexes of S, Cu, and Zn were generally around 50% or higher at three of the four sites, while harvest index for K was lower and for Ca was generally below 10% across the sites, implying that significant amounts of assimilated S, Cu, and Zn in the crop were exported via grain removal at harvest.

Differences in removal of these mineral elements related to crop species imply a need for different fertilization strategies when soybean, pea, and lentil are involved in rotation. Grain uptake and removal of K, Mg, and S that was frequently higher in soybean compared to pea and especially lentil suggest a greater potential for depletion of these elements in the soil over the long term when soybeans are grown over a long term. Site was an important factor so environmental and soil conditions will affect the removal and nutrient depletion potential, especially as it relates to yield. In addition to location-related factors, such as soil-climatic factors, agronomic practices including fertilization and crop rotation can affect the assimilation of mineral elements in crops. For example, Wang et al. (2008) reported that although N fertilization was vital for achieving satisfactory wheat yield and quality, excessive N

fertilization could lead to a decreasing Mg and Ca concentration in some crops, apart from increasing undesirable nitrate, titratable acidity, and acid to sugar ratio.

In 2015, wheat grown on the soybean stubble had higher grain K concentrations than that grown on the pea or lentil stubble at Saskatoon (9% higher on the soybean stubble) and Scott (8% higher on the soybean stubble), possibly due to the larger amount of K returned to soil in soybean straw, as indicated at the Saskatoon site. These findings point to the need to pay special attention to soil available K through soil testing when soybean is included in rotation over several years. In addition, although soybean had larger amount of straw Mg and Ca returned to soil in 2014 fall, neither wheat nor canola in 2015 showed response to different previous crops, probably because limitations of Mg and Ca are very rare for field crops in western Canada and thus the input difference among crop residue is not large enough to cause significant responses in crops grown in the subsequent year (Wang et al., 2008). Overall, soybean, pea, and lentil as previous crops produced similar impact on the following wheat and canola crops regarding K, Ca, Mg, S, Cu, and Zn in this study, while it is recommended that soil K and Mg levels be monitored when soybean is used in rotation due to its large removals of these elements.

#### **4.7 Conclusion**

Among the three pulse crops in this study, soybean grain had higher concentration of K, Ca, Mg and S than pea and lentil grain. The grain Cu and Zn concentration was similar among crops at a site. Straw mineral concentration showed inconsistent patterns among soybean, pea, and lentil. Soybean had consistently higher grain Ca removal than pea or lentil across the sites. Removal of K, Mg, and S in soybean grain was often higher or similar to pea, and typically higher than in lentil, suggesting a greater potential for depletion of these elements in the soil over the long term when soybean is grown. Site had some influence on the content of evaluated mineral elements in the harvest components of the grain legumes, especially in the straw, but generally crop type was a more important factor than site. In the subsequent year, wheat grown on the soybean stubble had higher grain K concentration at two of the four sites, but for other mineral elements evaluated, different stubbles from the previous year did not cause significant differences in the mineral concentration or uptake in the grain or straw of wheat or canola at any sites, indicating similar short-term rotational effects of soybean, pea, and lentil under Saskatchewan conditions in respect to K, Ca, Mg, S, Cu, and Zn.

## **5. BIOLOGICAL NITROGEN FIXATION BY SOYBEAN, PEA, AND LENTIL AS ESTIMATED BY THE <sup>15</sup>N DILUTION TECHNIQUE AND RECOVERY OF ABOVE-GROUND RESIDUE NITROGEN IN THE SUBSEQUENT CROP IN SASKATCHEWAN, CANADA**

### **5.1 Preface**

This chapter focuses on the N<sub>2</sub> fixation and N contribution from soybean above-ground residue to a following wheat crop in comparison to pea and lentil at the Rosthern site. As a grain legume, soybean is able to fix atmospheric N by forming symbiotic association with *Bradyrhizobium japonicum* bacteria and therefore, can meet at least part of its N requirement and potentially contribute to the soil N pool and subsequent crops in rotation. Therefore, information on N<sub>2</sub> fixation and the partitioning of fixed N in soybean is necessary for investigating the N benefits of soybean as a grain legume in crop rotations in comparison to pea and lentil. In addition, to investigate the N contribution of soybean above-ground residue to following crops in rotation in comparison to pea and lentil, this chapter also discusses the recovery of the above-ground residue N of soybean, pea, and lentil by a following spring wheat crop grown in the year after (2015). The <sup>15</sup>N dilution technique was used to measure the N<sub>2</sub> fixation soybean, pea, and lentil under field conditions, and the recovery of above ground residue N in above ground part of a following wheat crop.

### **5.2 Abstract**

Estimating soybean N<sub>2</sub> fixation, partitioning of fixed N, and residue N recovery by following crops is important in assessing the impact of soybean [*Glycine max* (L.) Merr.] production on N budgets and cycling. The objectives of the research described in this chapter were to (1) use the <sup>15</sup>N dilution technique to estimate the N<sub>2</sub> fixation and the partitioning of fixed N between the grain and the straw of a short-season soybean in comparison to pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.) under field conditions, and (2) to determine soybean

above-ground residue N recovery by a following spring wheat (*Triticum aestivum* L.) crop at a site in the Black soil zone of Saskatchewan. The grain N yield of soybean was lower than pea, while straw N yield was similar among the three crops. Percentage N derived from atmosphere (%Ndfa) was similar among the crops for both the grain (68-74%) and the straw (40-61%), with soybean having relatively more even partitioning between the grain and the straw compared to pea and lentil. The majority of total fixed N was retained in the grain, with 75% of it retained in the grain of soybean, 85% in pea, and 67% in lentil. The amount of fixed N comprised over 70% of total above ground N in soybean and over 60% in pea and lentil. In the subsequent year, wheat grown on soybean, pea, or lentil above-ground residues had similar yield and N uptake in both the grain and the straw, while wheat grown on the wheat crop residue had lower grain N uptake. Similarly, percentage of N derived from previous above-ground (straw) residue N in either the grain (8-13%) or the straw (9-12%) of a following wheat crop was not different among the legume residues. The total amount of N derived from residue in wheat grain was greater for wheat grown on lentil residue (13 kg ha<sup>-1</sup>) compared to pea (7 kg ha<sup>-1</sup>) and wheat (3 kg ha<sup>-1</sup>) residues. Overall, residue N recovery rates in the grain and the straw of wheat in the subsequent year were similar for the different pulse residues, indicating similar N benefits to following crops from soybean, pea, and lentil. The substantial amount of N yield and N<sub>2</sub> fixation by short-season soybean as well as residue N recovery in the following crop imply a significant external contribution to the soil N budget and availability, similar to pea and lentil, suggesting promising prospects for soybean production to contribute to N fertility under Saskatchewan soil-climatic conditions.

### **5.3 Introduction**

As an important macronutrient for crop nutrition and soil fertility, nitrogen (N) is added to agroecosystems mainly through application of commercial N fertilizers and N<sub>2</sub> fixation, while N added to soils via crop residue is generally considered as the internal N cycling within an agroecosystem (Janzen et al., 2003). Concerns have arisen related to application of inorganic N fertilizers, including the production of the greenhouse gas nitrous oxide (N<sub>2</sub>O), acidification caused by NH<sub>3</sub> deposition, water pollution by nitrate (NO<sub>3</sub><sup>-</sup>), and soil and water eutrophication (Mosier et al., 1998; Bouwman et al., 2013). These issues emerged because the application of inorganic N fertilizers normally occurs in a short time period, resulting in excessive inorganic N that is prone to losses in the soil system (Zhu and Chen, 2002). For leguminous crops, N<sub>2</sub>

fixation occurs via the symbiotic relationship between legumes and a group of soil bacteria known as rhizobia (Peoples et al., 1995b). Through the symbiotic relationship with soil rhizobia, legumes are able to partially meet their N demand for growth and reproduction, consequently taking up less N from the soil, and thus contributing to the soil N pool and to N nutrition of following crops in rotation (van Kessel and Hartley, 2000). Therefore, unlike inorganic N fertilization, N<sub>2</sub> fixation introduces atmospheric N into agroecosystems via accumulating N in legume biomass and exuding fixed N into the rhizosphere over the season possibly without causing environmental concerns related to excess inorganic N due to N fertilizer application (Crews and Peoples, 2004; Arcand et al., 2014b). In addition, decaying root cells and residue decomposition, including both above- and below-ground crop components, also contribute to the soil N pool in the year of growth and subsequent years (Gardner and Drinkwater, 2009; Lupwayi and Soon, 2009).

Through N<sub>2</sub> fixation processes, legumes are able to fix a substantial amount of N for their own use, and previous studies reported that 46-84% of the crop N yield in grain legumes was derived from the atmosphere (Ndfa) (Rennie et al., 1988; Walley et al., 2007; Yang et al., 2010). Salvagiotti et al. (2008) estimated that, on average, soybean [*Glycine max* (L.) Merr.] was able to meet upwards of 50-60% of its N demand by N<sub>2</sub> fixation. Although estimates of Ndfa vary considerably, generally legumes were reported to derive a substantial amount of N from fixation in the above-ground residue (Walley et al., 2007). Assessing N in the below-ground crop component is more difficult. Studies have reported that 11-56% of the total plant N is below-ground N in pulse crops (Khan et al., 2002; Anglade et al., 2015). In general, it was recognized that despite considerable proportion of Ndfa in the N yield of legume crops, removal of above-ground crop components, including grain and shoot residue, from the agroecosystems at harvest often resulted in a negative N-balance of the system (van Kessel and Hartley, 2000; Salvagiotti et al., 2008).

The N in crop residue left in the agroecosystems after harvest may be made available for crop uptake in following seasons through residue decomposition and mineralization, or be incorporated into the recalcitrant N pool, or be lost from the system through such pathways as denitrification and leaching (Janzen et al., 2003). Therefore, only a certain portion of legume residue N left in the field after harvest is available and recovered in crops in following years.

Indeed, the recovery of legume residue N from the previous rotational year by a subsequent crop was generally reported as 10-30% (Ehaliotis et al., 1998).

Quantification of N<sub>2</sub> fixation by legumes has been conducted using <sup>15</sup>N isotope dilution techniques because of its power to discriminate between soil-derived N and atmospheric N. In this method, <sup>15</sup>N-labelled fertilizers are applied to N-fixing crops, a corresponding non-fixing crop is chosen to serve as a reference crop, and the atom% <sup>15</sup>N of the N-fixing crops and the reference crop is used to calculate the N<sub>2</sub> fixation of the crops of interest (Fried and Middelboe, 1977; Chalk, 1985). The reference crop is crucial in obtaining accurate estimates of N<sub>2</sub> fixation, as the success of this technique relies on the similarity in the relative availability of soil N and fertilizer N to the non-fixing reference crop compared to the legume (Chalk, 1985). An ideal reference crop should have similar rooting patterns and N uptake profiles through the growing season to the legume crops of interest (Witty, 1983). Besides measuring N<sub>2</sub> fixation, <sup>15</sup>N application also provides a reliable way of estimating N recovery and tracing the fate of N added via either fertilizer or crop residue (Gardner and Drinkwater, 2009).

Despite the significant increase of soybean acreage in western Canada in recent years, little research has been conducted to systematically assess the N<sub>2</sub> fixation and N contribution of soybean to the soil N pool and succeeding crops under local soil-climatic conditions. One study in Manitoba reported limited N benefit from soybean to following crops, compared to pea, chickpea, and dry bean (Przednowek et al., 2004). This study, however, did not quantify the N<sub>2</sub> fixation of the legumes or N recovery rates by following crops, although this information is essential for accurate estimates of N benefits of legume crops. Therefore, the research presented in this chapter was conducted to measure the N<sub>2</sub> fixation of a short-season soybean under field conditions using <sup>15</sup>N isotope dilution and tracing methods, in comparison to two commonly grown pulse crops, pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.) at a site near Rosthern, SK in the Black soil zone in Saskatchewan, Canada. Hard red spring wheat (*Triticum aestivum* L.) was used as the non-N-fixing reference crop. In the subsequent year, N uptake and recovery of above-ground residue N in the grain and the straw of a spring wheat crop grown on the soybean, pea, or lentil stubbles were estimated. The overall goal of the work described in this chapter was to provide estimates of N<sub>2</sub> fixation and N distribution in different above-ground components of soybean and its N contribution to a following spring wheat crop in comparison to pea and lentil on a no-till Black Chernozem in Saskatchewan.



## 5.4 Materials and Methods

### 5.4.1 Experimental design and set up

The experiment was established at a site (Rosthern, SK) within the Black soil zone in Saskatchewan, Canada. The soil at the site is classified as an Orthic Black Chernozem, with a history of several years of no-till prior to establishment of the experiment (see Chapter 3). In the first rotational year (2014), N<sub>2</sub> fixation of soybean (cv. TH3303R2Y), pea (cv. CDC Meadow), and lentil (cv. CDC Maxim) was quantified using the <sup>15</sup>N isotope dilution technique, with hard red spring wheat (cv. CDC Abound) used as the non-fixing reference crop. In the subsequent year (2015), the N uptake and recovery rate of N from the above-ground residue (straw) by a spring wheat crop grown on soybean, pea, lentil, or wheat stubbles was determined. The experimental design was a Randomized Complete Block Design (RCBD), with legume crops randomized in 4 blocks and the reference crop planted beside the legume crop. Soybean, pea, lentil, and wheat were seeded on May 31<sup>st</sup>, 2014, and in the subsequent year wheat was seeded on May 13<sup>th</sup>, 2015. Detailed information on seeding rates, plot area, inoculation, weed control, harvest methods, residue return method, and harvest dates is provided in Chapter 3.

### 5.4.2 Nitrogen-15 application and analyses

When pea and lentil reached the fifth leaf stage (June 26<sup>th</sup>, 2014) and soybean the first-trifoliolate leaf stage (July 3<sup>rd</sup>, 2014), three 1m × 1m subplots (subplot 1, subplot 2, and subplot 3), were established in each of the legume plots and reference crop plots. A 10 atom% excess <sup>15</sup>N-(<sup>15</sup>NH<sub>4</sub>)(<sup>15</sup>NO<sub>3</sub>) fertilizer was uniformly applied to subplot 1 and subplot 2 at the rate of 20 kg N ha<sup>-1</sup> in a liquid form. The fertilizer was completely dissolved in 9L deionized water prior to application, following the same method as described by Knight (2012). A plastic frame was placed at the surface of the ground to confine the fertilizer solution within the plot area (Fig. 5.1). Following the fertilizer application, deionized water was applied over the plot area to rinse off the residual fertilizer contained on the leaves into the soil.

In fall 2014, crops were harvested by hand at maturity, with the whole crop, including grain, straw, and leaves, harvested from subplots to determine yield and N<sub>2</sub> fixation (see Chapter 3 for more details). After threshing, crop residue was returned to the plots, with residue from subplot 1 returned to subplot 1, residue from subplot 3 returned to subplot 2, and residue from subplot 2 returned to subplot 3 that did not receive <sup>15</sup>N-enriched fertilizer in the

spring of 2014. Using this approach, the only  $^{15}\text{N}$  input to subplot 3 was via the  $^{15}\text{N}$ -labelled above-ground crop residue after harvest. Samples from subplot 1 and subplot 2 from each plot were used to determine  $^{15}\text{N}$  concentration and fixation. In spring 2015, hard red spring wheat was seeded on the stubble of soybean, pea, and lentil on May 13<sup>th</sup> (see Chapter 3), and harvested on August 19<sup>th</sup> from subplots 1, 2, and 3 separately. The 2015 wheat N uptake, nitrogen derived from residue (N<sub>dfr</sub>) and residue N recovery rate (R) results provided in this chapter are thus from subplot 3 treatments.

Crop samples were dried at 30°C in the laboratory immediately after harvest and threshed using a stationary mechanical thresher. Grain and straw yields were determined and then sub-samples of grain or straw were taken after mixing of the main sample and collecting from several locations within the container. Sub-samples were ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA), oven-dried at 60°C to stable weight, and subsequently re-ground to fine powder using a roller ball mill (8000D Mixer/Mill, SPEX SamplePrep® LLC., Metuchen, NJ, USA). The re-ground grain and straw sub-samples were then weighed (2.5+/- 0.5 mg) using a micro-balance (Sartorius Microbalance, CPA2P, Bradford, MA, USA), and encapsulated using 8 × 5 mm tin capsules into an approximately spherical shape with air pressed out of the encapsulated sample. The encapsulated grain and straw samples were then analyzed for percent N and atom %  $^{15}\text{N}$  using a Costech ECS4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) coupled to a Delta V Advantage Mass Spectrometer (Isomass Scientific Inc. Calgary, AB), with chickpea at natural N abundance used as the standard for the spectrometry measurement.



