EFFECT OF FORAGE LEGUMES IN SHORT-TERM ROTATION ON PHOSPHORUS FERTILITY OF FOUR SASKATCHEWAN SOILS

A Thesis Submitted to the College of Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of Master of Science

in the Department of Soil Science

University of Saskatchewan

Saskatoon

By

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ABSTRACT

Legumes are becoming increasingly important in cropping systems due to their beneficial effects on soil nutrient availability. Including legumes into a cropping system not only positively affect many soil physical properties and increases soil nitrogen (N) supply, but is also reported to have a positive impact on soil phosphorus (P) availability. Although a series of studies have examined the effect of grain legumes in rotation on increasing soil N and P fertility after several years, the effect of forage legumes like alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) on soil P availability, the yield and P uptake of subsequently grown crops remain unknown in Western Canada. To address this gap, a four year field experiment was conducted in four soil zones of Saskatchewan: Dark Brown soil zone (Saskatoon), thin Black soil zone (Lanigan), Brown soil zone (Swift Current), and Gray soil zone (Melfort). The objectives of this study were (i) to evaluate the impact of including a two-year period of forage legumes alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) along with annual crops on soil P forms, amounts and availability and uptake of P by the crops in rotation and (ii) to evaluate the effect of the different crop rotations over the four years on soil P dynamics and P balance. After two years of forage legume and annual crop rotations, it was observed that alfalfa and red clover removed more P from the soil in the hay harvest as compared to the amount of P removed in grain in the barley (*Hordeum vulgare* L.) - pea (*Pisum sativum* L.) and barley (*Hordeum vulgare* L.) - flax (*Linum usitatissimum* L.) rotations at all four sites ($P < 0.10$). However, the four crop rotations did not significantly affect the amount of soil available P and P supply rate at all four sites ($P > 0.10$) despite greater P uptake and removal by forage legumes relative to annual crops in 2010 and 2011 growing seasons. Also, similar amounts of labile and stable P were measured in the different sequentially extracted chemical fractions after two years of different crop rotations at all sites ($P > 0.10$). Grain yields of wheat *(Triticum aestivum* L.) and canola (*Brassica napus* L.) crops grown following two years of forage legume and annual crop rotations were positively affected by forage legume rotations at Saskatoon, Lanigan and Melfort ($P < 0.10$). Wheat P uptake was improved significantly by two years of red clover at Lanigan, Swift Current and Melfort ($P < 0.10$) but canola P uptake was not affected by different crop rotations at all locations ($P > 0.10$). The amount of soil available P and P supply rate measured after wheat and canola harvest were not affected by different crop rotations at all sites ($P > 0.10$) despite the

greater P removal by forage legumes during the first two years of the four year crop rotation period at all sites and the enhanced P removal by wheat and canola crops following forage legume rotations at Lanigan and Melfort. This suggests that forage legumes are able to maintain soil P fertility in the face of greater P removal by crops in rotation, at least in the short-term. Four years of continuous cropping with the minimum amount of fertilizer P addition resulted in a significant reduction of soil P fertility over time at all locations ($P < 0.10$). The lack of a significant effect of rotation treatment on available P levels in the soil does not rule out that there is an effect but variability prevented its detection. Further research is needed to evaluate the effects of several cycles of this rotation over a number of years on soil P availability and the P uptake of crops in rotation.

ACKNOWLEDGMENTS

First of all, I would like to express my deepest appreciation to my supervisor Dr. Jeff Schoenau for his tremendous support, constructive guidance and persistent help throughout this research project; your guidance has been unwavering and I am fortunate to have mentored by such an excellent scientist and a teacher. I also greatly acknowledge the project co-investigator Dr. Paul Jefferson, who has contributed a lot to the accomplishment of this project. My thanks also go to the staff at the Western Beef Development Centre at Lanigan, the Agriculture and Agri-Food Canada Research Farm at Saskatoon, the Semiarid Prairie Agricultural Research Centre at Swift Current and the Melfort Research Farm at Melfort, for helping the field work of the project. I would also like to thank my committee members, Drs. Ken Van Rees and Bobbi Helgason for their invaluable suggestions and advice along the way. I would especially like to express my special appreciation to Dr. Bobbi Helgason for her help in soil microbial analysis for this project. My appreciation also goes to the Saskatchewan Agricultural Development Fund for the financial support to my research project.

In addition, I thank to the following people who have helped me to accomplish this research work: Cory Fatteicher, Ryan D. Hangs, Tom King, Hassan Ahmat, Gourango Kar, Ron Urton, Elliott Hildebrand, and Panchali Katulanda. Even though two years are considered enough time for finishing an MSc Research project, this time will never be enough to accomplish a project successfully without all of your kind help. Therefore, again I would like to say thanks to all of you who have helped me throughout the years.

I take the opportunity to also thank my friends: Dennis Will and Marie-Jeanne Will, Metin Atila and Aysa Demirkay Atila, Marvin Gennon for their care and help during my stay here in Canada. I am sincerely grateful to my parents, brothers and sisters for their endless encouragement and support, and always standing behind me. My thanks are also to my parents, brothers and sisters in law for their support during my study. Last, but not the least, my acknowledgement towards my husband Samat Amat and my beloved daughter Subina Samat for their continued love and inspiration throughout my study. All in all, without all of your kind help and contribution, this project would not be accomplished successfully!

DEDICATION

This dissertation work is dedicated to my family and friends who are most special in my life:

- My Husband Samat Amat and my daughter Subina Samat;
- My dad Rehmut Turap and my mum Riyanam Abla;
- My brothers Ablikim Rehmut and Zulkar Rehmut, and my sister Arzigul Rehmut;
- My best friends Dennis Will and Marie-Jeanne Will.

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LIST OF ABBREVIATIONS

A	Alfalfa
AAFC-Scott	Agri-Food Canada Research Farm at Scott
Al	Aluminium
AM fungi	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AWCD	Average well color development
B	Barley
C	Canola
C : P ratio	Carbon : phosphorus ratio
Ca	Calcium
CLPP	Community level physiological profiling
Co	Cobalt
CO_2	Carbon dioxide
Cu	Copper
DCP	Dicalcium phosphate
DNA	Deoxyribonucleic acid
EC	Electrical conductivity
Eh	Redox potential
Fe	Iron
FL	Flax
HAP	Hydroxyapatite
K	Potassium
Mg	Magnesium
MRF	Melfort Research Farm
N	Nitrogen
NH_4^+	Ammonium
NO_3^-	Nitrate
OCP	Octacalcium phosphate
P	Pea
P	Phosphorus
PBS	Phosphate-buffered saline
Pi	Inorganic P
Po	Organic P
RC	Red-clover
RCBD	Randomized complete block design
RNA	Ribonucleic acid

SEM	Standard error of mean
SOM	Soil organic matter
SPARC	Semiarid Prairie Agricultural Research Centre
W	Wheat
WBDC	Western Beef Development Centre
Zn	Zinc

1. INTRODUCTION

1.1 Soil P fertility

Phosphorus is a macronutrient essential for all living organisms (Raghothama, 1999). It is a component of key molecules including adenosine triphosphate (ATP), deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and phospholipids, playing a critical role in energy metabolism and the biosynthesis of nucleic acids and membranes (Lavelle and Spain, 2001; Brady and Weil, 2002). Inorganic P (Pi) is also involved in controlling key enzyme reactions and in the regulation of metabolic pathways (Theodorou and Plaxton, 1993).

In agricultural production, crop yield and quality rely on having adequate amounts of plant available P in the soil (Md Alamgir et al., 2012). In the soil, P is found in Pi and organic P (Po) forms which vary in their availability to plants (Hansen et al., 2004; Turner et al., 2007; Shen et al., 2011). Although many soils contain large reserves of total P (Holford, 1997), the concentration of plant readily available Pi is low in most agricultural soils. Much of the P in soil is not immediately available for plant uptake due to the adsorption and precipitation of P into less soluble and more stable P forms through various chemical reactions in the soil which vary with soil pH (Holford, 1997; Ticconi and Abel, 2004). Thus, P deficiency is the most common limiting factor in crop production after N in many soils (Holford, 1997; Wang et al., 2012), affecting 42 % of the cultivated land around the world (Liu et al., 2004). One remedy for the lack of available P is to apply large quantities of fertilizer P. However, this might lead to environmental pollution and is not economically sustainable (Withers et al., 2001). This is because overuse of P fertilizers above crop needs results in a build-up of soluble, labile Pi and residual P in the soil (Wang et al., 2007; Wu et al., 2008). Runoff of this element can increase the risk of eutrophication in nearby water bodies (Sharpley, 1995; Sharpley et al., 2000). Thus, the identification and incorporation of suitable crop species that are efficient in P mobilization into cropping systems as rotational crops is considered to be one of the most promising agronomic approaches to access insoluble native soil P reserves and to capitalize on the use of already accumulated residual soil P reserves (Ae et al., 1990; Lynch, 1998; Horst et al., 2001; Stutter et al., 2012).

1.2 Legumes in a crop rotation

Crop species vary in their ability to take up P from soils (Hocking et al., 1997; Nuruzzaman et al., 2005b). Among crop species, legumes are reported to possess a remarkable capacity to mobilize and take up sparingly soluble P from soils with both low and high P availability (Kamh et al., 1999, 2002; Nuruzzaman et al., 2006; Rose et al., 2010; Wang et al., 2011) compared to less P efficient cereal crops (Braum and Helmke, 1995; Hocking et al., 2001). Thus, incorporating these P mobilizing crop species into a cropping system can be employed as an effective way to access insoluble native soil P reserves (Ae et al., 1990; Horst et al., 2001). The ability of legumes to make P more readily available from various P pools has been attributed to (i) modification of rhizosphere pH due to root-induced acidification or alkalization (Gahoonia et al., 1992; Morel and Hinsinger, 1999; Hinsinger et al., 2003); (ii) the secretion of carboxylic acids such as malate and citrate (Veneklaas et al., 2003); and (iii) the exudation of root or microbially derived P solubilizing enzymes such as acid phosphatases and phytases (Richardson, 2001; Nuruzzaman et al., 2006). In addition, legumes are also capable of improving spatial access to soil P through their morphological root traits and symbiotic associations with mycorrhizal fungi (Hedley et al., 1982; Vance et al., 2003).

According to many research results, some legumes, particularly white lupin (*Lupinus albus* L.), chickpea (*Cicer arietinum* L.), field pea *(Pisum sativum* L.) and faba bean (*Vicia faba* L.), are not only capable of mobilizing available soil P for their own requirement, but they are also capable of enhancing the growth and P uptake of following crops such as wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) (Hens and Hocking, 2004; Nuruzzaman et al., 2005b). The enhanced growth of crops grown after legumes has been explained through various effects. First, the legume crop may solubilize soil P in excess of their own requirements and the excess amounts of P could be carried over and utilized by other less P efficient crops grown in rotation in the cropping system (Kamh et al., 2002). Furthermore, P release during the mineralization of legume residues can be another explanation for the elevated crop growth and P acquisition after legumes (Horst et al., 2001; Kamh et al., 2002; Nuruzzaman et al., 2005a,b; Oberson, 2011).

1.3 MSc research justification

Phosphorus deficiency is a major nutritional constraint for crop production in many agricultural soils around the world (Chen et al., 2008; Wang et al., 2011). Correcting P deficiency with the addition of P fertilizer is a common practice but complete reliance on chemical fertilizers is not economically sustainable and can have some negative consequences for the environment because of the following reasons (Withers et al., 2001). First, P fertilizer prices are increasing with the increase of P fertilizer use in agricultural production (Rose et al., 2010). Second, the crop fertilizer P use efficiency is relatively low. Only about 10 - 30% of applied P is taken up by plants in the first year (Holford, 1997; Syers et al., 2008; Sattari et al., 2012), as a considerable portion of the remaining P is converted to less soluble and available P forms and is accumulated in the soil as plant unavailable residual P (Halvorson and Black, 1985; Bolland, 1993; Holford, 1997; Richardson et al, 2001; Bünemann et al., 2006). Third, overuse of P fertilizers above crop needs increases the risk of environmental problems such as ground water contamination and eutrophication in some environments (Hodgkin and Hamilton, 1993). This has led to a search for both economically feasible and environmentally sustainable strategies for improving crop production that can reduce total reliance on chemical P fertilizer sources in P deficient soils. Incorporating P efficient legume species into a crop rotation is considered as a more sustainable way to access insoluble native soil P reserves and to enhance sustainable crop production around the world (Ae et al., 1990; Lynch, 1998; Horst et al., 2001).

To date, most studies have evaluated the effect of various grain legumes in rotation on soil N and P fertility and P mobilizing mechanisms of these crops (Nuruzzaman et al., 2006; Hassan et al., 2012a). Some studies have assessed the effect of grain legume crops on the growth and P uptake of following crops (Kamh et al., 1999; Horst et al., 2001; Hocking and Randall, 2001; Hens and Hocking, 2004; Nuruzzaman et al., 2005a,b; Hassan et al., 2012). However, no studies have examined the effect of forage legumes such as alfalfa and red clover in rotation on soil P availability, the yield and P uptake of subsequently grown wheat and canola crops under different soil-climatic conditions in Saskatchewan.

Forage legumes are considered an essential component of sustainable agro-ecosystems (Doran and Smith, 1991). Alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.)

are the predominant forage crops grown on a large scale worldwide (Taylor and Quesenberry, 1996; Morel et al., 2012), as well as in Western Canada because of their superior productivity and wide adaptation to various soil and environmental conditions when compared to other legumes (Martin, 1996; Sheaffer and Seguin, 2003). There are numerous benefits of including forage legumes into cropping systems (Sheaffer and Seguin, 2003; Ates et al., 2014) such as improved soil N fertility, organic matter and tilth, production of high quality forage for livestock feed, as well as reduced incidence of weeds, insects, and diseases in soil. In addition, rotational effects of forage legumes on P availability have also been observed (Campbell et al., 1993). Inclusion of these crops in rotation for only two years may increase the P availability and crop P uptake (Friesen et al., 1997). Wheat and canola are two crops widely grown in Western Canada (Friesenn et al., 2003). Therefore, evaluating the effect of two years of forage legume (alfalfa and red clover) and annual crop rotation (barley-pea and barley-flax) on soil P availability and the yield and P uptake of following wheat and canola crops can be a valuable approach in understanding how forage legumes in short-term rotation can affect soil P fertility.

1.4 Thesis arrangement

Following the introduction (chapter 1) and the subsequent literature review (chapter 2) this thesis follows a manuscript-style format, including chapters 3-5 and Appendix A. Each manuscript includes a detailed description of the research work examining the effect of two years of forage legume and annual crop on soil P fertility and the yield and P uptake of subsequently grown wheat and canola crops. Specifically, chapter 3 covers the yield and P uptake by alfalfa and red clover versus annual crops (barley, pea, and flax) during the first two years of a four year rotation. The objective of this study was to compare the yield and P uptake by the rotational crops alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), barley (*Hordeum vulgare* L.), pea (*Pisum sativum* L.) and flax (*Linum usitatissimum* L.) over two years of hay and grain production. Perennial and annual forage legumes are important component of sustainable agriculture, so it is important to assess the P uptake by these legumes included in crop rotations in comparison to annual crops under Saskatchewan soil-climatic conditions. In chapter 4, the soil P availability and changes in P fractions after two years of forage legume versus annual crops are reported. The objective of this study was to assess changes in soil test extractable P (modified Kelowna P), soil P supply rate (anion exchange membrane P) and distinctive labile and stable inorganic P (Pi) and organic P (Po) pools after two year of alfalfa (*Medicago sativa* L.); two year of red clover (*Trifolium pratense* L.); barley (*Hordeum vulgare* L.) followed by pea (*Pisum sativum* L.); and barley followed by flax (*Linum usitatissimum* L.) rotations in four Chernozemic soils in Saskatchewan. The information concerning P availability and changes in P fractions after two years of different crops, emphasizing the effect of perennial forage legumes versus annual crops is helpful in our understanding regarding P mobilizing ability and the overall effects of short-term forage legumes versus annual crops in rotation on soil P fertility. In chapter 5, the effects of the preceding two years of forage legume and annual crops on the yield and P uptake by wheat and canola crops grown in the last two years of the four year rotations is examined. The objectives of this study were (i) to assess the effect of two years of forage legume (alfalfa, red clover) and annual crop rotations (barley followed by pea, and barley followed by flax) on the yield and P uptake by wheat and canola grown in the next two years and (ii) to evaluate the effect of the four-year crop rotations on soil P dynamics and P balance at the four sites in Saskatchewan. To maximize the beneficial effects of legumes in crop rotation, it is important to understand the ability of various legume crops (both forage and grain legumes) in enhancing the yield and P uptake of different subsequent crops following in rotation. Next, overall synthesis and conclusion are included in chapter 6. Finally in Appendix A, the average well color development (AWCD) of Biolog EcoPlate^{TM} over the 7 days incubation period, P fertilizer recommendations for barley and spring wheat and soil test rating for plant available P (Kelowna P) level are displayed.