**Fig. 5.1.** Plastic frame used to confine applied fertilizer solution.

### 5.4.3 Calculations and statistical analysis

Atom% <sup>15</sup>N excess in the plant samples was calculated as the atom% <sup>15</sup>N in a material in excess of the natural abundance of atmospheric N<sub>2</sub> (0.3663 atom% <sup>15</sup>N). Percentage of N derived from atmosphere (%Ndfa) in total plant N was calculated for the grain and straw separately according to Rennie and Dubetz (1986) as:

$$\%Ndfa = \left[ 1 - \left( \frac{\text{atom } \%^{15}\text{N excess}_{N\text{-fixing crop}}}{\text{atom } \%^{15}\text{N excess}_{\text{non-fixing crop}}} \right) \right] \times 100 \% \quad (\text{Eq. 5.1})$$

The total amount of N derived from atmosphere (Ndfa, kg N ha<sup>-1</sup>) in plant biomass was calculated from the %Ndfa and total N yield of the crops according to Peoples et al. (2009) as:

$$Ndfa = \frac{\%Ndfa}{100} \times N \text{ yield (kg N ha}^{-1}\text{)} \quad (\text{Eq. 5.2})$$

Percentage of N derived from other sources, including fertilizer (%Ndff) and soil (%Ndff) in 2014, and from previous crop residue (%Ndf<sub>r</sub>) in 2015, as well as total amount of N derived from these sources (Ndff, Ndfs, Ndf<sub>r</sub>, in kg N ha<sup>-1</sup>) were calculated according to Ehaliotis et al. (1998) as:

$$\%Ndff = \frac{\text{atom } \%^{15}\text{N excess}_{\text{plant}}}{\text{atom } \%^{15}\text{N excess}_{\text{fertilizer}}} \times 100 \% \quad (\text{Eq. 5.3})$$

$$Ndff \text{ (kg N ha}^{-1}\text{)} = \%Ndff \times N \text{ yield}_{\text{plant}} \text{ (kg N ha}^{-1}\text{)} \quad (\text{Eq. 5.4})$$

$$Ndfs_{N\text{-fixing crop}} \text{ (kg N ha}^{-1}\text{)} = N \text{ yield}_{\text{plant}} - Ndfa - Ndff \text{ (kg N ha}^{-1}\text{)} \quad (\text{Eq. 5.5})$$

$$\%Ndf_r = \frac{\text{atom } \%^{15}\text{N excess}_{\text{plant}}}{\text{atom } \%^{15}\text{N excess}_{\text{residue}}} \times 100 \% \quad (\text{Eq. 5.6})$$

$$Ndf_r \text{ (kg N ha}^{-1}\text{)} = \%Ndf_r \times N \text{ applied (kg N ha}^{-1}\text{)} \quad (\text{Eq. 5.7})$$

Residue N recovery rate (R) was calculated according to Ehaliotis et al. (1998) as:

$$R = \frac{Ndf_r}{N \text{ applied}} \times 100 \quad (\text{Eq. 5.8})$$

All calculations were conducted for the grain and the straw separately from both 2014 and 2015 seasons. The N applied in fall 2014 was the amount of N returned to the subplots

(subplot 3) via return of the harvested straw residue, which was equal to the straw N yield of crops in subplot 2 from the same plot at 2014 harvest.

The standard deviation of the standards (chickpea) was within  $\pm 0.15\%$ . Data were analyzed using SAS 9.4 for Windows, and a significance level of 0.05 was used. Data distribution normality was tested using the Kolmogorov-Smirnov test by the PROC UNIVARIATE procedure, and the homogeneity of variance was tested using the Levene's test. The PROC GLM procedure was used to compare crop species followed by a Tukey's HSD test, and paired t-test was used to compare the grain and the straw plant components.

## 5.5 Results

### 5.5.1 N<sub>2</sub> fixation by soybean, pea, and lentil in 2014

The grain yield of soybean, pea, and lentil at the site (Table 5.1) was well above the average Saskatchewan levels in 2014, reported by the Government of Saskatchewan Ministry of Agriculture as 1278 kg ha<sup>-1</sup> of soybean, 2287 kg ha<sup>-1</sup> of pea, and 1537 kg ha<sup>-1</sup> of lentil (Government of Saskatchewan, 2014). Compared to soybean and lentil, pea had higher grain yield and grain N yield. The magnitude of grain N yield followed an order of pea > soybean ≥ lentil, with pea having almost twice the grain N yield as lentil, presumably due to superior pea grain yield at the Rosthern site in 2014. Straw N yield was similar among soybean, pea, and lentil. In general, N yield of the three crops was mainly allocated in the grain, with harvest N index of 72% for soybean, 78% for pea, and 60% for lentil.

The mean percent N derived from atmosphere (%Ndfa) in both the grain and the straw of soybean was higher than that of pea or lentil, although the difference was not statistically significant in either the grain or the straw (Table 5.1). Soybean had similar %Ndfa in the grain and the straw, whereas pea and lentil had significantly higher %Ndfa in the grain than in the straw. This component effect on %Ndfa was most evident for pea, with the %Ndfa in the grain being more than 1.5 times higher than that in the straw. Accordingly, pea had more grain N derived from atmosphere (Ndfa) than soybean and lentil, while in the straw soybean, pea, and lentil had similar amount of Ndfa. Similarly, pea had significantly more N derived from fertilizer (Ndff) and N derived from soil (Ndffs) in the grain than soybean and lentil, while in the straw the three crops had similar amount of Ndff and Ndffs. The majority of fixed N was allocated in the grain for all crops, with 75%, 85%, and 67% of above-ground fixed N retained in the grain

of soybean, pea, and lentil, respectively. This component effect on the partitioning of fixed N between the grain and the straw was statistically significant for all three crops. Unlike Ndfa, the partitioning of Ndff and Ndfs between grain and straw had a different trend, with different plant components having rather similar Ndff and Ndfs of soybean and lentil, whereas pea had higher Ndff and Ndfs in the grain than in the straw. Overall, pea had the highest amount of above-ground (grain + straw) N ( $\approx 188 \text{ kg ha}^{-1}$ ) derived from  $\text{N}_2$  fixation, followed by soybean ( $\approx 158 \text{ kg ha}^{-1}$ ), with lentil the least ( $\approx 133 \text{ kg ha}^{-1}$ ).

The contribution of different N sources to above-ground crop N yield followed the order of Ndfa > Ndfs > Ndff for all three tested legumes, with fixed N being the primary N source in the above-ground plant N. In the total above-ground (grain + straw) N, about 70% of soybean N was derived from atmosphere through  $\text{N}_2$  fixation ( $158.4 \text{ kg ha}^{-1}$ ) and approximately 27% from soil ( $61.8 \text{ kg ha}^{-1}$ ), with the rest ( $\approx 2\%$ ) derived from the applied fertilizer ( $5.1 \text{ kg ha}^{-1}$ ). Similarly, about 62% and 35% of above-ground plant N of pea was Ndfa ( $188.3 \text{ kg ha}^{-1}$ ) and Ndfs ( $107.8 \text{ kg ha}^{-1}$ ), respectively. For lentil, 62% and 35% of the above-ground plant N was Ndfa ( $133.5 \text{ kg ha}^{-1}$ ) and Ndfs ( $74.2 \text{ kg ha}^{-1}$ ), respectively. Results showed that for the three crops, especially for soybean,  $\text{N}_2$  fixation contributed a significantly higher amount of N to the total above-ground plant N compared to other N sources including the soil N pool and applied N fertilizer.

**Table 5.1.** Yield and N components (n=4) in soybean, pea, and lentil at harvest in 2014.

Previous crop	Yield	N yield	N	Ndfa <sup>§</sup>	Ndfa <sup>§</sup>	Ndff <sup>§</sup>	Ndfs <sup>§</sup>
	kg ha <sup>-1</sup>		%			kg ha <sup>-1</sup>	
	----- <b>Grain</b> -----						
Soybean	2649 B <sup>†</sup> b <sup>‡</sup>	161.7 Ab	6.1 Aa	73.7 Aa	118.9 Ab	3.2 Ab	39.7 Ab
Pea	6143 Aa	236.7 Aa	3.9 Ac	67.5 Aa	160.4 Aa	5.4 Aa	70.9 Aa
Lentil	2755 Bb	127.9 Ab	4.6 Ab	69.8 Aa	88.9 Ab	2.9 Ab	36.1 Ab
	----- <b>Straw</b> -----						
Soybean	6734 Aa	63.6 Ba	1.0 Bb	61.3 Aa	39.6 Ba	1.9 Aa	22.1 Aa
Pea	5422 Ab	67.7 Ba	1.3 Bab	40.4 Ba	27.9 Ba	3.0 Ba	36.9 Ba
Lentil	5727 Ab	86.2 Ba	1.5 Ba	50.7 Ba	44.6 Ba	3.5 Aa	38.1 Aa

<sup>†</sup> Within a column, means of the same crop followed by the same capital letter are not significantly different between the grain and the straw ( $p \geq 0.05$ ) according to a paired t-test.

<sup>‡</sup> Within a column, means of the same plant component followed by the same lowercase letter are not significantly different among crop species ( $p \geq 0.05$ ) according to a Tukey's HSD test.

<sup>§</sup> %Ndfa, Ndfa, Ndff, Ndfs denote percentage of N derived from atmosphere, amount of N derived from atmosphere, amount of N derived from fertilizer, and amount of N derived from soil, respectively.

### 5.5.2 Residue N recovery by the following wheat crop in 2015

In 2015, wheat grown on the  $^{15}\text{N}$  labelled above-ground residue soybean, pea, lentil, or the reference crop wheat from the previous year had similar atom%  $^{15}\text{N}$  levels in the grain and in the straw, respectively (Table 5.2). Different residues from the previous year did not result in significant differences in the atom %  $^{15}\text{N}$  of either the grain or the straw of wheat in the subsequent year. Similarly, atom %  $^{15}\text{N}$  in the surface soil profile (0-15cm) in both 2015 spring and 2016 spring showed no response to different residues from the previous growing season.

Wheat had similar N uptake on different residues (Table 5.3). Percent N derived from the above-ground crop residue (%Ndfr) of the previous year in wheat grain was similar among the legume residues, but significantly lower on the wheat residue than on soybean and lentil residues. Similarly, %Ndfr in the wheat straw was lower on the wheat residue than on the soybean residue. Total amount of N derived from previous residue (Ndfr) in the grain of wheat on different residues followed the order of lentil  $\geq$  soybean  $\geq$  pea  $\geq$  wheat, and wheat grain Ndfr was over fourfold on soybean stubble of that on wheat stubble. Wheat straw Ndfr was similar on soybean, pea, and lentil residues, but lower on wheat residue compared to soybean and lentil residues. Overall, N recovery rates from above-ground residues of the previous year were similar on soybean, pea, lentil, and wheat residues, although the grain N recovered appeared to be higher on soybean and lentil residues than on pea or wheat residues. Wheat in 2015 had more Ndfr in the grain than in the straw, no matter what the residue the previous year, which is consistent with the larger amount of N in wheat grain than in the straw (Table 5.3). However, the proportion of N derived from residue to applied N, which is the recovery rate (R), was similar on different residues in both the grain and the straw of wheat grown I 2015. Overall, Ndfr was mainly allocated in the grain, and recovery rates of above-ground residue were higher in the grain than in the straw of wheat, no matter grown on what residue of the previous year.

**Table 5.2.** Atom percent  $^{15}\text{N}$  (n=4) in wheat in 2015 and in the surface soil (0-15cm) (n=4) in May of 2015 and 2016<sup>†</sup>.

Previous crop <sup>‡</sup>	Grain	Straw	2015 spring soil	2016 spring soil
	%			
Soybean	0.44	0.43	0.38	0.38
Pea	0.43	0.43	0.38	0.38
Lentil	0.45	0.44	0.38	0.38

<sup>†</sup> No significant difference ( $p \geq 0.05$ ) detected among treatments in this table according to a Tukey's test.

<sup>‡</sup> Crop grown in 2014.

**Table 5.3.** N uptake and recovery (R) (n=4) in wheat in 2015.

Previous crop	Applied N <sup>†</sup>	N Uptake	Ndfr	Ndfr	R <sup>‡</sup>
	kg ha <sup>-1</sup>		%		%
	<b>Grain</b>				
Soybean	63.6 a <sup>§</sup>	73.0 A <sup>¶</sup> ab	13.2 Aa	10.1 Aab	15.7 Aa
Pea	67.7 a	81.5 Aab	8.0 Aab	6.8 Abc	10.4 Aa
Lentil	86.2 a	100.3 Aa	12.9 Aa	13.0 Aa	15.7 Aa
Wheat	30.5 b	64.3Ab	4.9 Ab	3.0 Ac	10.0 Aa
	<b>Straw</b>				
Soybean	63.6 a	22.5 Ba	12.3 Aa	2.8 Ba	4.3 Ba
Pea	67.7 a	21.9 Ba	9.3 Aab	2.1 Bab	3.2 Ba
Lentil	86.2 a	23.7 Ba	10.7 Aab	2.5 Ba	3.0 Ba
Wheat	30.5 b	18.5 Ba	4.7 Ab	0.9 Bb	2.8 Ba

<sup>†</sup> The amount of applied N as residue is equal to the N yield of straw returned to subplot 3 in fall 2014.

<sup>‡</sup> The above ground N recovery rate, calculated as  $R = \text{Ndfr} / N_{\text{applied}} \times 100$ .

<sup>§</sup> Within a column, means of the same plant component followed by the same lowercase letter are not significantly different among crop species ( $p \geq 0.05$ ) according to a Tukey's HSD test.

<sup>¶</sup> Within a column, means of the same crop followed by the same capital letter are not significantly different between the grain and the straw ( $p \geq 0.05$ ) according to a paired t-test.

## 5.6 Discussion

Soybean had slightly higher percent N derived from atmosphere (%Ndfa) in the grain and the straw than pea and lentil, although the difference was not statistically significant, indicating similar ability of the three crops to obtain N via N<sub>2</sub> fixation versus utilization of soil and fertilizer N under western Canadian soil-climatic conditions. The grain and the straw of soybean had similar %Ndfa, as opposed to pea and lentil, which had higher %Ndfa in the grain than in the straw, indicating different patterns among the crops regarding the partitioning of fixed N in different plant components and/or in different stages of plant growth. Lower %Ndfa in the straw of pea and lentil suggests greater early uptake and utilization of soil available N in the early vegetative growing stages before the onset of rapid fixation and/or reduced mobilization of this N to the seed in later growth stages when the plants started the reproductive stages, as also suggested by van Kessel (1994). Accordingly, compared to pea and lentil, the relatively even partitioning of fixed N in the grain and the straw of soybean possibly indicates a relatively steady fixation rate in soybean through the growing season from early vegetative stages to the reproductive stage later in the season.

The %Ndfa and total amount of biologically fixed N (Ndfa) of soybean, pea, and lentil in this study were in the high range compared to results of studies in other parts of the world or within Canada (van Kessel, 1994; Jensen, 1996; Salvagiotti et al., 2008; Peoples et al., 2009; Yang et al., 2010). For example, reviewing 61 studies of N<sub>2</sub> fixation by soybean conducted in different countries across the world, Salvagiotti et al. (2008) reported that on average, fixed N was able to meet 50% - 60% of the N demand of soybean and, if only N fixed in the above-ground biomass was considered, 80% of the studies had a negative N balance between the crop N removal and the N input via N<sub>2</sub> fixation. This is consistent with the results for soybean and pea in the present study, which showed lower above-ground Ndfa of soybean (159 kg ha<sup>-1</sup>) and pea (188 kg ha<sup>-1</sup>) than grain N removal in the two crops (162 kg ha<sup>-1</sup> of soybean and 237 kg ha<sup>-1</sup> of pea). However, lentil fixed about 134 kg ha<sup>-1</sup> in the above-ground biomass, as opposed to the 128 kg ha<sup>-1</sup> N removal via lentil grain harvest, resulting in a net N gain of 6 kg ha<sup>-1</sup> after harvest, provided that only the grain was exported from the field at harvest. Therefore, despite the smaller amount of grain Ndfa in lentil as opposed to soybean and pea, it indeed was associated with net N gain to the system after harvest, whereas soybean and pea resulted in slight negative N balance in the system. Similarly, a field study conducted in Saskatchewan also



showed a net N contribution of 59 kg ha<sup>-1</sup> from lentil to the soil (van Kessel, 1994). However, it should be noted that in these studies including the present study, the partitioning of fixed N in below-ground roots and rhizodeposition were not accounted for, which together could comprise up to more than 60% of total plant N and can be significant in estimating the N budget of the system (Arcand et al., 2013, 2014b). Therefore, including root N and below-ground N rhizodeposition in N<sub>2</sub> fixation estimation and N budgets would fill in the gap between total Ndfa in a crop and N export from the field in harvested seed (van Kessel and Hartley, 2000). However, it is still challenging to accurately quantify N<sub>2</sub> fixation completely due to the difficulty of measuring root N and N rhizodeposition under field conditions, which, we believe, could be the one of the important aspects of future studies on N<sub>2</sub> fixation by grain legume crops.

Despite the potential limits especially of heat unit in the Black soil zone of Saskatchewan compared to traditional soybean growing areas like eastern Canada or the Midwestern USA, soybean had good yield and N<sub>2</sub> fixation in the above-ground plant component in the present study, similar to pea and lentil crops that were traditionally grown in rotation in western Canada. In addition, the three crops had similar straw N yield, with soybean having a higher straw biomass yield, implying similar amount of N contribution to following crops via residue returning but higher C inputs via soybean residue. However, multiple factors other than only N and C inputs could affect N release from crop residues of the previous year through mineralization and thus its availability to succeeding crops in rotation. These factors include the C:N ratio and biochemical composition of the residue, soil microbial composition, and soil chemical and physical properties (Kumar and Goh, 2003; Ha et al., 2008; Jani et al., 2015). In addition, the contribution from below-ground biomass and N of legumes can be notably large as shown by previous studies (Khan et al., 2002; Arcand et al., 2013, 2014b). Arcand et al. (2013, 2014b) reported that below-ground N comprised over 60% of total crop N for pea, 70% for canola, 34% for lentil, and over 50% for wheat as estimated through greenhouse experiments. Similarly, Khan et al. (2002) suggested that the below-ground N could be substantial in total plant N in legumes, with 30-52% of N retained in the below ground plant part of fababean, chickpea, mungbean, and pigeonpea. Rochester et al. (1998) reported that in New South Wales, the below ground N in soybean was over 40% of the total plant N. In A Saskatchewan silt loam soil, the root biomass was 11% of the above-ground biomass in dry pea, and between 20-22% for wheat, canola, and lentil (Gan et al., 2009b).