2. LITERATURE REVIEW

2.1 Soil-climatic zones in Saskatchewan

Soil distribution in the prairie region is closely related to differences in climate and in the related natural vegetation across Western Canada (Fuller, 2010). The regional variations in climate and vegetation type across the prairies have resulted in the formation of soil zones which reflect the effect of precipitation, temperature and dominant vegetation type on soil-forming processes and hence on soil properties and types (Agriculture and Agri-Food Canada, 2000; Fuller, 2010). These soil zones are referred to as the Brown, Dark Brown, Black, Dark gray and Gray soil zones (Fuller, 2010). In Saskatchewan, there are four distinct soil zones: Brown, Dark Brown, Black, and Gray (Fig 2.1) (Agriculture and Agri-Food Canada, 2000). The Brown soil zone covers approximately 6.3 million hectares in southwest Saskatchewan, of which 69 % are cultivated (Agriculture and Agri-Food Canada, 2000). In this region, the relatively warm temperature, low soil moisture and soil organic matter (SOM) content limit crop growth; resulting in small grains and short-grasses for livestock production (Agriculture and Agri-Food Canada, 2000). Lying north and east of the Brown soil zone, the Dark Brown soil zone includes 7.28 million hectares of which approximately 82% of the soil is under cultivation. This soil zone is considered to be the most intensively farmed area in Saskatchewan (Agriculture and Agri-Food Canada, 2000). It is characterized by cooler and moister conditions with relatively high levels of organic matter at the surface (The Encyclopedia of Saskatchewan). There are 7.52 million hectares in the Black soil zone. About 73% of the Black soil zone is being cultivated for crop production (Agriculture and Agri-Food Canada, 2000). The Black soil zone is located to the north and east of the Dark Brown soil area. Although the growing season is shorter in this soil zone, the cooler temperature and increased moisture are appropriate for a wider variety of cropping practices (Agriculture and Agri-Food Canada, 2000). Further north, Gray, Dark Gray and Dark-Gray wooded soils encompass about 4.53 million hectares in the northern agricultural area, but only 45% of the Gray soils are cultivated (Agriculture and Agri-Food Canada, 2000). The characteristics of this region are lower soil organic matter content, higher moisture condition, but a shorter growing season compared to Black soil zone. Cereal crop yields are typically higher in the Black soil zones, whereas protein level tends to be higher in the Brown and Dark Brown soils (Agriculture and Agri-Food Canada, 2000).

Most soils in the prairie ecosystem belong to the Chernozemic soils based on the Canadian System of Soil Classification (Fuller, 2010). Chernozemic soils are not water-logged for extended period of time and have dark surface horizons that are high in organic matter. The high organic matter in the surface soil resulted from the SOM addition through the roots of grasses and limited decomposition of the organic matter due to dry soil moisture conditions (Soils of Saskatchewan; Fuller, 2010). Soil zones like Brown soil zone, Dark Brown soil zone, Black soil zone and Dark Gray soil zone are named according to the type of Chernozemic soils dominating the particular zone (Fuller, 2010). The Canadian System of Soil Classification recognizes Chernozemic soils at the broadest level of taxonomy, namely, the Order level. The Chernozemic Order is further divided into "Great Groups" that include the Brown Chernozem soils, Dark Brown Chernozem soils, Black Chernozem soils and Dark Gray Chernozem soils. In addition to Chernozem soils, the Gray Luvisolic and Brunisolic soils occur north of the prairie region within the boreal forest (Fuller, 2010; The Encyclopedia of Saskatchewan).

The nature and behavior of Chernozemic soils are mainly determined by soil organic matter accumulation, decomposition and transformation in the surface soil (or A horizon) (Fuller, 2010). The vegetation and climate have a significant influence on the amount and nature of organic matter accumulated in the soil. Therefore deposition of plant material belowground in the grassland system is critical for organic matter build-up in Chernozemic soils (Fuller, 2010). Agricultural management practices that manage crop yield and SOM and conserve soil moisture properly will most likely lead to the long-term sustainability of prairie soils.

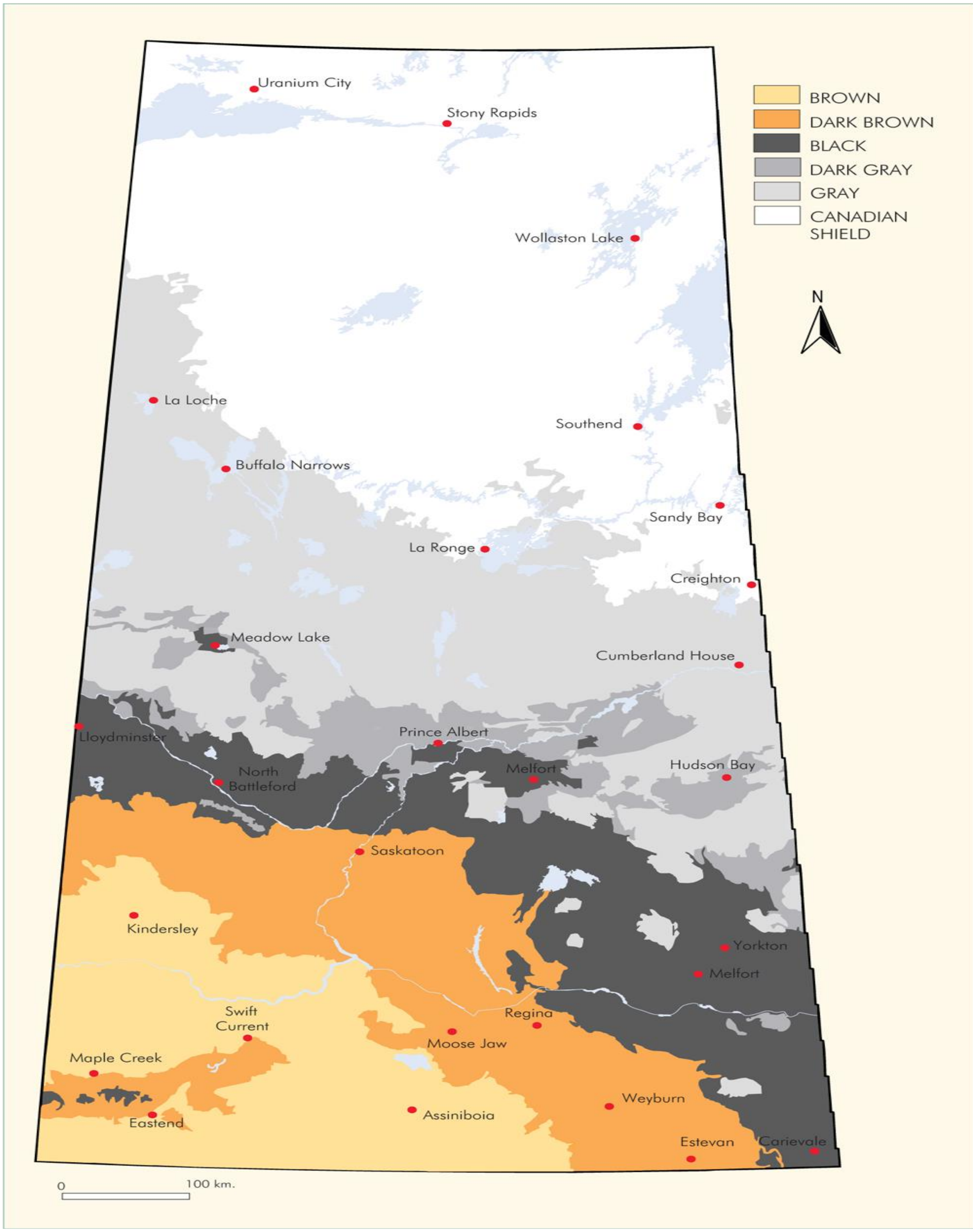

Fig 2.1. Soil Zones in Saskatchewan (Canadian Plains Research Center Mapping Division).

2.2 Phosphorus in the soil

In agricultural soils, phosphorus (P) originates from primarily two sources, the parent material from which the soil developed as well as inorganic and organic P fertilizers (Frossard et al., 1995). Soil P can be categorized into various chemical forms including inorganic P (Pi) and organic P (Po) which differ in their behaviour and fate in soils (Fig 2.1) (Hansen et al., 2004; Turner et al., 2007; Shen et al., 2011). Although many soils contain large reserves of total P (Holford, 1997), they are mainly present in plant unavailable forms. Only a marginal proportion of soil P (1-3% out of total P) is present in a form that is directly available to plants (Hinsinger, 2001). Plant roots mainly absorb P as orthophosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) from soil solution (Fig 2.2) (Brady and Weil, 2002), but the concentrations of those phosphate ions in the soil solutions is relatively low (0.1 and 10 μM), respectively at any one time (Ozanne, 1980; Mengel and Kirkby, 1987; Raghothama, 1999; Frossard et al., 2000). The plant available P level in the soil solution is preserved by solubilisation of solid P compounds, mobilization of adsorbed P, mineralization of organic P, decomposition of plant residues and turnover of microbial biomass (Hassan et al., 2012a).

Inorganic P constitutes 35 to 70 % of the total P in soil, depending upon the soil type and its organic matter content (Harrison, 1987). Soil Pi contains more than 170 different phosphate minerals which vary significantly in their solubility (Holford, 1997). These phosphate minerals are easily converted from sparingly soluble to increasingly insoluble forms over a period of time (Holford, 1997). In soil, Pi originates from the dissolution of the primary and secondary phosphate minerals; and the application of inorganic fertilizers. Primary phosphate minerals like apatite, strengite and variscite are very stable, so they release insufficient plant available P by weathering to meet the crop demand (Pierzynski et al., 2005). In contrast, secondary phosphate minerals such as calcium (Ca), magnesium (Mg), iron (Fe) and aluminium (Al) phosphates (Morgan 1997) vary in their dissolution rates according to the soil pH and size of mineral particles (Pierzynski et al., 2005, Oelkers and Valsami-Jones, 2008). The forms and solubility of soil P components are determined by various soil physical and chemical properties including soil pH (Fig 2.3), concentration of metal ions such as Ca, Mg, Fe and Al as well as the nature and surface areas of the soil particles (Holford, 1997). Generally, P is optimally available to plants in soils with pH values within the 6.5 to 7.5 range (Fig 2.3) (Hinsinger, 2001). In acidic soils, phosphate ions react with existing soluble Al and Fe to form various types of less soluble Al and Fe phosphates (Holford, 1997). In alkaline soils, phosphate ions tend to react readily with Ca and Mg to form less soluble Ca and Mg compounds or Pi is adsorbed on the surface of Ca and Mg carbonates (Holford, 1997). In addition, in soils with higher clay content Pi adsorbed on the surface of clay particles and become more immobile and unavailable.

Organic P (Po) generally accounts for 30 to 65 % of the total P in soils (Harrison, 1987). It is considered as a major and stable P component of most soils (Harrison, 1987). The main stabilized forms of Po are inositol phosphates (Richardson, 1994). There are some active forms of Po that includes orthophosphate diesters, labile orthophosphate monoesters, and organic polyphosphates (Turner et al., 2002; Condron et al., 2005). The soil Po pool is an important source for plant uptake; however, the plant roots can only access this pool after its mineralization into Pi (Richardson, 1994; Li et al., 2003). This is does not apply to water-soluble Po which may be absorbed by plants directly (Dalal, 1977). The mineralization of Po is highly influenced by soil moisture, temperature, surface physiochemical properties, pH, redox potential (Eh) and the activity of soil microorganisms in soil. Transformations of Po have a considerable influence on the overall availability of P in soil (Turner et al., 2007).

2.3 Fate of P added to soils

Inorganic and organic P (Pi and Po) fertilizers are commonly used worldwide to improve soil P fertility and increase agricultural crop production (Dalal, 1977; Cordell et al., 2009). In Western Canada, P fertilizer application is a common practice among crop growers due to the low plant available P status of most prairie soils, despite the high level of total P in some areas (Havlin et al., 1984; Malhi, 2010). The phosphate in inorganic and organic fertilizers are initially water-soluble and plant available (Bolan and Duraysamy, 2003), but when they dissolve in soil, various reactions begin to occur that make the phosphate less soluble and less available for plants (McNeil, 2008). The rate of these reactions and products produced are dependent on a series of factors including P level in soil, organic matter content, abundance and the activity of microorganisms, P adsorption capacity, as well as soil properties (Blake et al., 2000; Krishna, 2000; Bünemann et al., 2004). Important soil properties influencing the P conversion are pH, moisture, temperature, clay content and the minerals present in the soil.

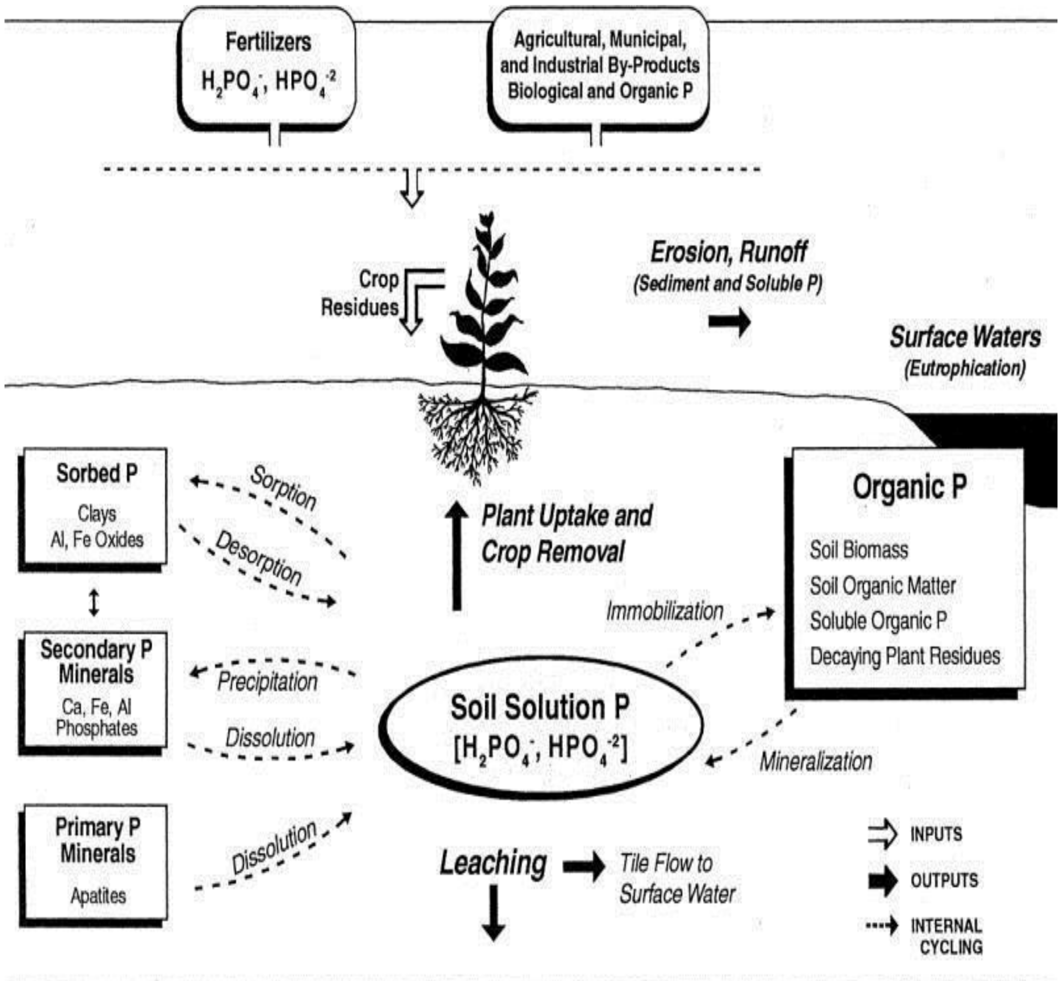

Fig 2.2. Phosphorus cycle in soil (From Pierzynski and McDowell, 2012).