Wheat grown on the lentil residue in 2015 had a larger amount of grain N derived from residue of the previous year, consistent with the slightly higher straw N content and lower C:N in harvested lentil straw compared to soybean and pea (see Chapter 3) and previous research on C:N ratios (Gan et al., 2011). The proportion of N derived from the residue however, appeared to be the highest in the grain of wheat grown on the soybean residue, possibly due to the higher C inputs via the soybean residue, which delayed the release of available N to later in the season. Overall though, soybean, pea, and lentil as previous rotational crops resulted in similar %N<sub>dfr</sub> both in the grain and the straw of the following wheat. As expected, wheat residue resulted in the lower %N<sub>dfr</sub> levels than legume residues, presumably due to the N and non-N benefits of legumes (Stevenson and van Kessel, 1996a), and especially the lower C:N ratios of the legume residue (57 for soybean, 40 for pea, and 34 for lentil), as opposed to the relatively higher C:N ratio of the wheat residue. It is noteworthy that despite the superior grain biomass yield and N yield of the pea, the wheat grown on the pea residue in the following year showed no higher %N<sub>dfr</sub>, and even lower total N<sub>dfr</sub> compared to wheat grown on the lentil residue. Therefore, the yield and total amount of N contained in the above-ground residue is not necessarily a reliable predictor of total available N contribution. The N contained in the residue is not necessarily all recovered by crop in the second year, and the contribution from below-ground is not necessarily related to above-ground contribution or similar for different crops. Long-term studies therefore, could possibly show different patterns regarding N cycling in the agroecosystem. Furthermore, rotational benefits of legumes to succeeding cereals include more aspects than only N contribution, such as improving soil structure, breaking the pest cycles, enhancing soil microbial activity, and stimulating soil nutrient availability and uptake of other nutrients (Stevenson and van Kessel, 1996a; van Kessel and Hartley, 2000; Kramer et al., 2002). For example, one research study conducted in the Black soil zone in Saskatchewan showed that N fertilizer alone was unable to increase the yield of succeeding crops grown on cereal residue to those on pulse residue (Wright, 1990). Overall, the recovery of residue N of the previous year by subsequent spring wheat (20% of N in the soybean residue, 13% of N in the pea residue, 18% of N in the lentil residue, and 13% of N in the wheat residue in the total above-ground after harvest) is similar to previous studies conducted in other regions of the world (Jensen, 1996; Ehaliotis et al., 1998).

## 5.7 Conclusion

This study is the first to systematically quantify N yield, N<sub>2</sub> fixation, partitioning by short-season soybean and subsequent recovery of above-ground straw N by a following crop in the northern Great Plains. Soybean had comparable above-ground biomass and N yield to pea and lentil in this two-year study conducted on a no-till Black Chernozem in Saskatchewan, Canada. Compared to soybean and lentil, pea had higher straw N yield but also the highest harvest N index. Nitrogen derived via fixation comprised over 60% of total above-ground plant N for soybean, pea, and lentil. At maturity, the amount of N derived from fixation in the grain was pea > soybean ≥ lentil, and the majority of fixed N was retained in the grain, especially for pea and to a lesser extent soybean. The three grain legume crops had similar straw N yield, which was consistent with the similar N recovery rates observed for wheat that was subsequently grown on the residue of soybean, pea, or lentil from the previous year. Further studies are required to develop sound estimates of N<sub>2</sub> fixation including both above-ground and below-ground N yield and rhizodeposition under field conditions. Our study shows that under Saskatchewan conditions, modern short-season soybean has good potential for N yield, N<sub>2</sub> fixation, and N contribution via residue decomposition to crops in the following season, in comparison to pea and lentil grown under similar conditions.

## **6. SOIL NUTRIENT SUPPLIES AND GREENHOUSE GAS EMISSIONS FROM TWO SASKATCHEWAN SOILS CONTAINING SOYBEAN, PEA, LENTIL, AND WHEAT RESIDUES**

### **6.1 Preface**

Following the important agronomic aspects such as crop yield, nutrient uptake, N<sub>2</sub> fixation, and the effects on following crops as discussed in previous chapters, this chapter turns to assessing impact of soybean production on soil nutrient supply rates and greenhouse gas emissions in comparison to pea and lentil. This was achieved through an incubation experiment conducted under controlled conditions over an 8-week period. Using intact soil cores containing soybean, pea, lentil, or wheat (the control) residue collected in fall of 2014 from the Rosthern and Saskatoon sites, I measured the soil N (NO<sub>3</sub><sup>-</sup>-N+ NH<sub>4</sub><sup>+</sup>-N) and PO<sub>4</sub><sup>3-</sup>-P supply rates in the cores along with CO<sub>2</sub> and N<sub>2</sub>O emissions, and <sup>15</sup>N<sub>2</sub>O enrichment in the gas fluxes measured through the incubation period. Results of this chapter can provide implications on the effects of soybean, pea, and lentil residues on soil nutrient supply rates and potential of greenhouse gas emissions in Saskatchewan soils.

### **6.2 Abstract**

While soybean acreage is expanding in western Canada, little is known about soil nutrient availability and greenhouse gas emissions in soils after soybean production. An 8-week incubation experiment was conducted using intact cores collected in October of 2014 after harvest from two field trials containing different crop stubbles located near Saskatoon and Rosthern, Saskatchewan. The cores were frozen and stored for five months at -18°C and then thawed prior to the incubation to simulate winter-spring conditions. Soil available N and P supply rates, CO<sub>2</sub> and N<sub>2</sub>O emissions, and δ<sup>15</sup>N-N<sub>2</sub>O enrichment in soil cores from plots that had <sup>15</sup>N-labeled soybean, pea, lentil, and wheat residue were assessed. Soil available N (NO<sub>3</sub><sup>-</sup>-N+ NH<sub>4</sub><sup>+</sup>-N) and PO<sub>4</sub><sup>3-</sup>-P supply rates decreased over the eight week incubation period in the Rosthern soil, with the supply rate of soil available N decreasing in the 5<sup>th</sup> week to about 25%

of that observed in the first week. This pattern was consistent with the pattern of  $\delta^{15}\text{N}\text{-N}_2\text{O}$  enrichment, reflecting the depletion of mineralizable N released from added residue and soil, and a transition from labile fractions to more recalcitrant fractions at around the 5<sup>th</sup> week. The Rosthern soil amended with the soybean residue had more available P released than the pea or lentil residue at week 6, and more than the wheat residue at week 8. The Saskatoon soil with the lentil residue had higher N supplies from the second week till the end of the incubation. The  $\text{CO}_2$  and  $\text{N}_2\text{O}$  fluxes did not differ significantly among different crop residue treatments in either soil, except that at the second week the  $\text{CO}_2$  flux was higher in the Saskatoon soil amended with the soybean residue than with the pea residue. Similar patterns in nutrient release and greenhouse gas fluxes in the two soils with soybean, pea, or lentil residues suggest similar agronomic and environmental impact among the grain legumes grown under Saskatchewan conditions.

### **6.3 Introduction**

Crop residue is linked to nutrient transformations and cycling in the plant-soil system in crop rotations by replenishing the soil nutrient pool through residue decomposition and providing carbon and mineral nutrients to promote soil microbial activity, thus affecting soil nutrient availability as well as gaseous emissions from soils (Álvarez et al., 2008; Muhammad et al., 2011). Nitrogen (N) and phosphorus (P) are macronutrients that are crucial to the yield and quality of agricultural crops, and N is of particular concern for large losses to the environment, resulting in adverse environmental effects (Mary et al., 1996; Mosier et al., 1998). Therefore, as crop residue is returned to soil at fall harvest, the N, P, and other nutrients released from the residue via biological decomposition and physical processes like leaching become part of the soil nutrient pool. These processes regulate the availability of N and P in soil, microbial community dynamics, and production of the greenhouse gases (GHG) including  $\text{CO}_2$  and  $\text{N}_2\text{O}$  (Aulakh et al., 2001; Ha et al., 2008).

Nutrient turn-over processes are affected by both biochemical composition of the residue and soil decomposing conditions (Redin et al., 2014; Jani et al., 2015). Biochemical characteristics of the crop residue include the N content, cellulose content, C:N ratio, lignin:N ratio, and concentration of lignin and polyphenol (Kumar and Goh, 2003; Walela et al., 2014). In addition, soil conditions and management practices also influence the decomposition and cycling of N and P released from added crop residue, mainly by regulating the decomposing

environment, which in turn, affects the composition, activity, and efficiency of the microbial community that is responsible for mineralizing, immobilizing, and transforming nutrients from added residue. These soil conditions and management practices include aeration, soil water content and temperature, tillage, residue placement, and crop rotation among others (Aulakh et al., 1991; Mary et al., 1996; Christensen, 2001; Khalil and Baggs, 2005; Malhi et al., 2006; Spargo et al., 2011). For example, Malhi et al. (2006) reported that compared to no-tillage and N fertilization, both tillage and adding N fertilizer resulted in higher N<sub>2</sub>O emissions on a Gray Luvisol soil in Saskatchewan, Canada. Nitrogen losses from soil through denitrification occurs due to a series of microbial reductions of NO<sub>3</sub><sup>-</sup> to N<sub>2</sub> through a series of intermediate N-gases including N<sub>2</sub>O (Cayuela et al., 2013). As a result, above-mentioned soil decomposing conditions and management factors regulating the microbial activity and composition can affect the emission of N<sub>2</sub>O in soil, including the availability of nitrate and soil organic C, soil aeration, pH, and temperature (Aulakh et al., 2001; Khalil et al., 2004). Previous studies showed over 30% of applied N could be lost from soil through denitrification, causing adverse environmental effects and depletion of soil fertility (Gregorich et al., 2008; van Kessel et al., 2013).

As a relatively new grain legume to Saskatchewan, soybean has received little research on its impact on soil-plant nutrient cycling and potential environmental impact when included in rotations under Saskatchewan soil-climatic conditions. In this chapter, we describe a study intended to reveal the impact of soybean in comparison to other pulse crops and wheat on short-term soil N and P availability as well as CO<sub>2</sub> and N<sub>2</sub>O emissions in two Saskatchewan soils: Saskatoon soil (Orthic Dark Brown Chernozem) and Rosthern soil (Orthic Black Chernozem) as described in Chapter 3. The 8-week incubation experiment was conducted under controlled environmental conditions with intact soil cores collected from the field in the fall after harvest of the different crops. The two soils contained <sup>15</sup>N-labeled soybean, pea, lentil, or wheat residue. The overall objectives were to compare the impact of the different crop residues on soil N and P availability and the greenhouse gas effects under controlled environmental conditions. This was accomplished by measuring nutrient supply rates to PRS resin membrane probes and CO<sub>2</sub> and N<sub>2</sub>O emissions during the incubation period.

## 6.4 Materials and Methods

### 6.4.1 Soil sampling

The soils were collected from two research sites located in the farming area in south-central Saskatchewan. In the spring of 2014, two sites were set up near Saskatoon (Orthic Dark Brown Chernozem) and Rosthern (Orthic Black Chernozem). Soybean [*Glycine max* (L.) Merr. cv. TH3303R2Y], pea (*Pisum sativum* L. cv. CDC Meadow), lentil (*Lens culinaris* L. cv. CDC Maxim), and hard red spring wheat (*Triticum aestivum* L. cv. CDC Abound) were grown at the two sites in 2014 (see Chapter 3). When soybean reached the first-trifoliolate leaf stage and pea and lentil reached the fifth leaf stage, a 10 atom% excess  $^{15}\text{N}$ -( $^{15}\text{NH}_4$ )( $^{15}\text{NO}_3$ ) fertilizer was uniformly applied at the rate of 20 kg N ha<sup>-1</sup> with the fertilizer dissolved in 10L deionized water prior to application (see Chapter 5). No fertilizers were applied for any of the crops as the soil test revealed adequate amount of available P, K, S, and micronutrients. Crops were harvested by hand at physiological maturity, with all above-ground parts collected and brought back to the lab for determining grain and straw yield and nutrient content.

After the grain and straw yield was determined and sub-samples were taken for analysis, the straw residue was returned to the plots where it was taken from, with surface soil samples taken from the 0-15cm soil depth prior to residue returning. Crop residue was incorporated into soil by shallow rotary tillage to a 5cm depth immediately after being spread evenly over the plot area with rakes, in order to anchor the residue into the soil (See Chapter 3). Intact soil core samples were taken from the plots after residue incorporation at each site, by inserting polyvinyl chloride (PVC) tubes (10cm diameter × 15cm height) into the soil and excavating the tubes with intact soil inside, as described by Hangs et al. (2013). The soil core samples were wrapped with plastic immediately after the excavation to prevent potential moisture loss during transportation back to the lab. Collected surface soil sample were air-dried and ground prior to analysis of soil available N and P supply rates. The intact soil PVC cores were placed into a freezer at -18°C and stored from November to March to mimic winter conditions in Saskatchewan prior to incubation.

#### 6.4.2 Experimental design and soil incubation: measuring soil nutrient supplies and GHG emissions

In April of 2015, soil cores were taken out of the freezer, and placed under 20°C to allow the soil cores to thaw slowly to reflect spring soil thawing. Soil water content was brought to field capacity by adding water to the point where free drainage began from the bottom of the cores. After ten days to allow for stabilization of conditions in the cores, Plant Root Simulator (PRS™) ion exchange resin membrane probes (Western Ag Innovations Inc., Saskatoon, SK, Canada) were inserted into each soil core, with one cation and one anion probe per soil core (Fig. 6.1). This was considered as the start of the incubation period. The PRS probes were replaced at the same time of the day weekly through the entire incubation period, by taking out the old probes from the soil cores and inserting a new set of regenerated probes into the same slots, with the new anion probe going to the same slot of the previous anion probe and the same for the cation probe in each soil core. Soil cores were incubated for 8 weeks. Soil water content was maintained at close to field capacity by adding water to the cores to the point where water began to exit the bottom of the cores at the start of the incubation, with cores then placed into trays with 1cm standing water between gas sampling periods throughout the incubation period.



**Fig. 6.1.** Soil nutrient supply measurement with Plant Root Simulator (PRS™) ion exchange resin membrane probes. One anion probe (orange) and one cation probe (purple) were inserted into each soil core with minimum disturbance and replaced weekly through the entire incubation period.



From the second week of the incubation onwards, gas samples were taken from the gas-sampling chambers, as described by Hangs et al. (2013). Briefly, soil cores were placed into chambers, with one soil core per chamber, prior to gas sampling, and gas samples were taken using a 20mL syringe at  $T_0$ ,  $T_{30}$ ,  $T_{60}$ , and  $T_{90}$ , which represented 0, 30, 60, and 90 minutes after placing the soil cores into the chambers and closing the chamber lids tightly, respectively. Samples taken at  $T_0$  represented the ambient air in the experimental environment (lab air) during the gas sampling period. Gas samples were emptied to 12mL pre-evacuated Exetainer™ vials (Labco Ltd., High Wycombe, UK) and stored under room temperature prior to further analysis. Gas samples were collected at the same time of the day weekly to the end of the incubation period.

#### 6.4.3 Analytical methods

Prior to use, PRS resin membrane probes were saturated with  $\text{HCO}_3^-$  for anion probes and  $\text{Na}^+$  for cation probes, according to the protocol of Hangs et al. (2013). Briefly, probes were shaken three times in a 0.5 M  $\text{NaHCO}_3$  solution for 4 h, and thoroughly rinsed with deionized water between each shaking. After use, probes taken out the soil cores were first washed using deionized water until the probes were free of soil, and then eluted with a 0.5 M HCl solution. The eluates were then analyzed for  $\text{NO}_3^-$ -N,  $\text{PO}_4^{3-}$ -P, and  $\text{NH}_4^+$ -N colorimetrically (Technicon AutoAnalyzer; Technicon Industrial Systems, Tarrytown, NY, USA), to determine weekly supply rates of  $\text{NO}_3^-$ -N,  $\text{PO}_4^{3-}$ -P, and  $\text{NH}_4^+$ -N. The supply rates are expressed as  $\mu\text{g}$  nutrient absorbed per  $\text{cm}^2$  of resin membrane surface area over time.

To determine  $\text{CO}_2$  and  $\text{N}_2\text{O}$  concentration in the gas samples, collected gas samples were analyzed using gas chromatography, with the  $\text{CO}_2$  analysis completed using a Varian Micro GC CP-2003 and the  $\text{N}_2\text{O}$  analysis using a Bruker 450 gas chromatograph (Bruker Biosciences Corporation USA). The fluxes of  $\text{CO}_2$  and  $\text{N}_2\text{O}$  were estimated from the concentration change in the headspace of the gas chamber.