Once soluble granular P fertilizers move into the soil solution, Pi can be taken up by plants and soil biota, converted into Po and immobilized or fixed by soil physicochemical reactions. Inorganic P (Pi) transformed into Po can be later mineralized again to soluble P by various soil microbial processes or plant root exudates (McLaughlin and Alston, 1986; McLaughlin et al., 1988). During the fixation process, the fertilizer phosphates in the soil solution readily become less soluble and less available to plants due to their precipitation and adsorption (Fig 2.3) (Wang et al., 2007b; Vu et al., 2008; Vu et al., 2010). First, P may be lost from the soil solution by precipitation reactions, where soluble phosphate anions combine with other metal cations (e.g., Ca, Al, Fe) in soil to form various insoluble solid compounds (Fig 2.4) (McNeil, 2008). Some of these P compounds dissolve over time and release soluble P again, whereas others remain considerably insoluble and are therefore "locked up" in the non-exchangeable pool and unavailable for plant uptake (McNeil, 2008). Second, as phosphate ions move towards the soil pores, they are removed from the solution and become attached to the surface of soil particles through a process called adsorption (Fig 2.4) (Arai and Sparks, 2007; McNeil, 2008). In this process, phosphate ions are adsorbed on the surface of clay mineral and Fe/Al oxides by forming various complexes (Arai and Sparks, 2007). Some of these adsorbed phosphate ions on the surface remain in a plant available forms by desorption reactions. Remaining phosphate ions are occluded in nanopores that frequently occur in Fe/Al oxides by forming a very strong bond and permanently removed from plant available pools into the non-exchangeable pool (Arai and Sparks, 2007; McNeil, 2008).

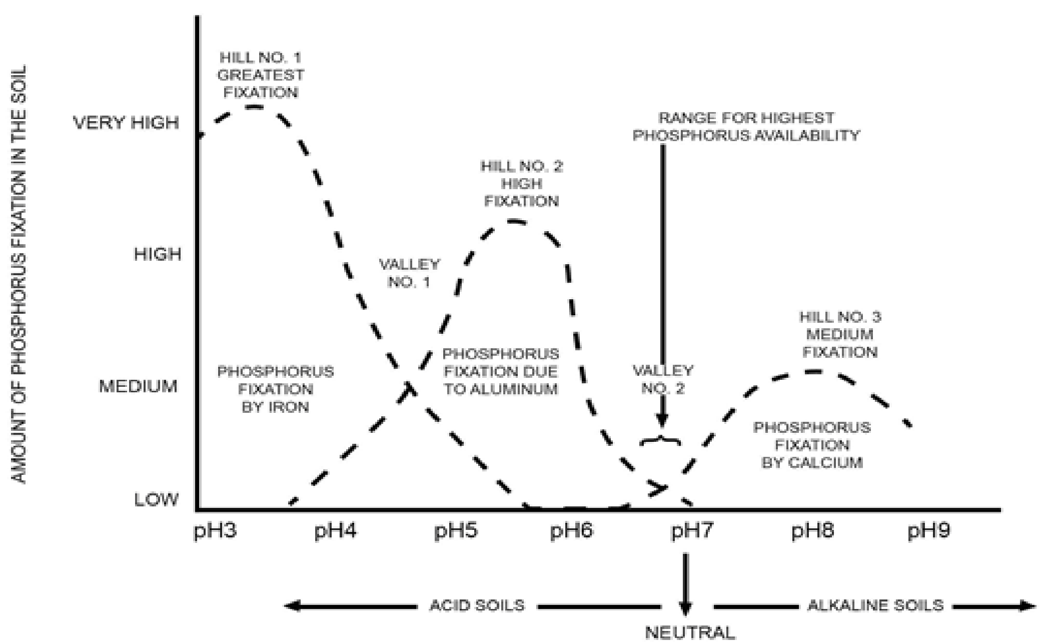

Fig 2.3. The availability of phosphorus is affected by soil pH (From Michigan State University Extension, 2012).

In neutral to calcareous pH soils, P retention is dominated by precipitation reactions (Lindsay et al., 1979), even though P can also be adsorbed by clay minerals and Ca carbonates

(Devau et al., 2010). Phosphorus can precipitate with Ca to form dicalcium phosphate (DCP), octocalcium phosphate (OCP) and hydroxyapatite (HAP) (Arai and Sparks, 2007). The formation of each product results in a decrease in solubility and availability of phosphates in the soil (Arai and Sparks, 2007). In acidic soils, P is adsorbed mainly on clay particles and hydrous oxides of Al to form amorphous Al and Fe phosphates (Frossard et al., 1995). With further reactions, the amorphous Al and Fe phosphates gradually diffuse into the interior of the Fe and Al oxides and become more unavailable to plants (Arai and Sparks, 2007).

In soil, applied P can modify three major P pools in the soil- plant system: soil solution P, available soil P and fixed soil P (Shen et al., 2011). All these P pools exist in complex equilibria with each other, representing from very stable, sparingly available, to plant available P pools such as labile and solution P (Shen et al., 2011). The equilibrium reactions of three major P pools after the fertilizer application can be summarized in the following:

Solution P ⟷ Labile P ⟷ Non-labile P

Crops receive their P from the soil solution that is in equilibrium with the adsorbed P in the soil and from P compounds that can readily dissolve (Barber, 1980).

Adding to the active P pool through fertilization will eventually increase the amount of fixed P, therefore, decreasing the efficiency of P fertilizer. The availability of applied P in soil becomes very low within the growing season (Vu et al., 2007). In the first year, plants take up about only 10-30 % of the phosphorus fertilizer applied (Holford, 1997; Syers et al., 2008; Sattari et al., 2012), as a considerable portion of remaining P becomes immobile and unavailable for plant uptake (Vu, et al., 2007). It is accumulated in the soil as sorbed P in the form of various P compounds, termed residual P (Halvorson and Black, 1985; Bolland, 1993; Holford, 1997). Although this P retained in the soil may have some residual benefits in subsequent years, a continued annual supply of P-based fertilizers is required to maintain adequate labile P levels in soil and to increase the productivity of agricultural land (Condron, 2004). However, the level of soil soluble P from fertilisers, especially from manures, needs to be monitored carefully to lessen potential P losses and negative environmental impacts (Lehman et al., 2004).

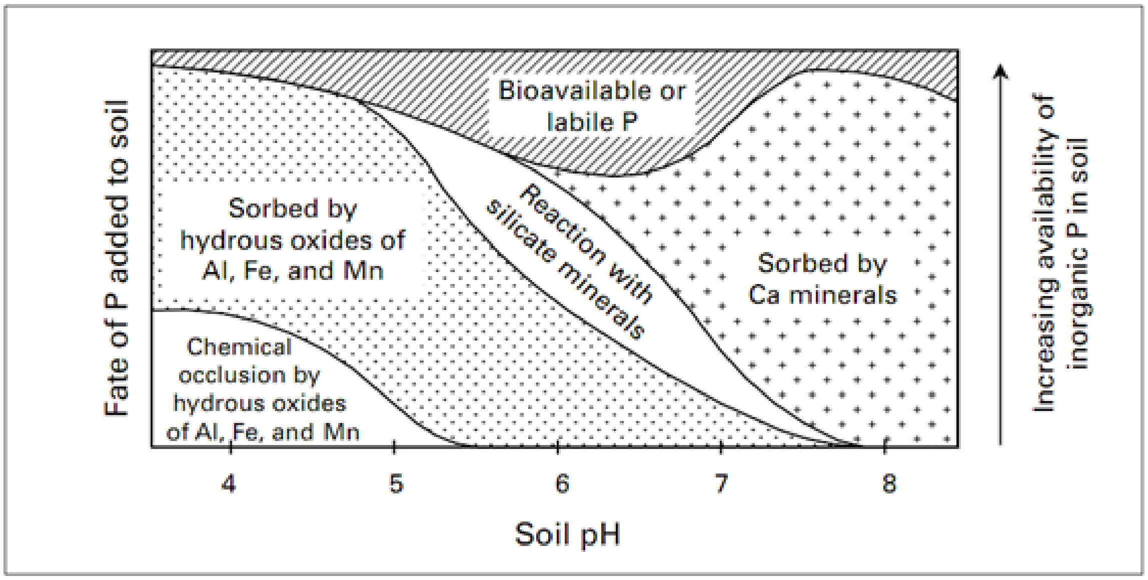

Fig 2.4. Fate of phosphorus added to soil (From Michigan State University Extension, 2012).

2.4 Plant available soil P and its uptake by plants

Crop response to P depends mainly upon two processes: soil P supply in an available form and the ability of the crop to take up P (Föhse et al., 1988). Due to the low concentration of dissolved P in the soil solution, plants often face significant challenges in acquiring an adequate supply of P to meet the demands of basic cellular requirements (Schachtman et al., 1998). Plants only absorb P from the soil solution in the form of phosphate anions, either in the form of $H_2PO_4^-$ or HPO_4^{2-} (Holford, 1997; Hansen et al., 2004; Turner et al., 2007). Although there are large quantities of P stored in most soils, the amount of plant readily available, dissolved P in the soil solution is very low at any one time (Holford, 1997). This immediately available P pool must be replenished regularly during the growing season to meet crop P demands (Bieleski, 1973). Phosphate concentrations in the soil solution (1~10 μM) are in equilibrium with the adsorbed solid phase P in the soil (this process is called desorption) and are maintained by various physiochemical reactions (Barber, 1980). As the soil solution P is depleted by crop uptake, unavailable P can slowly be released to more available forms to replenish the soil solution. Although this slow release can supply adequate P for plant growth in many natural systems, it is generally considered to be too slow to provide sufficient amount of available P in intensively managed agricultural systems without additional supply of inorganic P fertilizers and other organic P amendments (Kucey et al., 1989).

The uptake of P is a difficult process for plants because the available P in the bulk soil is low but plant requirements are high (Schachtman et al., 1998). Phosphate moves into plant cells mainly by diffusion rather than mass flow (Schachtman et al., 1998) due to the fact that the concentration of P in the soil solution is very low, rarely exceeding 10 μM (Bieleske, 1973). Higher plant P uptake creates a Pi-depletion zone around the root, due to low diffusion rates of Pi (10^{-12} to 10^{-15} m^2 s^{-1}) in soils (Marschner, 1995; Jungk, 2001). In order to adapt to P deficient soil conditions and increase P uptake efficiency, plants have evolved a series of mechanisms including morphological, physiological and biochemical changes to utilize previously unavailable soil P (Shen et al., 2011). Morphological changes include an increase in root growth, total root length, root/shoot ratio, root branching and the abundance and distribution of root hairs to explore larger soil volume in order to maximize P acquisition (Fig 2.5) (Gaume et al., 2001; Jungk, 2001). In addition, some physiological and biochemical processes that occur at the root surface further influence the availability of soil P to plants (Vance et al., 2003; Richardson, 2009). These processes are increased production of phosphatase enzymes, modification of rhizosphere through secretion of chemical compounds that are capable of freeing P from metallic P compounds or organic complexes (Marschner, 1995; Johnson et al., 1996a, b) and better symbiosis with soil microbes such as arbuscular mycorrhizal fungi (AM fungi) (Fig 2.5) (Richardson, 1994; Raghothama, 1999; Vance et al., 2003; Lambers et al., 2006).

2.5 Forage legumes for a sustainable cropping system

Legumes have been used in cropping systems for centuries (Sheaffer and Seguin, 2003). Forage legumes have been considered an essential component of sustainable cropping systems throughout history although incorporation of legumes into the cropping system has declined following the introduction of relatively cheaper chemical fertilizers, herbicides and pesticides (Doran and Smith, 1991). After agricultural industrialization in the 20th century, the agricultural production system in many countries has become mainly dependent on the use of chemical fertilizers and herbicides and pesticides because of the better crop yield through chemical fertilization than legume rotations. However, this approach has led to concerns about the environmental and economic sustainability of the system over time. In agricultural production, heavy dependence on the use of commercial fertilizer inputs to enhance crop yield results in a rising cost of growing a crop, soil degradation, and environmental pollutions (McCartney and Fraser, 2010). Therefore, there is a renewed interest in diversifying agricultural production systems through the use of forage legumes (McCartney and Fraser, 2010).

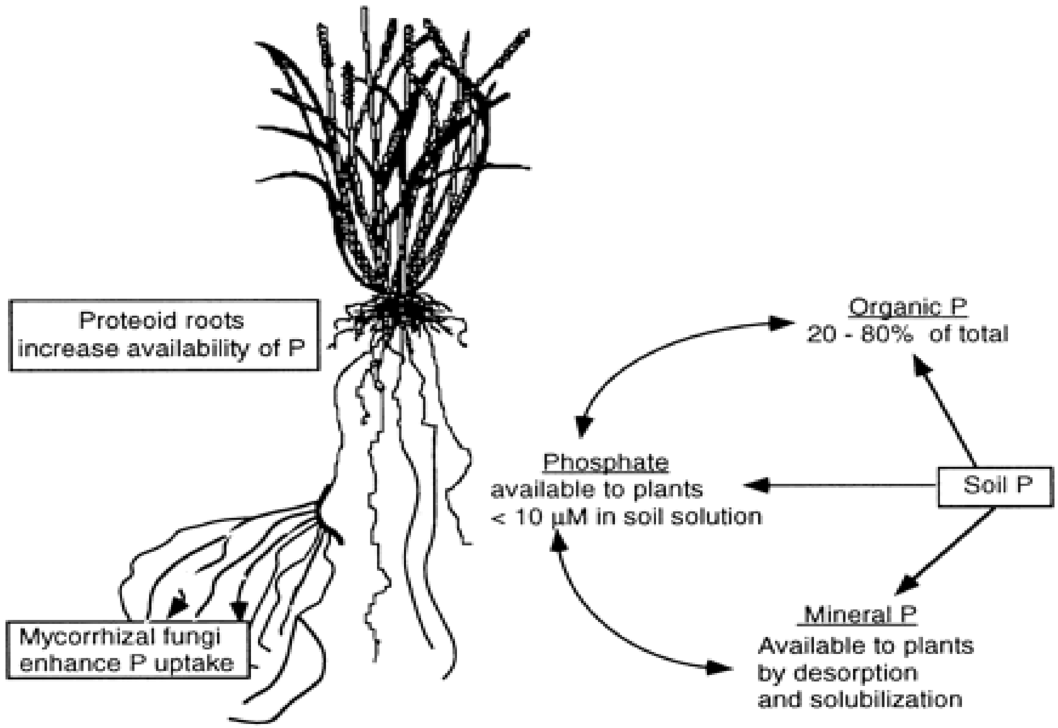

Fig 2.5. Plant acquisition of soil P (From Schachtman et al., 1998)

The nitrogen-fixing perennial and annual forage legumes are members of Fabaceae family that include important forage crops like alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.), and food crops like edible beans (*Phaseolus spp* L.) and peas (*Pisum sativum* L.). Forage legumes are an essential component of sustainable agro-ecosystems. First, they are a primary source of forage to supply protein and fiber for livestock rations (Buxton and Mertens, 1995). Forage legumes can be fed as hay or silage or grazed directly (Sheaffer and Seguin, 2003). In addition, legumes are also grown for land conservation and enhanced soil fertility when incorporated into the cropping system (Sheaffer and Seguin, 2003). There are numerous benefits of including forage legumes in cropping systems (Sheaffer and Seguin, 2003). These benefits include contribution of nitrogen (N), soil and water conservation, production of high quality forage for livestock feed, as well as reduced incidence of weeds, insects, and diseases in soil, and providing a habitat for wildlife.

2.5.1 Biological nitrogen (N_2) fixation

The N benefits of forage legumes have been recognized for a long time (Hoyt and Leitch, 1983; Badaruddin and Meyer, 1989). A distinctive feature of legumes is their ability to fix atmospheric N_2 (Sheaffer and Seguin, 2003; Ates et al., 2014). It is achieved by establishing a symbiotic relationship between legumes and soil bacteria, collectively known as rhizobia (Sheaffer and Seguin, 2003). The amounts of N_2 fixed by legumes are varied depending on legume species and biotic and abiotic factors in the soil (Ledgard and Steele, 1992; Sheaffer and Barnes, 1997; Seguin et al., 2000, 2001). Biotic and abiotic factors affecting N_2 fixation include stresses such as pests, insufficient soil nutrients, soil acidity, salinity, drought and defoliation. These conditions diminish legumes' N_2 fixation ability by interrupting the molecular communication between rhizobia and legumes and reducing legume plant's photosynthetic capacity (Graham, 1992; Zhang and Smith, 2002). High N status of soil is another factor that decreases biological N_2 fixation rates of legumes (Sheaffer and Seguin, 2003). A major proportion of fixed N_2 is removed from the soil by plant harvest; however, a considerable amount of this N is still returned to the soil by residue decomposition and N leaking during root exudation (Sheaffer and Seguin, 2003). For example, according to Ferguson and Gorby (1971), grain crops grown after alfalfa on the prairies can benefit from legume derived soil N in the first two years after forage termination. Because of the legume contribution in soil N nutrition, chemical N fertilizer rates for crops grown following legumes can be reduced (Peterson and Russelle, 1991).