The enrichment of  $\delta^{15}\text{N}$  - $\text{N}_2\text{O}$  in the gas samples was determined in samples taken at  $T_{90}$ , using a Picarro G5131-*i* cavity ring-down spectroscopy (CRDS) Analyzer for  $\delta^{15}\text{N}$ - $\text{N}_2\text{O}$  (Picarro Inc., Santa Clara, CA, USA) in Professor Richard Farrell's lab in the Soil Science Department, University of Saskatchewan, Canada. To ensure the best performance of the Picarro instrument, gas samples were diluted when necessary so as to keep the bulk  $\text{N}_2\text{O}$  concentration below

1.5ppmv, by injecting N<sub>2</sub>O-free Ultra High Purity (UHP) air which was stored in a tank into the gas vials, and taking out the same amount of air as injected. This way, by adjusting the amount of air to inject, the air left in the vials was diluted to contain N<sub>2</sub>O concentration no higher than 1.5 ppmv. Due to the lack of an internationally recognized standard for  $\delta^{15}\text{N}$ -N<sub>2</sub>O, the accuracy and drift in  $\delta^{15}\text{N}$ -N<sub>2</sub>O was determined with diluted  $\delta^{15}\text{N}^{\alpha}$ -N<sub>2</sub>O and  $\delta^{15}\text{N}^{\beta}$ -N<sub>2</sub>O reference gases (98%+) supplied by Cambridge Isotope Laboratories, Inc., of which the enrichment of  $\delta^{15}\text{N}^{\alpha}$ -N<sub>2</sub>O and  $\delta^{15}\text{N}^{\beta}$ -N<sub>2</sub>O was tested by the Stable Isotope Facility (SIF), University of California, Davis, USA.

#### 6.4.4 Calculations and statistical analysis

Cumulative N and P supply rates were calculated according to the PRS handbook manual, by adding weekly supplies to obtain the total supply during the whole measuring period (8 weeks) (Western Ag Inc., 2006). The CO<sub>2</sub> and N<sub>2</sub>O fluxes were calculated using the concentration at three sampling points, including T<sub>0</sub> (ambient air), T<sub>30</sub>, and T<sub>60</sub>. The minimum detectable concentration difference (MDCD) was calculated for each sample using the sample pairs and the standard deviation, as described by Yates et al. (2006). When the N<sub>2</sub>O concentration of subsequent sampling intervals was smaller than the MDCD, the flux was considered linear and the flux during the sampling period was calculated using a linear regression. However, when it was larger than the MDCD, then the flux was determined according to the model proposed by Hutchinson and Mosier (1981). Cumulative GHG emissions were calculated by linearly interpolating the intermittent flux measurements made during the incubation period (Pennock et al., 2006).

Data were analyzed using SAS 9.4 for Windows, and a significance level of 0.10 was used in the analysis of variance (ANOVA) and subsequent post hoc tests. Data distribution normality was tested using the Kolmogorov-Smirnov test by the PROC UNIVARIATE procedure, and homogeneity of variance was tested using the Levene's test. Data of CO<sub>2</sub> and N<sub>2</sub>O were transformed using log transformation prior to ANOVA analyses, and data presented in the thesis were back - transformed. Comparison of group means of each soil was conducted by using the PROC GLM procedure followed by a Tukey's HSD test.

## 6.5 Results

### 6.5.1 Soil initial nutrient supply rates

Initial soil supply of available N and P before residue incorporation reflects the baseline amount of ions from the readily available nutrients in the soil solution and the labile pool. Prior to return of the crop straw residue in the fall of 2014, the Rosthern soil with the different crops grown in the 2014 growing season had  $\text{NO}_3^-$ -N,  $\text{PO}_4^{3-}$ -P, and  $\text{NH}_4^+$ -N supply rates among the crop stubble plots that were not significantly different (Table 6.1). At the Saskatoon site, soil with the lentil plots had twice the soil  $\text{NO}_3^-$ -N supply compared to other treatments, while soil  $\text{PO}_4^{3-}$ -P and  $\text{NH}_4^+$ -N supply rates were similar among the soybean, pea, lentil, and wheat plots. Overall, soil supply rates of readily available N in soybean plots were similar to the other crop plots after the 2014 growing season and before residue incorporation, with the exception that at the Saskatoon site lentil plots had significantly higher soil N supplies. In both Rosthern soil and Saskatoon soil, the mean soil P supply rates appeared to be highest in soybean plots, although the difference was not statistically different.

**Table 6.1.** Soil available N ( $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N) and  $\text{PO}_4^{3-}$ -P supply<sup>†</sup> (n=4) in Rosthern soil and Saskatoon soil in fall 2014 prior to straw residue incorporation.

Site	Crop residue <sup>†</sup>	$\text{NO}_3^-$ -N	$\text{PO}_4^{3-}$ -P	$\text{NH}_4^+$ -N
		$\mu\text{g cm}^{-2} (24 \text{ h})^{-1}$		
Rosthern	Soybean	11.1 a <sup>‡</sup>	0.7 a	0.6 a
	Pea	8.5 a	0.5 a	0.4 a
	Lentil	12.8 a	0.5 a	0.7 a
	Wheat	12.5 a	0.8 a	0.5 a
Saskatoon	Soybean	18.9 b	0.3 a	0.1 a
	Pea	18.4 b	0.1 a	0.1 a
	Lentil	36.0 a	0.2 a	0.1 a
	Wheat	15.4 b	0.1 a	0.2 a

<sup>†</sup> Residue returned to the soil in fall 2014.

<sup>‡</sup> Within a column, means within a site followed by the same letter are not significantly different from each other ( $p \geq 0.10$ ) according to a Tukey's HSD test.

### 6.5.2 Soil N and P supply rates in the incubation

During the 8-week incubation period, weekly supply rates of N ( $\text{NO}_3^-$ -N+  $\text{NH}_4^+$ -N) in the Rosthern soil showed a decreasing trend, and from the 5<sup>th</sup> week of incubation onward, the supplies stayed rather low until the end of the incubation (Fig. 6.2). The ANOVA revealed that there were no significant differences in soil N supplies among crop stubbles in any week over the incubation period in the Rosthern soil. Weekly soil N supplies in the Saskatoon soil cores,

similarly, also showed a decreasing trend with time during the 8-week incubation period, although not as great as that in the Rosthern soil. Additionally, N supply rates in the Saskatoon soil significantly varied with residue types, with N supplies being significantly higher in the lentil residue than in the wheat residue from the second week to the end of the incubation, and the soybean and pea residues having intermediate N supplies. In the last week of the incubation, N supplies in the Rosthern soil were about one twentieth of that in the first week, whereas N supplies in the Saskatoon soil were about one third to two thirds of that in the first week of incubation, reflecting different soil N mineralization patterns in the two soils. Weekly supply rates were initially lower in the Saskatoon soil than in the Rosthern soil, consistent with lower organic matter content in the Dark Brown soil and a lower mineralization rate compared to the Black soil.

Soil P supply rates also showed different trends in the Rosthern soil than in the Saskatoon soil (Fig. 6.3). In the Rosthern soil, the weekly P supplies showed a decreasing trend with incubation time. For the week 6 measurement, which encompasses the supply of available P from week 5 to week 6, the P supply was significantly higher in the soybean residue than in pea and lentil residues, and for week 8 it was higher in the soybean residue than in the wheat residue, whereas during the rest of the incubation time P supplies were similar among residues in the Rosthern soil. P supplies in the Saskatoon soil, on the other hand, did not show a clear increasing or decreasing trend with time during the incubation period, with no statistically significant difference among different residue treatments through the entire incubation period.

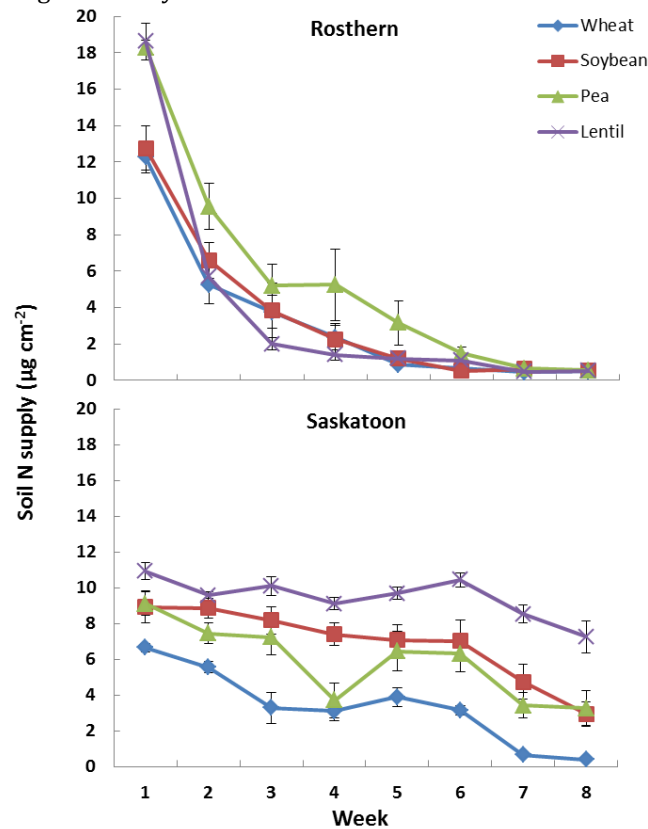
Cumulative supplies of available nutrient released over the incubation were calculated by summing the weekly supply rates (Table 6.2). By the end of the 8-week incubation, Rosthern pea residue soil had the highest mean cumulative N supply, followed by soil from lentil and soybean residues, with wheat residue having the lowest cumulative supply as would be expected, but the differences in cumulative soil N or P supplies among residue types were not significantly different at this site. The Saskatoon soil had the highest cumulative  $\text{NO}_3^-$ -N supply in the soil cores collected from the lentil plots and the lowest from wheat plots, with soybean and pea plots having intermediate cumulative  $\text{NO}_3^-$ -N supplied in the incubation. The cumulative  $\text{PO}_4^{3-}$ -P and  $\text{NH}_4^+$ -N supplies were similar among residue types in the Saskatoon soil.

**Table 6.2.** Cumulative soil available N ( $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N) and  $\text{PO}_4^{3-}$ -P supply (n=4) in the Rosthern and Saskatoon soils at the end of the 8-week incubation.

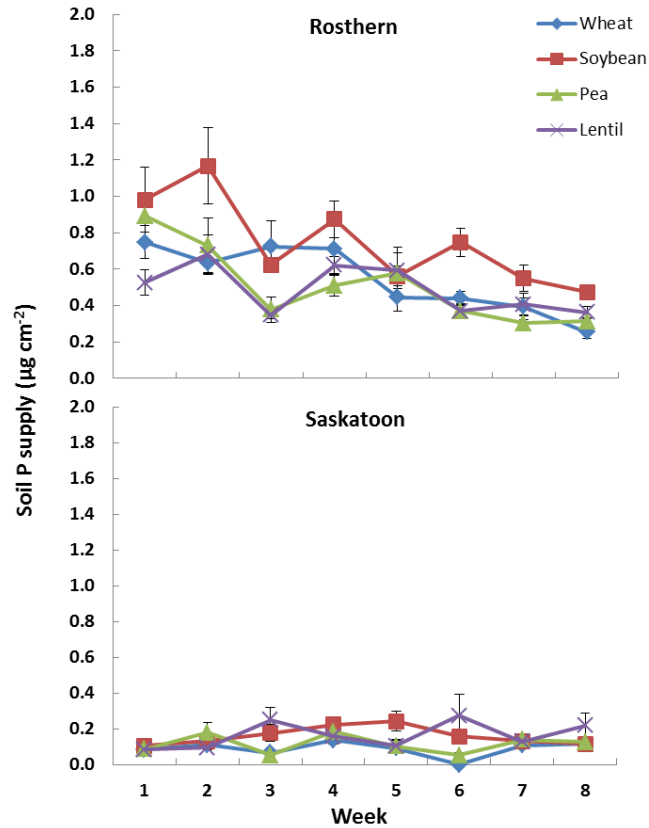
Site	Crop residue <sup>†</sup>	$\text{NO}_3^-$ -N	$\text{PO}_4^{3-}$ -P	$\text{NH}_4^+$ -N
		$\mu\text{g cm}^{-2}$		
Rosthern	Soybean	26.2 a <sup>‡</sup>	6.0 a	2.1 a
	Pea	42.5 a	4.1 a	1.6 a
	Lentil	29.1 a	3.9 a	1.8 a
	Wheat	23.3 a	4.2 a	1.5 a
Saskatoon	Soybean	52.7 ab	1.3 a	0.3 a
	Pea	46.5 ab	0.9 a	0.5 a
	Lentil	75.3 a	1.3 a	0.4 a
	Wheat	26.2 b	0.7 a	0.5 a

<sup>†</sup> Residue returned to the soil in fall 2014.

<sup>‡</sup> Within a column, means within a site followed by the same letter are not significantly different from each other ( $p \geq 0.10$ ) according to a Tukey's HSD test.



**Fig. 6.2.** Weekly soil available N ( $\text{NO}_3^-$ -N+  $\text{NH}_4^+$ -N) supply (n=4) in Rosthern and Saskatoon intact soil cores from different crop stubbles collected in the fall of 2014 and used in a laboratory incubation. Error bars represent one standard error. Rosthern soil had no significant differences in soil N supply among different crop residue treatments in any week during the entire incubation period; while Saskatoon site lentil stubble had significantly higher N supply than that the wheat stubble from week 2 to the end of the incubation according to a Tukey's HSD test ( $p \geq 0.10$ ).



**Fig. 6.3.** Weekly soil available  $\text{PO}_4^{3-}\text{-P}$  supply ( $n=4$ ) in Rosthern and Saskatoon intact soil cores from different crop stubbles collected in the fall of 2014 and used in a laboratory incubation. Error bars represent one standard error. In the Rosthern soil, soil P supplies in soybean stubble were significantly higher than in the pea or lentil stubble soil in week 6; in week 8 soybean stubble had higher P supply than wheat stubble residue soil. The P supplies in the Saskatoon soil were not significantly different among crop stubble types through the entire incubation period according to a Tukey's HSD test ( $p \geq 0.10$ ).

### 6.5.3 CO<sub>2</sub> and N<sub>2</sub>O emissions

CO<sub>2</sub> fluxes from different crop residues at Rosthern were similar over the entire incubation period (Fig. 6.4). CO<sub>2</sub> fluxes from different Rosthern crop stubble residue soils showed a similar trend over time, with CO<sub>2</sub> fluxes remaining relatively low in the first 5 weeks of the incubation, and then becoming higher towards the end of the incubation. For the Saskatoon soil, in the second week of incubation, the CO<sub>2</sub> flux from the soybean stubble soil was significantly higher than that from the pea stubble soil, with lentil and wheat stubble soils having intermediate CO<sub>2</sub> fluxes. During the rest of the incubation period, soil with different crop stubble residues had similar CO<sub>2</sub> fluxes. There was also a trend for the CO<sub>2</sub> fluxes at the Saskatoon site to increase towards the end of the incubation, possibly reflecting a shift in decomposer community and/or replenishment of substrate in the soils.

Similarly, N<sub>2</sub>O fluxes were similar among crop stubble residues throughout the entire incubation period in the Rosthern soil, remaining very low for all stubble types from the 5<sup>th</sup> week to the end of the incubation period. This coincided with a period of low soil available N supply rates (Fig 6.2), suggesting substrate limitations for nitrous oxide production. Similarly, N<sub>2</sub>O fluxes from different crop stubble types at Saskatoon were not significantly different for any week of the incubation period. While Saskatoon soybean and pea stubble soils appeared to have some fluctuations after being incubated for 4 weeks, N<sub>2</sub>O fluxes from Saskatoon soil generally remained at rather low levels throughout the incubation period.

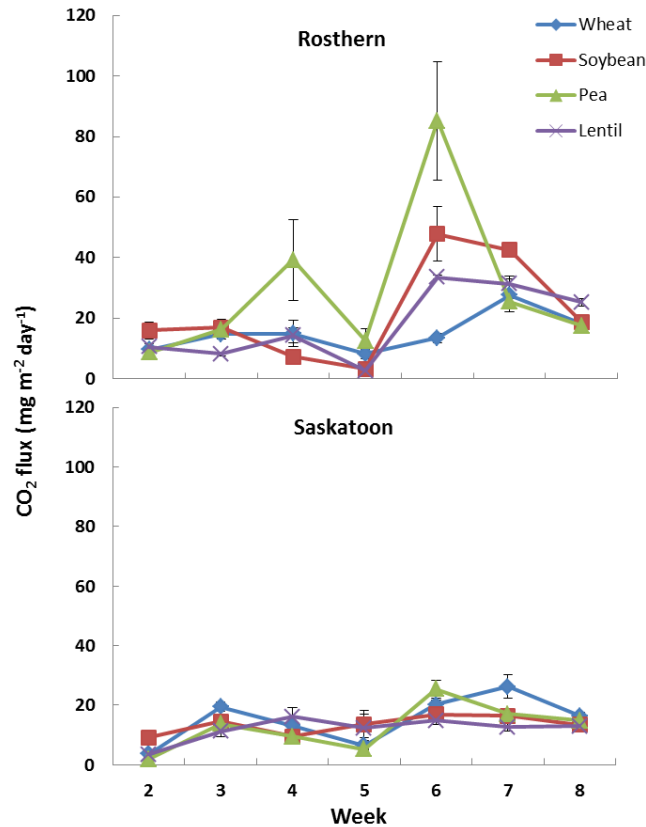
Overall, at the end of the 8-week incubation, cumulative CO<sub>2</sub> emissions were not significantly different among crop stubble residues in the Rosthern soil (Table 6.3). Rosthern soybean stubble soil appeared to have higher mean CO<sub>2</sub> emissions than other stubble residue types, but the difference was not significant. Similarly, the Saskatoon soil had similar cumulative CO<sub>2</sub> emissions among different crop stubble residues. Compared to CO<sub>2</sub> emissions, cumulative N<sub>2</sub>O emissions over the 8-week incubation differed greatly in mean values among the stubble types at the Rosthern site but were not significantly different, reflecting high variability among replicate cores for N<sub>2</sub>O emissions at this site. The Saskatoon soil exhibited less variability, with the soybean stubble having significantly higher cumulative N<sub>2</sub>O emissions than the lentil and wheat stubble residues by the end of the incubation.