2.5.2 Soil and water conservation

Perennial and annual legumes incorporated in cropping systems can reduce soil erosion by decreasing surface runoff and increasing soil water infiltration rate (Hargrove and Frye, 1987; Zemenchik et al., 1996). Root and shoot residues of legumes can increase SOM and microbial activity which improve soil structure by binding more soil particles together into aggregates and forming more pore spaces, thereby increasing the rate of water infiltration and soil water retention (Angler, 1992; Campbell et al., 1993; Rasse et al., 2000). Also, the active growing legume and its residues on the soil surface can protect the soil from the impact of intensive precipitation, which reduces the formation of surface crust that causes surface pore blockage, decrease the velocity of runoff, and provide shading which helps reduce soil water evaporation (Hargrove and Frye, 1987).

Forage legumes are also quite effective in improving soil quality due to their large and deep root system, longer growth period and greater capacity for nitrogen fixation. Underseeding a cereal crop with legumes helps in the conservation of soil moisture, soil physical characteristics and soil fertility (Campbell et al. 1992). This is because, under wetter soil conditions, perennial and annual forage legumes can produce large quantities of organic matter and nitrogen in the second year after under seeding in cereals (Elbasha et al., 1999). Green manure is a type of growing plant that is ploughed under to improve soil fertility. On degraded soils with low organic matter content, regular green manure application with forage legumes enhances soil N and organic matter over time by adding N and organic residue in the soil. Legumes that improve SOM and provide soil coverage may enhance water available for crop growth by reducing evaporation, improving water infiltration, and increasing soil water holding content (Frye et al., 1988). In contrast, legumes may also deplete soil water on wet soils enabling earlier planting. In some moisture limited conditions, use of legumes in rotations can sometimes be undesirable because of depletion of soil water reserves (Voss and Schrader, 1984; Frye et al., 1988; Huggins et al., 2001).

2.5.3 Weed, disease and pest control

Inclusion of forage legumes in a crop rotation is considered an effective approach to weed, disease and pest control (Liebman and Dyck, 1993; Entz et al., 1995). Generally, diversifying crop rotation with legumes prevents the development of specific weed populations adapted to monoculture crops (Liebman and Dyck, 1993). Legumes like alfalfa (*Medicago sativa* L.) are especially effective in weed control for the following reasons. First of all these legumes are periodically harvested, so weeds can be removed before seed production. In addition, development of new weed populations from seed is restricted by competition with existing plants (Sheaffer and Seguin, 2003). It is also reported that legume residues from cover crops are able to suppress weed emergence and growth (Teasdale, 1993; Fisk et al., 2001). Intercropping legumes with another crop can be a third approach in weed control. Intercropping can greatly suppress weed growth due to the competition between legumes and weeds for water, light and nutrients (Liebman and Dyck, 1993). It is important to note that including legumes in a crop rotation may decrease populations of certain weeds in subsequent crops, but populations of other weeds adapted to the legume system may actually increase (Sheaffer and Seguin, 2003).

Adding forage to crop rotations helps break the life cycle of crop specific pathogens by growing a non-host crop in sequence (Kirkegaard et al., 2008). Rotating crops with non-host or less suseptable plants could be a way to minimize the specific pathogenic populations due to their natural mortality and the antagonistic activities of other microorganisms (Entz, 2004; Kirkegaard et al., 2008). But it is important to recognize that disease cannot be eliminated because some important soil-borne diseases have a wide host range and may not be adequately controlled by forage legume break crops. In addition, perennial forages are sometimes continuous monoculture; therefore host-specific pathogen and insect populations can build up in the crops (Entz et al., 2002).

2.5.4 Feed value

Legume forages have an important role in ruminant production due to their high energy, protein and fibre contents (Dewhurst et al., 2009). They contain high levels of fibre that can be converted to energy by ruminant animals (Buxton and Mertens, 1995). Fibre is essential in rumen function, but providing too much fibre in a ruminant diet slows down digestion and limits dry matter intake (Sheaffer and Seguin, 2003). Legumes have a high concentration of forage protein derived from the biological N_2 fixation of the plant, so they can be a reliable and inexpensive protein source for animal nutrition (Sheaffer and Seguin, 2003; Dewhurst et al., 2009). Generally, compared to grasses, legumes are also a superior source of other essential minerals including Ca, Mg, K (potassium), Zn (zinc), Co (cobalt), and Cu (copper) (Spears, 1994).

2.6 Phosphorus mobilization by legumes

In addition to the aforementioned benefits, rotational effects of legumes on P availability have also been observed (Campbell et al., 1993). Legumes are reported to possess a remarkable capacity to mobilize sparingly soluble P from soils with both low and high P availability (Kamh et al., 1999, 2002; Nuruzzaman et al., 2006; Rose et al., 2010; Wang et al., 2011) compared to less P efficient cereal crops (Braum and Helmke, 1995; Hocking et al., 1997). Kamh et al. (1999) indicated that some legume species such as white lupin (*Lupinus albus* L.) are able to solubilize P even in excess of their own requirements. Legumes can mobilize P from various P pools through the following mechanisms: (i) modification of rhizosphere pH due to root-induced acidification or alkalization (Gahoonia et al., 1992; Morel and Hinsinger, 1999; Hinsinger et al., 2003); (ii) secretion of carboxylic acids such as malate and citrate (Veneklaas et al., 2003); and (iii) exudation of root or microbially derived P solubilizing enzymes such as acid phosphatases and phytases (Richardson, 2001; Nannipieri et al., 2003; Nuruzzaman et al., 2006). In addition, legumes are also capable of improving spatial access to soil P through its special morphological root traits and symbiotic associations with arbuscular mycorrhizal fungi (AM fungi) even though those strategies are not specific to legumes and have also been reported for members of other families such as Brassicaceae or Asteraceae (Hedley et al., 1982; Bolan, 1991). Thus, incorporating legumes into cropping systems as inter crops or in rotation is considered to be one of the promising agronomic approach to access insoluble native soil P reserves and to ensure sustainable crop production around the world (Ae et al., 1990; Lynch, 1998; Horst et al., 2001).

2.6.1 Rhizosphere acidification or alkalization

Modification of rhizosphere pH results mainly from atmospheric N_2 fixation (Tang et al., 1998; Hinsinger et al., 2003) and unbalanced cation/anion uptake ratios (Neumann and Römheld, 2002). Plants' preferential uptake of anions (nitrate NO_3^-) over cations (ammonium NH_4^+) generally maintains electrical neutrality by releasing excess anions such as OH^- or HCO_3^-, thereby inducing an alkalization of the rhizosphere. In contrast, excess uptake of cations, NH_4^+, over anions, NO_3^-, enhances H^+ extrusion, and thereby causing rhizosphere acidification (Gahoonia et al., 1992). In acidic soils, rhizosphere alkalization may enhance P solubility by mobilization of P adsorbed onto Fe and Al oxides (Gahoonia and Nielsen, 1992; Jungk et al., 1993), whereas in neutral and alkaline soils, rhizoshere acidification may improve P availability by increasing the solubility of Ca-phosphate (Gahoonia and Nielsen, 1992). Acidification also occurs in the rhizosphere of N_2 fixing legumes. In this process, the exudation of H^+ in response to the imbalance of cation over anion uptake increases the availability of sparingly soluble soil P (Jarvis and Robson, 1983; Loss et al., 1993; Tang et al., 1999). Root-induced changes of rhizosphere pH may have some other origins. The exudation of organic acids is believed to be one factor that contributes to rhizosphere acidification, but it is a controversial subject (Hinsinger, 2001). According to previous studies, organic acids exuded as anions rather than acids (Haynes, 1990; Jones, 1998), so the net release of H^+ is likely to occur to compensate for this net efflux of negative charges and maintain cation-anion (or charge) balance (Hinsinger, 1998). However, considering the overall cation-anion balance this still means that the release of the organic anion will result in a rhizosphere acidification. Yet, compared with the uptake of major nutrients, the fluxes of exudated organic anions are rather small in many plant species (Jones, 1998). Thus, it can be neglected when considering their potential contribution to changes in rhizosphere pH (Petersen and Böttger 1991). Another possible origin of pH changes in the rhizosphere is the coupling of redox potential and pH as induced by plant roots in the rhizosphere (Van Breemen, 1987; Ahmad and Nye, 1990). In addition, carbon dioxide (CO_2) production by plant root and microbial respiration is considered to be another source of rhizospere acidification (Gollany et al., 1993).