**Table 6.3.** Cumulative CO<sub>2</sub> and N<sub>2</sub>O emissions (n=4) from Rosthern and Saskatoon soils at the end of the 8-week incubation.

Site	Crop residue <sup>†</sup>	CO <sub>2</sub> -C	N <sub>2</sub> O-N
		g m <sup>-2</sup>	mg m <sup>-2</sup>
Rosthern	Soybean	946 a <sup>‡</sup>	458 a
	Pea	580 a	24 a
	Lentil	757 a	899 a
	Wheat	650 a	97 a
Saskatoon	Soybean	576 a	192 a
	Pea	556 a	127 ab
	Lentil	526 a	30 b
	Wheat	669 a	60 b

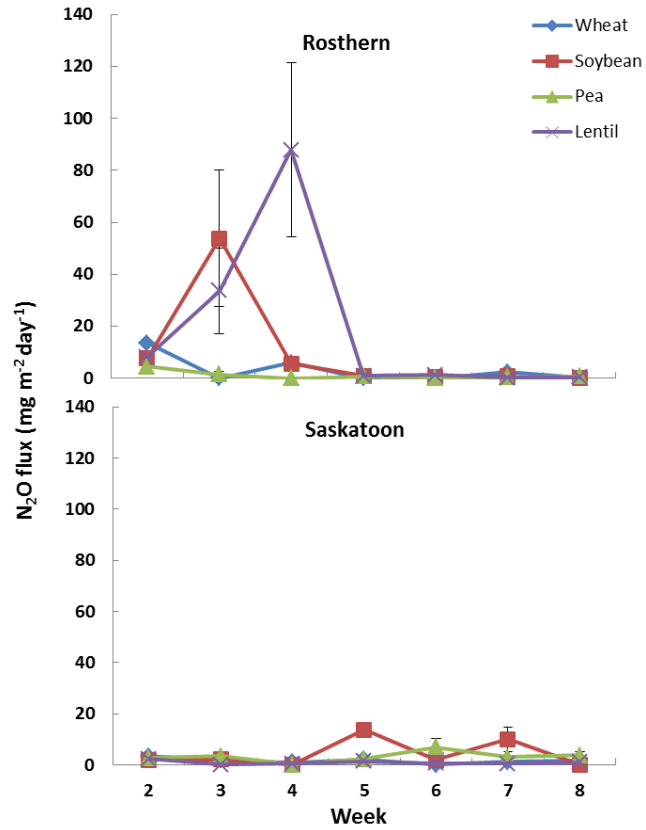
<sup>†</sup> Residue of the crop that was grown on the plot in 2014.

<sup>‡</sup> Within a column, means within a site followed by the same letter are not significantly different from each other ( $p \geq 0.10$ ) according to a Tukey's HSD test.



**Fig. 6.4.** CO<sub>2</sub> fluxes (n=4) during the incubation of intact cores collected from different crop stubbles from the Rosthern and Saskatoon sites. Values are mean daily CO<sub>2</sub> fluxes measured on the same day each week throughout the 8-week incubation period. Error bars represent one standard error. The Rosthern soil CO<sub>2</sub> fluxes were not significantly different among stubbles through the entire incubation period, whereas Saskatoon soil had significantly higher CO<sub>2</sub> flux from soybean stubble than from pea stubble at week 2 according to a Tukey's HSD test ( $p \geq 0.10$ ).



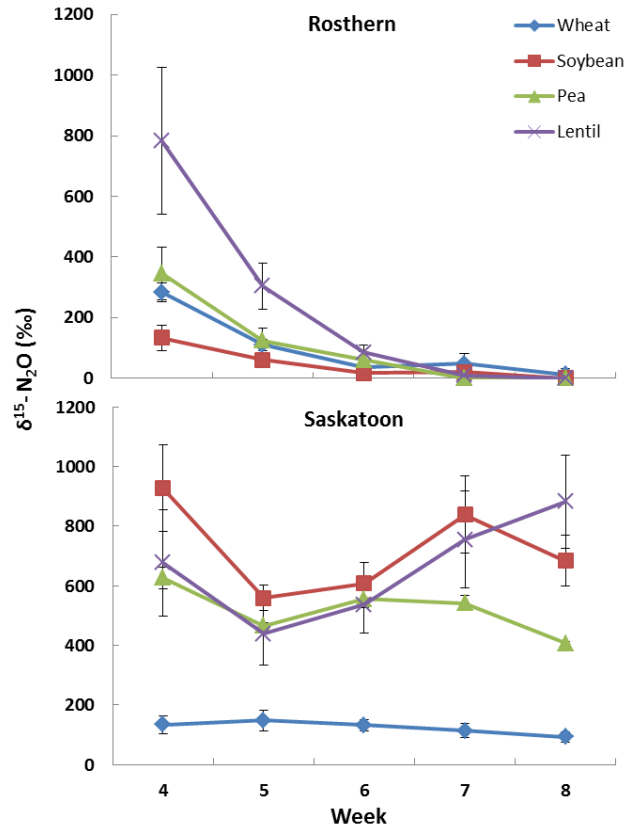


**Fig. 6.5.** N<sub>2</sub>O fluxes (n=4) during the incubation of intact cores collected from different crop stubbles from the Rosthern and Saskatoon sites. Values are mean daily N<sub>2</sub>O fluxes measured on the same day each week throughout the 8-week incubation period. Error bars represent one standard error. N<sub>2</sub>O fluxes from the different crop stubbles were not significantly different during the incubation period for both Rosthern and Saskatoon sites according to a Tukey's HSD test ( $p \geq 0.10$ ).

#### 6.5.4 $\delta^{15}\text{N-N}_2\text{O}$ enrichment

The  $\delta^{15}\text{N-N}_2\text{O}$  is a measure of the degree of N-15 enrichment in the  $\text{N}_2\text{O}$  produced in the incubation. The N-15 found in the  $\text{N}_2\text{O}$  from the incubation originates from the N-15 that was incorporated into the crop plants when it was added to the plots at the beginning of the 2014 growing season. As the N in the stubble residue (straw, roots, and root exudates) is transformed during decomposition, some of the N-15 label makes its way into the  $\text{N}_2\text{O}$ . A declining amount of N-15 in the nitrous oxide indicates a lower proportion of the  $\text{N}_2\text{O}$  originating from the stubble residue and a greater amount coming from non-labelled soil N. At the Rosthern site, the lentil stubble had the highest  $\delta^{15}\text{N-N}_2\text{O}$  at the beginning of incubation period, indicating greater contribution of the lentil residue to  $\text{N}_2\text{O}$  compared to the other crop residues (Fig. 6.6). At this site, the  $\delta^{15}\text{N-N}_2\text{O}$  decreased over the incubation, reflecting less contribution of stubble residue N and more contribution from soil N such as humus N as the incubation proceeded. From the 6<sup>th</sup> week,  $\delta^{15}\text{N-N}_2\text{O}$  in the gas emissions from Rosthern remained rather low, approaching zero  $\delta^{15}\text{N-N}_2\text{O}$  by the end of the 8-week incubation period. This pattern was similar to that observed for the soil N supplies and  $\text{N}_2\text{O}$  fluxes in the Rosthern soil over the incubation period. However, no statistically significant differences were detected in  $\delta^{15}\text{N-N}_2\text{O}$  among different stubble residues in the Rosthern soil at any sampling time during the incubation period.

In contrast to the Rosthern soil,  $\delta^{15}\text{N-N}_2\text{O}$  in the Saskatoon soil stubble residues did not show any clear trends over time (Fig. 6.6). Throughout the incubation, the wheat stubble residue had the lowest  $\delta^{15}\text{N-N}_2\text{O}$ , indicating that least amount of  $\text{N}_2\text{O}$  was derived from the stubble residue of wheat. This is consistent with wheat straw residue having the lowest N content and widest C:N ratio which would increase the amount of residue N that was initially immobilized rather than being converted to inorganic N and ultimately contributing to  $\text{N}_2\text{O}$  production. At the 6<sup>th</sup> week, the  $\delta^{15}\text{N-N}_2\text{O}$  from the soybean stubble residue soil was significantly higher than that from the wheat stubble soil, with pea or lentil stubble soil having intermediate  $\delta^{15}\text{N-N}_2\text{O}$  in the gas emissions. Additionally, in the last week of the incubation, Saskatoon lentil stubble residue soil had significantly higher  $\delta^{15}\text{N-N}_2\text{O}$  than that with the wheat stubble residue, while soil with soybean and pea stubble residues had similar  $\delta^{15}\text{N-N}_2\text{O}$  enrichment in the gas emissions.



**Fig. 6.6.** N-15 enrichment of nitrous oxide ( $\delta^{15}\text{N-N}_2\text{O}$ ) ( $n=4$ ) of the Rosthern and Saskatoon soils during the incubation. Values are the mean  $\delta^{15}\text{N}$  measured for the  $\text{N}_2\text{O}$  on the same day weekly throughout the 8-week incubation period. No significant differences were detected except for the Saskatoon soil at week 6 and week 8, with  $\delta^{15}\text{N-N}_2\text{O}$  being significantly higher from soybean stubble compared to wheat at week 6, and  $\delta^{15}\text{N-N}_2\text{O}$  being significantly higher from lentil stubble than from wheat stubble at week 8, according to a Tukey's HSD test ( $p \geq 0.10$ ).

## 6.6 Discussion

Nitrogen and phosphorus exist in soils in various organic and inorganic forms, with mineral N ( $\text{NO}_3^- + \text{NH}_4^+$ ) and  $\text{PO}_4^{3-}$  as the forms that are readily available for plant uptake and microbial assimilation (Cruz et al., 2009). Soil microbial community is responsible for decomposing the above- and below-ground crop residue that is added to soil during crop growth as well as that which remains after harvest. Through decomposition, nutrients are converted into various forms before being released as inorganic ions, and any factors, biotic or abiotic, that affect the microbial activity can influence the availability of N and P in soils (Ha et al., 2008; Malik et al., 2012; Noack et al., 2014). In the present study, N ( $\text{NO}_3^- + \text{NH}_4^+$ ) and P ( $\text{PO}_4^{3-}$ ) supplies in the Rosthern soil in the fall of 2014 before straw residue return, were similar with soybean, pea, lentil, or wheat grown during the 2014 growing season, implying similar impact on post-harvest soil N and P supplies from the different stubbles, consistent with results shown in Chapter 3. Furthermore, after crop straw residues were returned and intact soil collected and incubated, this similarity continued through the entire 8-week period of incubation for soil available N with different residue additions, indicating comparable impact of soybean, pea, lentil, and wheat stubble residues on the availability of soil N in the Rosthern soil. The Rosthern wheat stubble soil had comparable levels of available N supplies to the legume stubble soils, indicating similar direct N benefits from wheat as the previous crop as opposed to grain legumes over the short-term to a following crop at this site.

Results of the incubation are consistent with the soil N supply rates and plant N uptake assessments that were made in the field the following season as described in Chapter 3, which showed similar soil available N and crop yield effects of different legume stubble residue types. In the Saskatoon soil, the wheat stubble had the lowest soil available N supplies and lentil the highest over the incubation period. This is consistent with the field data for wheat grown in the field at this site the following rotational year, in which wheat on wheat stubble had lower crop N uptake compared to wheat grown on the pulse stubbles (see Chapter 3). Higher soil available N supply rates observed with legume stubbles as opposed to the wheat stubble suggest an improved soil N status with legume stubble residues, presumably due to the N contribution (Stevenson and van Kessel, 1996a; Beckie and Brandt, 1997) and narrower C:N ratios (Arcand et al., 2014a) of legume stubble residues, which is referred to as the N benefits of legumes. For example, by comparing yield responses of barley and canola grown on wheat, canola, and field

pea stubbles on two Chernozem soils in Saskatchewan, Canada, Beckie et al. (1997) estimated that the N contribution of pea were 12, 27, and 28 kg ha<sup>-1</sup> in the three sub-trials in this study, confirming the predominant N benefits as opposed to non-N benefits of legumes and to non-legumes. Similarly, the N contribution by soybean to a succeeding non-legume crop was reported as about 30 kg ha<sup>-1</sup> in a two-year rotation experiment comprising corn-corn and soybean-corn sequence conducted in central Ontario, Canada (Ding et al., 1998).

The Rosthern soil with different crop stubble residue types generally had similar soil P supplies during the incubation, with the exception that the soybean stubble soil had significantly higher available P supplies two times during the incubation (week 6 and 8 periods), which may be explained by higher P concentration in the soybean straw residue. Furthermore, higher soil P supply rates in the Rosthern soybean stubble soil only became statistically significant after 6 weeks of incubation, implying a slow release or turn-over rate of available P from the residue. As P in added crop residue can convert into labile P fractions such as soluble inorganic and organic P forms, as well non-labile fractions like inorganic P in the solid phase or stable organic forms in soils (Malik et al., 2012), the results of the present study suggest that adding P-rich soybean residue can be beneficial to improving soil P availability in the short-term in rotations. Similarly, in a 16-week incubation experiment with an Vertisol soil amended with soybean residue, wheat residue, or P fertilizer, Reddy et al. (2001) found that adding soybean residue increased the soil labile inorganic and organic P levels and caused dissolution of recalcitrant P fractions during the incubation, improving soil P fertility overall. As a result, the stimulation of recalcitrant P dissolution by adding soybean residue, together with removal of larger amount of P in soybean grain as opposed to pea or lentil grain, as shown in Chapter 3, are likely to cause greater depletion of soil P fertility over the longer term when soybean is included in crop rotations in comparison to pea and lentil.

The trend in soil N and P supplies over time in the two soils was a tendency towards decreasing supply rates as the incubation proceeded, especially with soil N in the first five weeks. This decrease of soil N supplies likely reflects a reduced amount of available N released from the recent crop residues over time and is supported by the  $\delta^{15}\text{N-N}_2\text{O}$  data which shows reduced residue N-15 contribution over time. The grain legume stubbles would have sufficiently low C:N ratios to result in net mineralization over the incubation period. In an incubation experiment using an Australian Calcarosol soil, Ha et al. (2008) found in an

incubation that soil soluble C, N, and P decreased during the first 15 days and remained stable thereafter till the end of the 61-day incubating period. The difference between the results of Ha et al. (2008) and the current study may reflect different soil properties (e.g., 81% sand in their study in comparison to 27% sand in Rosthern soil), crop stubble type and rotation history, amount of added residue, and different soil-climatic conditions. All these factors can influence the microbial activity, nutrient requirements, residue decomposition, nutrient release rate, and GHG emissions over time in soils (Spargo et al., 2011; McDaniel et al., 2014).

Not unexpectedly, the Saskatoon wheat stubble soil had significantly less soil available N supplies than the lentil stubble soil from the second week till the end of the incubating period, indicating greater direct soil N benefits of the lentil stubble as opposed to wheat in the Saskatoon soil. This agrees with the findings of previous studies conducted in Saskatchewan soils (Stevenson and van Kessel, 1996a; Beckie and Brandt, 1997; Beckie et al., 1997). For available P, different crop stubbles resulted in similar soil available P release during the incubating period in the Saskatoon soil, supporting comparable effects on soil supplies of available P measured in the field in 2015 on the different crop stubbles as covered in detail in Chapter 3. Similar to the Rosthern soil, the Saskatoon soil showed a decreasing trend in soil available N with the incubation time but to a much less degree, and the soil P supply rates in the Saskatoon soils at the end of the incubation period were quite similar to the levels at the beginning of the incubation. Different patterns in available N and P dynamics in the Saskatoon soil as opposed to the Rosthern soil indicated that soil N mineralization and soil microbial activity in the two soils are influenced to a different degree. Organic matter levels in the 0-15cm depth of the two soils are similar (~3.5% organic carbon at both sites) despite the Saskatoon site being located in the Dark Brown soil zone and the Rosthern site being located in the Black soil zone, because the Saskatoon site is located in a lower elevation depression in the landscape which tends to have higher organic matter. However, salinity at the Saskatoon site as described in Chapter 4 and reflected in the high EC of this soil (see Table 4.2 in Chapter 4) may also be a factor affecting microbial activity and decomposition rates. Conditions of high soil salinity can result in smaller, more stressed, and less metabolically efficient soil microbial communities, hence different soil mineralization and immobilization patterns in comparison to soils without salinity (Rietz and Haynes, 2003). Correspondingly, Wichern et al. (2006) reported that increasing soil salinity was related to reduced decomposition of maize residue

added into the soil, and a microbial community that is less efficient in decomposing added substrate, which might have been the case at the Saskatoon site. Additionally, the N<sub>2</sub>O emissions from the Saskatoon soil after week 5 showed an opposite trend to that from the Rosthern soil, tending to increase, suggesting a different nature of the mineralization-immobilization processes.

Available N supplies in Rosthern soil reached near zero at around week 5 and remained low thereafter, consistent with the pattern of CO<sub>2</sub> and N<sub>2</sub>O fluxes, and  $\delta^{15}\text{N-N}_2\text{O}$  in this soil, no matter with what type of residue amendments. The higher initial release rates of available N and P, CO<sub>2</sub> and N<sub>2</sub>O and the more rapid decrease in ion supply rates and greenhouse gas production over time in the Rosthern soil compared to Saskatoon soil for all stubble treatments is likely a consequence of more rapid and intense transformation and decomposition of residues in the Rosthern soil. This is consistent with favourable conditions for decomposition in this soil compared to the salt-affected Saskatoon soil.

The CO<sub>2</sub> fluxes demonstrated a decrease at week 5, and increased again after week 5, underlining a transition from reaching low levels of soil microbial respiration to recovering to higher levels possibly because of a transition of microbial community composition from species that were consuming and depleting labile fractions of the substrate to one with species effective at decomposing more recalcitrant pools. Although Marschner et al. (2011) reported that the composition of the soil microbial community changed the fastest within the first 1-2 weeks of incubation and slowed down thereafter, in later stages of decomposition the dominant microbial community decomposition was reported to experience a transition from copiotrophs and r-strategists to oligotrophs and K-strategists (Bastian et al., 2009). Microbial factors such as species composition at the later stage were reported to have a more important role in affecting decomposition of added residue than other factors such as biochemical composition of the residue and the initial composition of the soil microbial community (Bray et al., 2012). In the present study with the Rosthern soil, from week 5 the population of microbes that were capable of decomposing more recalcitrant fractions in the soil likely started to expand, consuming soil available N and resulting in less soil available N and N<sub>2</sub>O fluxes but higher soil respiration, i.e. more CO<sub>2</sub> fluxes but less N<sub>2</sub>O fluxes (van Groenigen et al., 2005). Moreover,  $\delta^{15}\text{N-N}_2\text{O}$  also decreased around week 5, approaching zero thereafter, strongly

supporting the depletion of residue-introduced nutrients in the soil with time and that the depletion reached a threshold point at around week 5.

Overall, different legume crop stubble residues present in the two Saskatchewan soils did not result in consistent differences in soil supplies of N and P, or CO<sub>2</sub> and N<sub>2</sub>O emissions from either of the two soils during the incubating period, although in the Saskatoon soil leguminous residues amended did result in higher cumulative soil N supplies compared to wheat residue as expected, reflecting the N benefits of grain legumes as opposed to non-legumes, due to decomposition of both above- and below- ground residue as well as N rhizodeposition (Lupwayi and Kennedy, 2007). For example, in a greenhouse study, N derived from rhizodeposition, which refers to N released into the rhizosphere from living and decaying roots, was reported to be 9% greater from pea than from canola (Arcand et al., 2013). The N contribution from pea as the previous crop as opposed to canola, was estimated as high as 27 kg N ha<sup>-1</sup> in a field experiment conducted in Saskatchewan (Beckie and Brandt, 1997), presumably due to factors such as higher levels of postharvest soil available N after legumes (Campbell et al., 1992), narrower C:N ratios of the legume residue (Kumar and Goh, 2003), and stimulation of N mineralization with legume residue incorporation (Birch and Dougall, 1967). Furthermore, by reviewing publications on N<sub>2</sub> fixation by legumes commonly grown on the northern Great Plains, Walley et al. (2007) suggested that despite the great variation of N benefits of legumes, legume species with higher levels of N<sub>2</sub> fixation, including faba bean, field pea, and lentil, tended to have more N benefits in comparison to species with lower levels of N<sub>2</sub> fixation. However, only one study on soybean was included in this review paper, reflecting limited number of studies in evaluating the N contributions of soybean in this region. The current study showed that levels of N<sub>2</sub> fixation were relatively similar among the three pulse crops, including soybean (see chapter 5). Although pulse grain yield has been suggested as a parameter for providing N credits in fertilizer recommendations for following crops in rotation (Beckie and Brandt, 1997), above-ground straw yield and composition or harvest index is likely a better indicator for the contribution. However, it is difficult to practically obtain straw yield values for grower fields, and below-ground contributions would be very challenging to predict.

## **6.7 Conclusion**

This study revealed that under controlled environment conditions of an 8-week incubation of intact soil cores collected from two field research sites (Rosthern and Saskatoon),



legume crop stubbles, including soybean, pea, and lentil, had similar impact on soil supplies of available N ( $\text{NO}_3^-$ -N+  $\text{NH}_4^+$ -N). This is in agreement with results obtained in the field in 2015 as described in Chapter 3. In one of the two soils (Saskatoon), the soybean stubble residue resulted in more available  $\text{PO}_4^{3-}$ -P release than pea, lentil and wheat stubbles, explained by a greater P content in the soybean residue. Soil available N and P supplies decreased with time in the Rosthern soil regardless of stubble types. In the Saskatoon soil the lentil stubble had higher N supplies from the second week of incubation till the end, whereas soil P supplies in different stubbles were similar through the entire incubating period. Different patterns of soil supplies of available N and P are attributed to differences in soil conditions at the two sites, particularly the salinity conditions at the Saskatoon site which appears to have reduced and delayed the decomposition of the crop residues. Correspondingly,  $\text{CO}_2$  fluxes from both soils demonstrated a decrease at around week 5 and started to recover thereafter, suggesting a transition of soil decomposing mechanisms at around this time. The Rosthern soil had similar  $\text{CO}_2$  fluxes among stubble types through the entire incubation period, whereas the Saskatoon soil amended with the soybean residue had a higher  $\text{CO}_2$  flux than that amended with the pea residue at the second week of the incubation. By the end of this 8-week incubation, cumulative  $\text{CO}_2$  and  $\text{N}_2\text{O}$  emissions in the different stubble types were not significantly different among residue stubbles in either soil, except that Saskatoon soybean stubble residue soil had higher cumulative  $\text{N}_2\text{O}$  emissions than lentil and wheat stubble residues. In the Rosthern soil,  $\delta^{15}\text{N}$ - $\text{N}_2\text{O}$  results were consistent with dynamics of soil N supplies and  $\text{N}_2\text{O}$  fluxes, reflecting the depletion of residue-derived substrate and a transition of the composition of the microbial community at around week 5. In contrast, the Saskatoon soil did not show any clear decreasing or increasing trends in  $\delta^{15}\text{N}$ - $\text{N}_2\text{O}$  with time, possibly reflecting a stressed microbial community due to high salt conditions. The impact of different stubble types over a greater number of rotational cycles deserves attention in the future.

## 7. SYNTHESIS AND CONCLUSIONS

The research presented in this Ph.D. dissertation is the first to systematically investigate several important agronomic, plant nutrition and soil fertility implications of soybean production under western Canadian soil-climatic conditions. In particular, this research investigates grain and straw yield, nutrient (N, P, K, S, Mg, Ca, B, Cu, and Zn) concentration and uptake, and N<sub>2</sub> fixation of three modern short-season soybean varieties, in comparison to three pea varieties and lentil varieties, and the effects on the yield, nutrient uptake, and recovery of N from crop residue by a following wheat crop. To cover the range of anticipated growing conditions on the prairies, the field research trials were conducted at four sites: two in Dark Brown and two in Black soil zone in Saskatchewan, Canada. In addition, greenhouse gas emissions and soil supply rates of N and P in soybean, pea, and lentil stubbles were also assessed in this research. The <sup>15</sup>N methods were used to assess the N<sub>2</sub> fixation by the legumes in the first study year (2014), the recovery of crop residue N by a spring wheat crop grown in the following year (2015), and presence of <sup>15</sup>N label in soil N fractions and N<sub>2</sub>O emissions from soils containing <sup>15</sup>N-enriched residues. The results can provide researchers, producers, and policy makers with new information on implications of including soybean in rotation on nutrient cycling, balance and availability, short and long-term fertilizer requirements, and environmental impact in the northern Great Plains.

### 7.1 Key findings and conclusions

The three short-season soybean varieties (cv. P001T34R, TH3303R2Y, and NSC Moosomin) produced similar grain yield (929-3534 kg ha<sup>-1</sup>) and straw yield (3799-6956 kg ha<sup>-1</sup>), grain uptake of N (40-168 kg N ha<sup>-1</sup>) and P (6-21 kg P ha<sup>-1</sup>), and high levels of K, Ca, Mg, and S uptake in comparison to three pea varieties (cv. CDC Meadow, CDC Amarillo, and CDC Limerick) and lentil varieties (cv. CDC Impower, CDC Invincible, and CDC Maxim) grown at the four sites (Rosthern, Saskatoon, Scott, and Yorkton sites). Soybean grain yield is comparable to results of

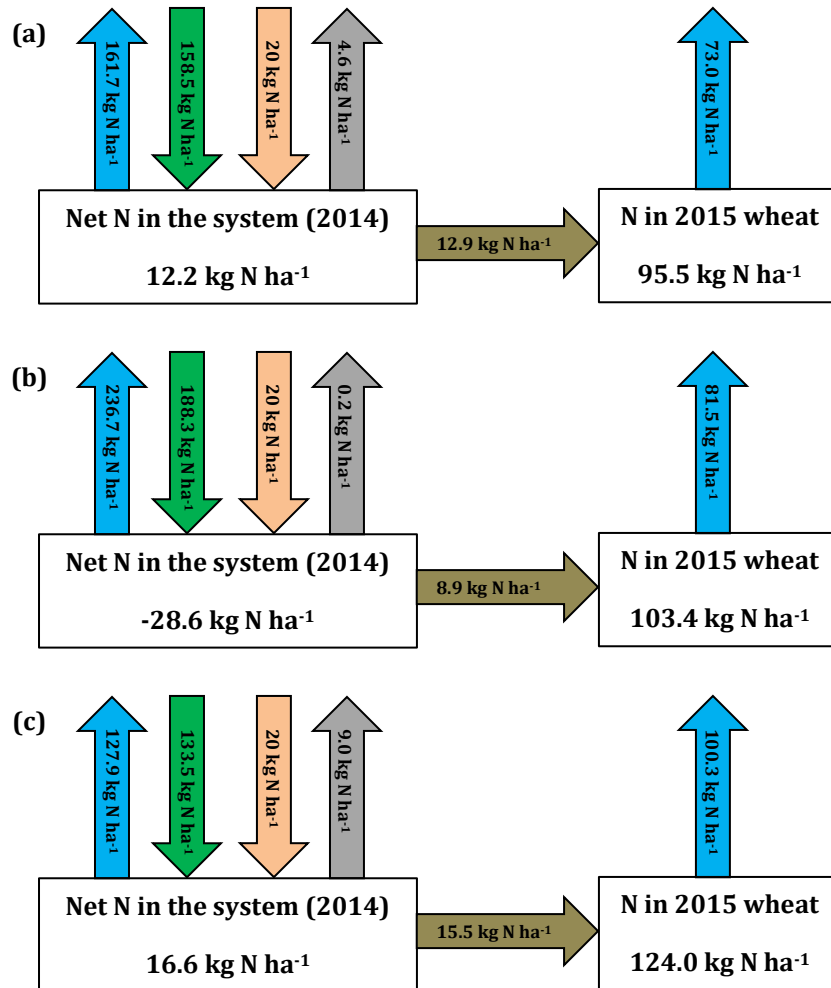
previous studies conducted in western Canada, but about 20% lower than results from eastern Canada or mid-western USA. At the Rosthern site, pea had particularly high grain yield in 2014 compared to soybean and lentil, resulting in high levels of the other parameters measured for pea, including nutrient uptake, N<sub>2</sub> fixation, and harvest indexes at this site. Lentil generally had lower grain yield and harvest indexes compared to soybean and pea across the sites.

Soybean generally had higher grain but similar straw N and P concentration compared to pea and lentil, indicating a different allocation pattern of N and P between different plant components of soybean, and considerable removal of N and P via soybean grain harvest. Indeed, soybean had higher harvest N indexes (HNI) and harvest P indexes (HPI) at two of the four sites. Lentil generally had low HNI and HPI, reflecting relatively even partitioning of N and P in its grain and the straw. Unlike the partitioning of plant N, the partitioning of fixed N between grain and straw was relatively more even in soybean than in lentil, implying different patterns among the crops regarding soil-N-assimilating, N-fixing, and remobilization mechanisms through different growth stages. In addition, the C:N ratios in soybean straw were higher than that in pea or lentil straw at two sites (Scott and Yorkton sites). The generally lower C:N ratio in lentil straw may have contributed to observed greater release of available N in the early part of the following growing season. Overall, soybean, pea, and lentil as previous crops did not produce large, consistent differences in the yield, N and P uptake in wheat or canola crops grown the year after. This indicates that fertilizer N and P recommendations for cereal or oilseed crops immediately following soybean are not likely to require significant adjustments compared to other pulse crops, although greater P concentration in soybean seed suggests greater potential for P depletion from this crop grown in long-term rotation compared to other pulses.

Nutritional value apart from N and P was evaluated in this research as little or no information exists for soybeans grown under prairie conditions. Soybean had higher concentration of K, Ca, Mg, and S, and similar Cu and Zn in the grain than pea and lentil across the sites. For the straw, soybean, pea, and lentil had inconsistent patterns. Accordingly, soybean had large removal of K, Mg, Ca, and S in the grain, and lentil often had significantly lower grain removal of these elements, reflecting greater potential for depletion of these elements from the soil when soybean is grown, although the Ca depletion is not a big concern for prairie soils. In the subsequent year, spring wheat generally showed no response to

different legume residues in terms of these elements. Therefore, soybean has good nutritional value regarding the evaluated elements, while monitoring soil element levels, especially K and Mg along with P is necessary when soybean is grown for several rotation cycles.

Considering  $N_2$  fixation, soybean proved to be a good N fixer in this study. Nitrogen derived from atmosphere (Ndfa) ( $159 \text{ kg ha}^{-1}$ ) comprised over 70% of the total above-ground plant N in soybean, as opposed to 62% in pea ( $188 \text{ kg ha}^{-1}$  Ndfa) and 62% in lentil ( $134 \text{ kg ha}^{-1}$  Ndfa). The majority of fixed N was retained in the grain, with  $119 \text{ kg N ha}^{-1}$  (75% of total above-ground fixed N) in soybean grain,  $160 \text{ kg N ha}^{-1}$  (85%) in pea grain, and  $89 \text{ kg N ha}^{-1}$  (67%) in lentil grain. At maturity, the amount of fixed N in the straw was similar among the crops, but in the grain it was higher in pea than in soybean and lentil. Percentage of N derived from atmosphere (%Ndfa) in the grain was slightly higher in soybean (74%) than in pea (68%) and lentil (70%), but the difference was statistically insignificant. The straw had a similar pattern regarding the %Ndfa levels. It is noteworthy that unlike pea or lentil, which had higher %Ndfa levels in the grain than in the straw, soybean had similar %Ndfa between the grain and the straw, suggesting a more uniform fixation rate in soybean through the growing season from early vegetative stages to the reproductive stage in comparison to pea and lentil. In the subsequent year, spring wheat grown on soybean, pea, and lentil stubble had similar N uptake and percentage of N derived from the above-ground residue (%Ndf<sub>r</sub>) of the previous year, while wheat grown on the wheat residue (the control) generally had lower levels of N uptake and %Ndf<sub>r</sub>. The total amount of N in wheat recovered from previous above-ground residue was higher from the lentil residue than the pea residue, possibly due to the slightly higher straw N in lentil ( $86 \text{ kg ha}^{-1}$  with  $45 \text{ kg ha}^{-1}$  derived from  $N_2$  fixation) than in pea ( $68 \text{ kg ha}^{-1}$  with  $28 \text{ kg ha}^{-1}$  derived from  $N_2$  fixation) and lower C:N ratio. Overall, residue N recovery rates in wheat were similar among the three legume above-ground residues from the previous year, reflecting similar N release ability and N contribution to following crops from soybean, pea, and lentil above-ground residue under Saskatchewan conditions. Results of  $N_2$  fixation support the observed lack of differences among pulse crop stubbles in yield and N uptake of wheat grown in the following year at the four sites. Predictions of N contribution/credits from pulses as previous crop in rotation may be better made from straw yield or harvest index rather than just grain yield. Estimates would be further improved with a below-ground component. An illustration of N budgets synthesized from the results of this research is shown in Fig. 7.1.



**Fig. 7.1.** N budgets for (a) soybean, (b) pea, and (c) lentil as synthesized from results of the Rosthern site. The system is defined as the plant-soil system, so the straw N of the crops, which was returned to the field after harvest, was not considered as N flow across the boundaries of the system. Arrows with different colors represent different net N flows across the boundaries of the system: Blue=N exports via grain harvest of legumes in 2014 and of wheat in 2015; green=N fixed in the above-ground plant part via N<sub>2</sub> fixation; orange=N inputs via 15N-enriched fertilizer; grey=N losses via N<sub>2</sub>O emissions over an 8-week period; brown=above-ground legume residue N recovered by the above-ground plant parts (grain and straw) of wheat in 2015. Note that below-ground component is not included in this model.

Consistent with the similar residue N recovery rates and total N uptake by spring wheat grown on soybean, pea, and lentil residues, the cumulative soil N supplies under the wheat crop with soybean, pea, or lentil residues were similar by the end of the 2015 growing season at the Rosthern site. Similarly, in the 8-week incubation experiment conducted under controlled conditions, intact soil cores taken after harvest from the soybean, pea, and lentil stubbles had similar soil available N supplies through the incubation period. The soil P supply rates were higher with soybean residue than with lentil residue in the field through most of the growing season at the Rosthern site but similar with different residue additions in the incubation experiment, possibly due to different environmental conditions between the field and the incubation experiment. Conditions of the field such as wet-dry cycles possibly have resulted in particular enhancement of short-term P release from the soybean residue by physical leaching or microbial immobilization-mineralization processes. Soil P supply rates measured at the Saskatoon site during the growing season of 2015 showed no response to different residues. The presence of salts near the soil surface at the Saskatoon site due to a high water table appeared to reduce and delay residue decomposition and thus nutrient release from residues of the previous year.

The CO<sub>2</sub> and N<sub>2</sub>O gas emissions were generally similar with different legume residue additions in this research. Isotopic N measurements of the N<sub>2</sub>O gas fluxes from the Rosthern soil in the incubation experiment implied the depletion of residue-derived substrate and a transition of the composition of the microbial community during residue decomposition, consistent with the dynamics of soil N supplies and N<sub>2</sub>O fluxes. The Saskatoon soil did not show any clear patterns regarding  $\delta^{15}\text{N-N}_2\text{O}$  enrichment, possibly reflecting a stressed microbial community due to presence of salinity at this site. Overall, results of the greenhouse gas emission assessment suggest a similar effect from the soybean residue regarding greenhouse gas emissions in Saskatchewan soils compared to pea or lentil residue.

Overall, selected soybean varieties showed comparable agronomic and environmental impacts compared to pea and lentil in this research. Soybean is a good yielder and N fixer with similar contribution to nutrition of following crops compared to pea and lentil grown under similar conditions. This research suggests promising prospects for soybean production under the soil-climatic conditions in the northern Great Plains, with considerations for extra soil P and K depletion in the long term when soybean containing rotations are used for several years.

## 7.2 Future research

The research presented in this dissertation found general similarity in short-season soybean compared to pea and lentil regarding the grain and straw yield, nutrient uptake, N<sub>2</sub> fixation, partitioning of nutrients and fixed N, and in patterns, although different patterns within the season were observed. The rotational benefits of legumes can vary with different cropping systems, such as legume species, crop sequences, and the frequency of legumes included in a crop rotation (Wright, 1990; Soon and Arshad, 2004b; Knight, 2012). Although not observed in this study, differences in relative growth and yield among pulse crops that are more different and extreme may produce significantly different contributions to nutrition of following crops. For example, environmental conditions such as a very wet growing season may result in very poor lentil growth and yield while favoring soybean, such that N<sub>2</sub> fixation, nutrient uptake and removal and harvest would be vastly different among the crops, and significantly affect the nutrient contributions to following crops. Therefore expansion of the database to include nutrient uptake, removal and predicted contributions of the crops under more extreme environmental conditions would be desirable. It seems likely that yield itself could be a valuable predictor of nutrient removal and contribution. In addition, larger C:N ratios of soybean straw at two of the four sites may have not caused significant differences regarding the short-term rotational benefits of soybean compared to pea and lentil, but in the long term the different ratios of C:other nutrients can cause significant impact on soil organic matter and soil nutrient availability to crops in rotation. In addition, it should be noted that, in the research presented in this dissertation, the site factor generally had significant influence on the evaluated parameters. Therefore, future research on soybean production in the northern Great Plains should include a greater number of rotational cycles and compare a variety of crop sequences with different legume species grown with various frequencies at different sites.

In the present study, only the above-ground plant parts (grain + straw) were assessed for yield, nutrient uptake, and N<sub>2</sub> fixation. However, as reported by Arcand et al. (2014), the below-ground plant N can comprise a considerable amount of total plant N and therefore, future studies should integrate measurements of N<sub>2</sub> fixation, nutrient uptake, and nutrient release in both above-ground and below-ground plant parts, including rhizodeposition during the year when legumes are grown. Although this integration is still rather challenging, especially under field conditions, application of <sup>15</sup>N methods, possibly combined with <sup>13</sup>C

methods, might provide a way to achieve this goal. Likewise, using stable isotopic methods will also provide researchers with the possibility to close the N cycle in an agroecosystem by accurately assessing N losses from the systems via pathways such as nitrification, denitrification, ammonia volatilization, and leaching. So far these pathways remain difficult to quantify due to measurement challenges and great spatial and temporal variation, but they should be accounted for as they are an important part of the N cycle in agroecosystems.

Regarding the potential limitations of heat units and precipitation that may be anticipated in western Canada, soybean varieties are likely to demonstrate various levels of tolerance during the growing season, resulting in different yield, N<sub>2</sub> fixation, and rotational benefits, as suggested by Purcell et al. (1997). In the current study, varietal effects were generally small. However, knowledge on the relationship between varietal tolerance to environmental stress, differences in growth pattern and biomass accumulation to nutrient uptake, N<sub>2</sub> fixation, N partitioning, and water use efficiency, is required to make sound recommendations for suitable varieties for a particular region. Warmer, wetter conditions encountered in many regions on the Canadian prairies in the past few years and the development of more suitable varieties for short growing season conditions have fueled grower interest in soybean production, and contributed to expansion of acres. This expansion seems likely to continue. With the assistance of data from cropping system research conducted over several years with soybean as a rotational crop, models including crop, soil, and social-economic parameters can be developed to provide comprehensive information on the agronomic, environmental, and economic implications of expanded soybean production in the northern Great Plains.



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## **APPENDIX A: YIELD, NITROGEN AND PHOSPHORUS UPTAKE OF WHEAT GROWN ON TWO SASKATCHEWAN SOILS AMENDED WITH SOYBEAN, PEA, AND LENTIL STRAW RESIDUE**

### **A.1 Introduction**

Nutrient recovery efficiency, such as nitrogen recovery efficiency (NRE) and phosphorus recovery efficiency (PRE), refers to the amount of nutrient in the crop as a ratio of the amount applied or available. It is commonly calculated by the nutrient difference between the control group and the fertilizer-receiving group. Many factors regulate the nutrient recovery efficiency and decomposition processes of crop residues, such as soil microbial, soil aeration conditions, and initial biochemical composition of the added crop residue (Kumar and Goh, 2003; Walela et al., 2014). Under the same environmental conditions, the biochemical composition of crop residue serves as the most important factor influencing the nutrient recovery efficiency, by restraining the microbial access to breaking down and decomposing the residue and thus releasing the available nutrients for plant use (Lupwayi and Soon, 2009). The objective of this incubation study was to assess the nutrient release and recovery efficiencies of soybean, pea, and lentil above-ground residues in two Saskatchewan soils under controlled environmental conditions in comparison to N fertilizer applied at different rates.

### **A.2 Materials and methods**

Soybean, pea, and lentil dry residues were collected in the first week of October, 2014, from the Crop Development Center pulse crop field research plots located at south Preston Avenue, Saskatoon. The soybean, pea, and lentil were grown in the same small plot area under the same fertility regime. The residue collected was the above-ground material, including mature stalks, pods, and leaves left after the seed had been removed. The stalk material was cut into pieces of about 2cm lengths, as what would normally happen when harvesting with a combine and the stalks are passed through a straw chopper. The top 15cm of two contrasting Brown Chernozemic soils (wheat stubble), including (1) Echo association sandy loam soil (Echo) from the upslope of a field in cereal-fallow rotation with little or no fertilizer added for the past 80 years, and (2) Ardill association clay loam soil (Ardill) from a low slope that has a history of fertilization and grain legume crops grown in rotation were collected near Central Butte, Saskatchewan. The processed residues were mixed with the soils thoroughly, with the application amount of each of the residues calculated according to the typical harvest index of

each crop under Saskatchewan conditions (Table A.1). Additionally, to compare the impact of the residue amendments with commercial fertilizer amendment on wheat yield and nutrient uptake, urea was applied to soils at three rates, namely, 50 mg N kg<sup>-1</sup> soil, 100 mg N kg<sup>-1</sup> soil, and 200 mg N kg<sup>-1</sup> soil. To avoid deficiencies of other nutrients, sulphate and phosphorus fertilizers were added in the form of calcium phosphate monobasic (Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>) and potassium sulfate (K<sub>2</sub>SO<sub>4</sub>) at the rate of 25 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> soil and 25 mg S kg<sup>-1</sup> soil to all pots.

One kilogram of soil was weighed out and put into plastic 15cm diameter pots, with each treatment having four replicates. Before seeding, the soil water content was brought up to field capacity by slowly adding water until water freely drained out the bottom of the pots followed by an overnight equilibration. Wheat was seeded by placing 7 seeds per pot approximately 3 mm below the soil surface, and thinned to four plants per pot after emergence. Growing conditions were set as 18h/6h for day/night lengths, and 22°C/16°C for day/night temperature. The soil water content was maintained at around field capacity throughout the experiment, and plants were harvested seven weeks after seeding. Pre-seeding available nutrient concentration and nutrient content in soybean, pea, and lentil residues were analyzed by ALS Laboratory Group Agricultural Services. Wheat dry matter yield of each pot (4 plants), total N, and total P of the wheat plants (above-ground part) were determined. Total N and P in the above-ground plant were determined by sulfuric acid peroxide digestion (Thomas et al., 1967) followed by Technicon automated colorimetry. SAS 9.4 programme was used to conduct statistical analyses.

**Table A.1.** Calculation of residue rates applied in 1<sup>st</sup> incubation study.

Crop type	Grain yield	Straw yield	Harvest index <sup>†</sup>	Pot area	Residue rate
	kg ha <sup>-1</sup>			cm <sup>2</sup>	g pot <sup>-1</sup>
Soybean	2000	3000	0.40	182.42	5.47
Pea	2500	5080	0.33	182.42	9.27
Lentil	1500	2350	0.39	182.42	4.29

<sup>†</sup> Harvest index (HI) were obtained from published data (Bolinder et al., 2007; Gan et al., 2009a).

### **A.3 Results and discussion**

The Ardill soil had more extractable N, P, and other soil nutrients than the Echo soil (Table A.2), reflecting the Ardill soil's position in the lower slopes of the field landscape. Soybean, pea, and lentil residues differed in nutrient content regarding different nutrients (Table A.3). For example, soybean residue had the highest K concentration, whereas pea residue had the highest total N and lentil residue had the highest P concentration. This is likely a consequence of genetic differences among the crops in relative nutrient requirements that are subsequently expressed in the nutrient content of the residue.

The dry matter yield was similar among residues after 7 weeks of growth in either soil, with wheat yield on lentil residue reaching a comparable level to 50 mg N kg<sup>-1</sup> fertilizer (Table A.4). Overall, adding soybean, pea, or lentil residues at the rate of what is typically returned to the field on the prairies did not improve wheat dry matter yield within the first seven weeks of growth compared to the control group. In contrast, the addition of N fertilizer resulted in large and significant yield increases compared to the control group, indicating that N availability was a major limitation on dry matter yield in these two soils. Soybean residue had similar wheat N uptake to the control in the Ardill soil, whereas pea and lentil residues increased wheat N uptake to a similar level to 50 mg N kg<sup>-1</sup> fertilizer. In the Echo soil, pulse residues had similar wheat N uptake to the control. Pulse residues did not improve the wheat P uptake compared to the control in either soil, possibly due to the basal addition of P fertilizer to all treatments. Wheat from both the control and residue-amended groups might have sufficient P for their growth in the first seven weeks, resulting in no response to additional P added in the residues.

Wheat generally had comparable NRE with pulse residues and urea additions in the Ardill soil, with the highest NRE from the lentil residue. In the Echo soil, only the lentil residue improved wheat NRE compared to the control. Wheat NRE on pulse residues in the Echo soil was lower than in the Ardill soil, possibly reflecting the overall lower biological activity and N recycling ability of the Echo soil. In the Echo soil, soybean and pea residues had negative NRE, possibly due to net N immobilization and a corresponding decrease of soil available N, especially when the soil had a low available N content to begin with (Table A.2). Correspondingly, N and P uptake by wheat on pulse residues were higher in the Ardill soil than in the Echo soil.



**Table A.2.** Selected characteristics of Ardill soil and Echo soil

Soil	Depth cm	Texture	pH	NO <sub>3</sub> -N	P	K	SO <sub>4</sub> <sup>2-</sup> -S Cu Mn Zn B Fe					
							kg ha <sup>-1</sup> 0-15cm					
Ardill	0-15	Loam	7	9	>67	>672	>54	3	35	8	3	496
Echo	0-15	Loam	8	4	40	>672	4	1	13	2	3	19

**Table A.3.** Nutrient concentration of soybean, pea, and lentil residue

Residue	TN	P	K	S	Ca	Mg	B	Cu	Fe	Na	Zn	Mn
	%				mg kg <sup>-1</sup>							
Soybean	0.76	0.05	1.11	0.09	1.44	0.87	45.1	6.9	2410	<100	10	86.8
Pea	1.27	0.06	0.57	0.13	2.45	0.60	28.9	3.5	531	180	6.2	37.1
Lentil	1.11	0.09	0.42	0.11	1.13	0.48	25	6.2	722	290	10.7	31.4
D.L.†	0.05	0.01	0.01	0.01	0.01	0.01	3	2	5	100	5	3

†The reporting limits

**Table A.4.** Wheat yield, N uptake, P uptake, N recovery efficiency (NRE), and P recovery efficiency (PRE) (n=4) in the Ardill and the Echo soil.

Soil	Treatment	Added N	Added P	Yield	N uptake	P uptake	NRE	PRE
		mg kg <sup>-1</sup> soil						
Ardill	Urea_1	50	25	1256 A <sup>†</sup> bc <sup>‡</sup>	11.0 Ab	4.1 Ab	15 Aa	7 Ab
	Urea_2	100	25	1608 Ab	11.8 Ab	4.2 Ab	9 Aab	3 Ab
	Urea_3	200	25	3072 Aa	17.9 Aa	7.8 Aa	10 Aab	5 Ab
	Soybean	42	28	700 Acd	7.4 Adc	2.4 Ac	3 c*	3 b*
	Pea	118	31	873 Acd	9.4 Abc	3.4 Abc	4 c*	34 a*
	Lentil	48	29	1033 Abcd	10.3 Ab	3.4 Abc	13 Aab	52 Aa
	Control	-	-	678 Ad	6.8Ad	2.4 Ac	-	-
Echo	Urea_1	50	25	853 Abc	5.3 Bbc	2.2 Bb	10 Aa	5 Aa
	Urea_2	100	25	1476 Ab	8.1 Bb	2.9 Bb	10 Aa	4 Aa
	Urea_3	200	25	2708 Aa	15.4 Ba	4.4 Ba	12 Aa	3 Ba
	Soybean	42	28	393 Bd	2.5 Bd	0.6 Bc	0 **	-6 **
	Pea	118	31	344 Bd	2.3 Bd	0.6 Bc	0 **	-3 **
	Lentil	48	29	463 Bcd	3.4 Bcd	0.8 Bc	3 Bb	4 Ba
	Control	-	-	367 Bd	2.6 Bd	0.7 Bc	-	-

† Within each column, means followed by the same capital letter are not significantly different (p≥0.05) between the two soils with the same fertilizer treatment according to Tukey's HSD test

‡ Within each column, means followed by the same lowercase letter are not significantly different (p≥0.05) among different fertilizer treatments in the same soil according to Tukey's HSD test

\* Tukey's HSD test was not conducted for practical reasons.

## **APPENDIX B: GREENHOUSE GAS EMISSIONS FROM TWO SASKATCHEWAN SOILS AMENDED WITH SOYBEAN, PEA, AND LENTIL RESIDUES**

### **B.1 Introduction**

Soil microbial activity is affected by many factors, such as soil water content, pH, temperature, redox conditions, and the quantity and quality of available substrate (Walela et al., 2014). In agricultural systems, crop residues returned to the soil constitute a large part of the substrate for microbial growth. Fresh residues added into soil generally undergo a series of decomposition and mineralization processes performed by different groups of enzymes and soil microbes, through which the nutrients contained in the residues are transformed and ultimately released as inorganic ions, thereby becoming available for plant uptake and soil microbial assimilation, or prone to losses from the plant-soil system (Mary et al., 1996). During the decomposition, some of the C and N in the substrate are used for microbial cell synthesis and a portion is evolved from the soil system as through respiration (Ha et al., 2008). Some added N can be lost from the soil system through denitrification, a process of nitrate reduction involving production of a series of intermediate gaseous oxide products as well as dinitrogen gas (Miller et al., 2008). Among these gaseous products, N<sub>2</sub>O is a greenhouse gas with a large global warming potential. Therefore, estimating CO<sub>2</sub> and N<sub>2</sub>O emissions from soils with different crop residues can help tracking the N released from the residues and assessing the environmental impact of the residues. As a complement to the first incubation study that examined the release of available nitrogen and phosphorus and its uptake by wheat plants (see Appendix A), this incubation study was conducted to provide more insight into the nutrient release from soybean, pea, and lentil residues and the production and evolution of gaseous compounds including CO<sub>2</sub> and N<sub>2</sub>O.

### **B.2 Materials and methods**

Soybean (cv. TH3303R2Y), pea (cv. CDC Meadow), and lentil (cv. CDC Maxim) were grown in plastic 15cm diameter pots containing 1 kg soil with four plants per pot under controlled conditions in 2014. <sup>15</sup>N-labeled Ammonium Nitrate (<sup>15</sup>NH<sub>4</sub>)(<sup>15</sup>NO<sub>3</sub>) fertilizer with an enrichment of 10% was applied at the rate of 20 mg N kg<sup>-1</sup> soil for three times before the flowering stage of the plants. Soil water content was maintained at field capacity through the

growth period and plants were harvested at maturity. After harvest, grains were removed and the remainder of the crop residue was dried in an oven for 48 hours at the temperature of 40°C and ground using a Wiley Mill.

An Ardill clay loam soil with wheat grown in the preceding year was used (see Appendix A). The air-dried soil was mixed thoroughly with ground soybean, pea, or lentil residues at a rate of 2.8g residue kg<sup>-1</sup> soil, respectively. One kilogram of the soil amended with each residue was weighed and placed into plastic 15cm diameter pots inside the incubation chambers as described by Nelson et al. (2007), with six replicates of each residue treatment. The soil was incubated in the growth chamber for six weeks at a stable temperature of 25°C day and night, with the soil water content maintained at 75% of field capacity to ensure that anaerobic conditions did not occur. Gas samples were collected after 1, 3, 7, 14, 28, and 42 days of incubation, and N<sub>2</sub>O and CO<sub>2</sub> concentration was measured using gas chromatography and the gas fluxes were calculated as described in Chapter 6.

### **B.3 Results and discussion**

Results showed considerable variability in CO<sub>2</sub> and N<sub>2</sub>O fluxes with different residues through the six-week incubation period (Table B.1). By the end of the incubation, soybean, pea, and lentil residues showed significantly different impact on cumulative CO<sub>2</sub> emissions but not cumulative N<sub>2</sub>O emissions, with the cumulative CO<sub>2</sub> being the highest from the soybean residue and the lowest from the pea residue (Table B.2). Cumulative CO<sub>2</sub> and N<sub>2</sub>O emissions by the incubation period ranged from 294.6-408.1 g m<sup>-2</sup> and 2.3-12.6 mg N m<sup>-2</sup>, respectively. The CO<sub>2</sub> emissions are well in line with results from previous studies, whereas the N<sub>2</sub>O emissions are rather low compared to other studies (Hangs et al., 2013). This is possibly because the amount of available N in the soil was low, coupled with limited release of available N from the residues as observed in the incubation study described in Appendix A, resulting in insufficient substrate for denitrifiers to conduct denitrification and release significant amount of N<sub>2</sub>O from the soil. Furthermore, the soil water content in this study was kept at around 75% of field capacity, which was relatively low compared to other studies in which the soil water content was often maintained at near field capacity. Lower soil water content leads to higher oxygen levels and thus impairs the facultative anaerobic denitrification process, resulting in rather low N<sub>2</sub>O emissions in this study.

As the same amount of each residue was added, these findings suggest that soybean residue is more readily degraded and/or stimulates humus breakdown to a greater extent than pea or lentil residue. Mean nitrous oxide emissions appeared to be the highest from the pea residue, consistent with its higher N content and lower C:N ratio as shown in Appendix A. The N<sub>2</sub>O recovery, namely, cumulative N<sub>2</sub>O per added N from residues, over the incubation period showed the similar trend, with pea residue appearing to result in the highest N<sub>2</sub>O recovery, and soybean residue the lowest. This is consistent with the low C:N ratio of the soybean residue. However, the difference among N<sub>2</sub>O recoveries from different residues was not statistically significant, possibly due to the large variation observed with N<sub>2</sub>O fluxes through the incubation period. Overall, none of the pulse residues increased the nitrous oxide emissions compared to the control over the six-week incubation period. Further assessment of CO<sub>2</sub> and N<sub>2</sub>O emission patterns from residue-amended soils through incubation intact cores was carried out following this trial and presented in Chapter 6.

**Table B.1.** Daily CO<sub>2</sub> and N<sub>2</sub>O fluxes (n=6) from soil amended with soybean, pea, or lentil residues<sup>†</sup>.

Day	Daily CO <sub>2</sub> flux (g)				Daily N <sub>2</sub> O flux (mg)			
	Soybean	Pea	Lentil	Control	Soybean	Pea	Lentil	Control
1	6.4	5.4	6.2	21.3	0.4	0.2	0.2	-0.3
3	18.6	9.9	17.8	11.7	0.5	0.1	0.3	0.1
7	9.0	8.1	9.4	8.2	0.0	1.7	0.0	0.6
14	1.7	2.4	1.6	1.4	0.0	0.0	0.0	0.1
28	10.4	7.6	8.5	8.3	0.2	0.5	0.5	-0.2
42	16.6	9.5	11.2	17.2	0.1	0.0	0.1	0.1

<sup>†</sup> No significant difference ( $p \geq 0.15$ ) was detected according to Tukey's HSD test

**Table B.2.** Cumulative CO<sub>2</sub> and N<sub>2</sub>O emissions and N<sub>2</sub>O recovery (n=6) at the end of the 6-week incubation.

Treatment	CO <sub>2</sub> g m <sup>-2</sup>	N <sub>2</sub> O mg m <sup>-2</sup>	N <sub>2</sub> O recovered mg m <sup>-2</sup> (g added-N) <sup>-1</sup>
Soybean	408.1 a	3.0 a	140.2 a
Pea	294.6 c	12.6 a	354.3 a
Lentil	340.5 b	8.2 a	263.8 a
Control	329.3 bc	2.3 a	n/a

<sup>†</sup> Means followed by the same letter in the same column are not significantly different from each other ( $p \geq 0.15$ ) according to Tukey's HSD test

**APPENDIX C: ATOM % <sup>15</sup>N IN SOYBEAN, PEA, LENTIL, WHEAT, AND THE SUBSEQUENT  
WHEAT CROP AT THE ROSTHERN AND SASKATOON SITES**

**Table C.1.** Atom % <sup>15</sup>N (n=4) in the grain and the straw of soybean, pea, and lentil at the Rosthern and Saskatoon sites in 2014.

Site	Crop	Grain		Straw	
		%		%	
Rosthern	Soybean	0.56	A <sup>†</sup> b <sup>‡</sup>	0.67	Ab
	Pea	0.59	Bb	0.81	Ab
	Lentil	0.59	Bb	0.78	Ab
	Wheat	1.09	Aa	1.11	Aa
Saskatoon	Soybean	0.96	Aa	1.07	Aa
	Pea	0.88	Aa	1.08	Aa
	Lentil	0.96	Aa	1.10	Aa
	Wheat	0.89	Aa	0.91	Aa

<sup>†</sup> Means of the same crop followed by the same capital letter are not significantly different between the grain and the straw (p≥0.05) according to paired t-test

<sup>‡</sup> Means of the same plant component followed by the same lowercase letter are not significantly different among crop species (p≥0.05) according to Tukey's HSD test

**Table C.2.** Atom % <sup>15</sup>N (n=4) in the grain and the straw of wheat grown on soybean, pea, and lentil residues at the Rosthern site in 2015.

Residue	Grain		Straw	
	%		%	
Soybean	0.43	Aa	0.43	Aa
Pea	0.43	Aa	0.43	Aa
Lentil	0.45	Aa	0.44	Aa
Wheat	0.44	Aa	0.44	Aa

<sup>†</sup> Means of the same crop followed by the same capital letter are not significantly different between the grain and the straw (p≥0.05) according to paired t-test

<sup>‡</sup> Means of the same plant component followed by the same lowercase letter are not significantly different among crop species (p≥0.05) according to Tukey's HSD test

## **APPENDIX D: SELECTED SOIL PROPERTIES AT THE FOUR SITES**

In the spring and fall of 2014, post-harvest soil samples were taken prior to residue incorporation from three depths across the plot area at each site (Table D.1). Soil available  $\text{NO}_3\text{-N}$  after the growing season was generally lower in soybean plots across the sites, consistent with the higher N removal in soybean grain compared to pea and lentil as shown in Chapter 3. However, pre-seeding soil test in 2015 and the N uptake of crops grown in 2015 on the stubbles of soybean, pea, or lentil showed no response to different previous pulse crops from 2014, possibly because residue additions and following mineralization processes mitigated the difference in soil available N among different pulse stubbles. Soil available P after the first growing season was generally similar among different pulse crops, consistent with the lack of response in crop yield and P uptake as shown in Chapter 3, suggesting similar impact on post soil available P levels from soybean, pea, and lentil in a short-term. In addition, soybean plots had less soil water content compared to pea and lentil plots at the Rosthern site, possibly indicating more water consumption of soybean during the growing season, although in the second year the wheat crop showed similar yield and nutrient uptake on different previous pulse stubbles. At the Saskatoon, Scott, and Yorkton sites, post-harvest soil water content showed no response to different pulse crops grown in the season.

**Table D.** Post-harvest soil properties (n=4) at the four sites in September, 2014.

Site	Crop <sup>†</sup>	Depth cm	NO <sub>3</sub> -N	SO <sub>4</sub> -S	MK-P <sup>‡</sup>	MK-K <sup>‡</sup>	pH	Moisture	EC
			mg kg <sup>-1</sup>			%		dS m <sup>-1</sup>	
Rosthern	Soybean	0-15	7.2 b <sup>¶</sup>	4.9	13.4 a	221	6.8	17.1 b	0.11
		15-30	1.0 b	3.0	4.4 a	118	7.2	14.9 b	0.11
		30-60	0.9 b	3.4	- <sup>§</sup>	-	7.7	16.1 a	0.20
	Pea	0-15	10.5 a	4.1	11.9 a	196	6.9	21.2 a	0.15
		15-30	1.8 a	3.3	5.0 a	111	7.3	17.7 a	0.14
		30-60	1.4 a	3.1	-	-	7.7	16.4 a	0.20
	Lentil	0-15	10.7 a	5.2	9.9 a	182	6.9	19.7 ab	0.16
		15-30	1.9 a	3.2	4.5 a	113	7.3	17.7 a	0.13
		30-60	1.5 a	3.1	-	-	7.8	15.6 a	0.20
Saskatoon	Soybean	0-15	6.2 b	538.0	17.8 a	709	6.3	29.7 a	2.11
		15-30	1.1 b	174.2	5.6 a	256	6.8	25.3 a	0.83
		30-60	1.2 a	212.5	-	-	7.4	23.2 a	1.04
	Pea	0-15	11.2 a	696.6	17.7 a	674	6.5	30.3 a	2.73
		15-30	2.6 a	391.6	6.6 a	290	6.8	25.4 a	1.58
		30-60	1.4 a	450.3	-	-	7.3	23.3 a	1.85
	Lentil	0-15	9.1 a	611.6	18.0 a	639	6.2	31.2 a	2.39
		15-30	1.6 b	202.7	5.5 a	249	6.8	26.4 a	0.97
		30-60	1.3 a	382.2	-	-	7.4	23.8 a	1.72
Scott	Soybean	0-15	5.3 b	11.4	8.6 a	223	7.2	16.5 a	0.23
		15-30	3.5 a	31.2	3.6 a	98	7.6	16.5 a	0.33
		30-60	4.7 a	22.2	-	-	7.8	15.9 a	0.48
	Pea	0-15	13.9 a	13.4	8.6 a	224	7.3	15.6 a	0.30
		15-30	4.9 a	23.6	3.6 a	104	7.7	15.4 a	0.35
		30-60	8.1 a	62.5	-	-	8.0	16.4 a	0.59
	Lentil	0-15	17.9 a	15.5	9.1 a	240	6.9	16.0 a	0.28
		15-30	4.4 a	61.7	3.3 a	101	7.5	15.8 a	0.45
		30-60	8.6 a	96.3	-	-	7.8	16.7 a	0.78
Yorkton	Soybean	0-15	7.7 b	19.6	7.5 b	213	7.9	18.9 a	0.33
		15-30	2.1 b	5.9	3.7 a	124	7.9	22.2 a	0.24
		30-60	1.7 b	2.8	-	-	8.2	21.7 a	0.29
	Pea	0-15	16.4 b	5.7	10.3 a	292	7.9	18.4 a	0.39
		15-30	3.2 a	2.1	4.2 a	139	8.0	18.5 a	0.21
		30-60	2.8 a	3.1	-	-	8.1	22.8 a	0.26
	Lentil	0-15	12.8 b	6.0	12.5 a	253	7.8	19.0 a	0.34
		15-30	3.5 a	2.0	4.9 a	112	7.9	18.6 a	0.20
		30-60	3.1 a	3.0	-	-	8.1	18.9 a	0.23

<sup>†</sup> Crop grown in the plots during 2014 growing season.

<sup>‡</sup> Modified Kelowna extractable available P and K.

<sup>§</sup> Not determined.

<sup>¶</sup> Within a column, means within a site for the same depth followed by the same letters are not significantly different from each other (p≥0.05) according to a Tukey's HSD test.

## APPENDIX E: HARVEST C:N RATIO OF SOYBEAN, PEA, AND LENTIL IN 2014

**Table E.** Harvest C:N ratio (n=4) in the straw of soybean, pea, and lentil in 2014.

Site	Crop	Crop variety	C:N†
			%
Rosthern	Soybean	TH3303R2Y	50 a
	Pea	CDC Meadow	39 a
	Lentil	CDC Maxim	33 a
Saskatoon	Soybean	TH3303R2Y	38 a
	Pea	CDC Meadow	25 a
	Lentil	CDC Maxim	32 a
Scott	Soybean	TH3303R2Y	110 a
	Pea	CDC Meadow	23 b
	Lentil	CDC Maxim	26 b
Yorkton†	Soybean	TH3303R2Y	86 a
	Pea	CDC Meadow	27 b
	Lentil	CDC Maxim	17 b

† Within a column, means within a site followed by the same letter are not significantly different from each other ( $p \geq 0.05$ ) according to a Tukey's HSD test.



## APPENDIX F: GRAIN AND STRAW YIELD OF CANOLA AND WHEAT IN 2016

In the last week of April, 2016, Roundup Ready canola (*Brassica napus* L. cv. Nexera 1016RR) was seeded at the Rosthern, Saskatoon, and Scott sites by the Crop Development Centre, University of Saskatchewan. The seeding rate was 5.6 kg ha<sup>-1</sup>, and no fertilizers were applied at any of the sites throughout the season. At the Yorkton site, hard red spring wheat (cv. CDC Abound) was seeded in the beginning of May, 2016, according to the regular rotation sequence of the producer. Odyssey™ (imazamox-imazethapyr) was sprayed across the plots at each site in the last week of May for weed control in wheat and glyphosate for canola. In fall 2016, canola and wheat crops were harvested by hand at maturity in the fourth week of August, and above ground plant samples were brought back to the lab for determining the grain and straw yield.

Canola in 2016 showed no consistent significant response to different grain legume stubbles from 2014 (Table F.1), indicating similar year 2 rotational impact on the yield of following crops from stubbles of soybean, pea, and lentil. Although some responses were observed at Saskatoon, this site was affected by subsoil salinity during the growing season and crop growth was distressed in 2016. Likewise, the grain and the straw yield of wheat were similar on soybean, pea, and lentil stubbles at the Yorkton site. Overall, in this 3-year grain legume-cereal-oilseed crop rotation experiment, selected short-season varieties of soybean showed similar impact on following cereal and oilseed crops in comparison to selected varieties of pea and lentil grown under Saskatchewan conditions.

**Table F.** Grain and straw yield (n=4) of canola at the Rosthern, Saskatoon, and Scott sites, and of wheat at the Yorkton site in 2016.

Site	Stubble	Stubble variety	Yield <sup>†</sup>	
			Grain	Straw
			kg ha <sup>-1</sup>	
Rosthern	Soybean	P001T34R	3760 a	5457 a
		TH3303R2Y	3234 a	4594 a
		NSC Moosomin	3906 a	5724 a
	Pea	CDC Meadow	3365 a	5077 a
		CDC Amarillo	4188 a	5502 a
		CDC Limerick	4045 a	5975 a
	Lentil	CDC Impower	2898 a	4224 a
		CDC Invincible	3321 a	4719 a
		CDC Maxim	3672 a	5251 a
	Wheat	CDC Abound	2883 a	4479 a
Saskatoon	Soybean	P001T34R	1307 ab	1928 ab
		TH3303R2Y	1303 b	1796 b
		NSC Moosomin	2346 a	3527 a
	Pea	CDC Meadow	736 b	1283 b
		CDC Amarillo	1222 b	1999 ab
		CDC Limerick	1538 ab	2647 ab
	Lentil	CDC Impower	1388 ab	2122 ab
		CDC Invincible	1157 b	1795 b
		CDC Maxim	674 b	1052 b
	Wheat	CDC Abound	892 b	1418 b
Scott	Soybean	P001T34R	2095 a	3290 a
		TH3303R2Y	2163 a	3781 a
		NSC Moosomin	1826 a	3146 a
	Pea	CDC Meadow	2559 a	3876 a
		CDC Amarillo	2905 a	3785 a
		CDC Limerick	2824 a	3780 a
	Lentil	CDC Impower	2450 a	3670 a
		CDC Invincible	2449 a	4391 a
		CDC Maxim	2192 a	3338 a
	Yorkton <sup>†</sup>	Soybean	P001T34R	3002 a
TH3303R2Y			2578 a	2732 a
NSC Moosomin			3088 a	3568 a
Pea		CDC Meadow	2755 a	3084 a
		CDC Amarillo	3276 a	3699 a
		CDC Limerick	2841 a	3339 a
Lentil		CDC Impower	2583 a	3046 a
		CDC Invincible	2891 a	3150 a
		CDC Maxim	3186 a	3575 a

<sup>†</sup> Within a column, means within a site followed by the same letter are not significantly different from each other ( $p \geq 0.05$ ) according to a Tukey's HSD test.