2.6.2 Exudation of carboxylates

It is well documented that nutrient deficiency, particularly, P, Fe and Zn deficiency, stimulates the excretion of carboxylates from both cluster and non-cluster rooted crop species (Hassan et al., 2012). Carboxylates are low molecular weight organic anions including monocarboxylic, (e.g., lactic, gluconic, acetic, formatic) dicarboxylic, (e.g., oxalic, tartaric, malic, fumaric, malonic) and tricarboxyclic acids (e.g., citric acids) (Ryan et al., 2001). The effectiveness of these compounds to solubilize soil P depends upon the numbers and arrangements of carboxy1 and hydroxy1 groups (Ryan et al., 2001). Substantial exudation of carboxylates is well documented amongst a number of grain legume crops including white lupin (*Lupinus albus* L.), pigeon pea (*Cajanus cajan* L.) and chick pea (*Cicer arietinum* L.) (Ae et al., 1991; Neumann and Romheld, 1999; Veneklaas et al., 2003). The exudation rates and composition of carboxylates vary considerably according the crop species and soil type (Dinkelaker et al., 1989; Ae et al., 1990; Veneklaas et al., 2003). For example, white lupin (*Lupinus albus* L.) releases large quantity of citrate from the cluster roots which results in the solubilisation of soil P, and hence increasing the availability of P to plants (Dinkelaker et al., 1995; Hocking et al., 1997). Carboxylates released in the rhizosphere can mobilize soil P pools through the following mechanisms (i) reducing the number of binding sites for fixation via chelation of Fe and Al (Gerke, 1992) and (ii) suppression of re-adsorption and precipitation of Pi into soil and P fixing minerals (Braum and Helmke, 1995; Nziguheba et al., 2000).

2.6.3 Phosphorus solubilizing enzymes

The exudation of plant and microbially derived P solubilizing enzymes such as acid phosphatases and phytases is another process of P solubilisation in many soils, especially in P deficient soils (Jungk et al., 1993; Nuruzzaman et al., 2006; Richardson et al., 2009). The availability of Po is low due to its low solubility in many soils, so it needs to be hydrolyzed by acid phosphatases or phytases before the Pi is released into rhizospere from Po pools for plant uptake (Balemi and Negisho, 2012). Acid phosphatases and phytases are a group of plant and microbial produced enzymes that can enhance soil available P from unavailable P pools by hydrolyzing a variety of Po forms (Tarafdar and Claassen, 1988; Richardson et al., 2001) in response to P deficiency. Li et al. (1997) reported that the amount of acid phosphatases and phytases secreted in response to P limitation differ between plant species. Low P concentration in roots as a result of P deficiency stimulates the synthesis and release of extracellular and intracellular acid phosphatases that function through the hydrolyzing and mobilizing Pi from soil organic phosphates (Duff et al., 1994). Various acid phosphates are identified in plant roots and soil miroorganims (Richardson, 1994), and their activities are dependent upon soil type, plant species and age, as well as microbial activity and community composition (Tarafdar and Jungk, 1987). For example, in P stressed conditions, white lupin (*Lupinus albus* L.) roots exudates about 20 times more acid phosphatases in comparison to P sufficient control plants (Tadano and Sakai, 1991). Plants also improve plant P nutrition by secreting phytases into the rhizospere to hydrolyze the inositol-phosphate and degrading phytic acid with intracellular phytase (Balemi and Negisho, 2012). Edriss et al. (2002) reported that phytases are able to improve the P nutrition of peas by releasing extracellular adenosine triphosphate (ATP) and other organic phosphate molecules into the rhizospere. In plant germination, seedlings can utilize Pi from phytase-induced hydrolyzation of phytic acid (Duff et al., 1994).

2.6.4 Morphological root traits

Deep rooting legumes such as alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), and Egyptian pea (*Sesbania sesban* L.) are able access nutrients in deep soil layers, e.g., N from a depth of 1m (Gathumbi et al., 2003). This mechanism is efficient in P uptake because deep-rooted legume species can improve spatial access to soil P and they also bring P solubilized at depth in the root rhizosphere to the surface in organic forms where it can be recycled through chemical, physical and microbial activity (Campbell et al., 1993). The availability of indigenous soil P as well as residual fertilizer P added in previous years may be enhanced by legumes. The formation of proteoid roots or cluster roots is another important trait of root systems that significantly enhance the P acquisition by legume crops (Fig 2.5) (Schachtman et al., 1998). Some legume species e.g., white lupin (*Lupinus albus* L.), are able to develop proteoid roots to effectively scavenge P from soils and to solubilize insoluble P resources in P deficient soils (Shane and Lambers, 2005). Proteoid roots are closely packed roots covered with dense mat of root hairs, which significantly increase the surface sorption area of the root system to enhance P uptake (Playsted et al., 2006; Lambers et al., 2006). Proteoid roots are specialized in efficient synthesis and secretion of organic anions and acid phosphatases, which are able to mobilize recalcitrant P pools and mineralize Po fraction for plant uptake (Playsted et al., 2006; Lambers et al., 2006).

2.6.5 Symbiotic relationship with arbuscular mycorrhizal fungi (AM fungi)

The symbiotic association between plant roots and AM fungi is known to be as an important mechanism for plant P acquisition from soil (Marschner and Dell, 1994; Smith and Read, 1997). This mechanism plays an important role in P uptake and growth of many legumes (Smith and Read, 1997) in P deficient soil conditions. In this symbiotic association, plants supply carbohydrates to the AM fungi and in return AM fungi develop an extensive hyphal network that is highly efficient in increasing the sorption area of plant roots (Fig 2.6) (Smith and

Read, 1997). Enhanced P absorption by mycorrhizal hyphae is generally related to the increased physical exploration of the soil, but it also can be attributed to the modification of the root environment (Bolan, 1991; Joner and Jacobsen, 1995; Smith and Read, 1997). Increased nutrient absorption through physical exploration of the soil arises when hyphae extend several centimeters out into the surrounding soil allowing considerably larger volume of soil to be explored (Smith, 2003). The mycorrhizae may also solubilize normally insoluble P sources through excretion of various organic acids and acid phosphatases (Ahonen-Jonnarth et al., 2000; Koide and Kabir, 2000). It is also considered that faster movement of P into mycorrhizal hyphae may increase the plant P uptake by increasing the affinity for P ions and by decreasing the threshold concentration required for absorption of P (Shenoy and Kalagudi, 2005). Under low P supply, AM fungi can contribute up to 77% of P uptake; however, only 49% of P uptake is achieved by AM fungi under high P supply (Thingstrup et al., 2000).

2.7 Legume effects on following crops

Certain legume crops, particularly white lupin (*Lupinus albus* L.), chickpea (*Cicer arietinum* L.), field pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.), are not only capable of mobilizing available soil P for their own requirement, but they are also capable of enhancing the growth and P uptake of following crops such as wheat, canola or maize (Hens and Hocking, 2004; Nuruzzaman et al., 2005b). In a rotation study conducted in Western Australia, compared to other legumes, faba bean (*Vicia faba* L.) showed a promising effect on following cereal growth and P uptake in low available P soils (Nuruzzaman et al., 2005a). Also, the beneficial effects of legumes on the growth of following cereal crops in subsequent years have been well documented in Western Canada (Wright, 1990; Stevenson and Kessel, 1996). Although the positive effects of legumes to the following crops are generally attributed to (i) an improved N status of soils through the symbiotic N_2 fixation of legumes (Peoples et al., 1995), (ii) breaking soil borne diseases, and (iii) improving soil physical characteristics (Imai, 1991), there is some evidence that enhanced P solubility is another factor that is responsible for the beneficial effects of legumes on subsequent annual crops (Nuruzzaman et al., 2005). Several studies have demonstrated that the growth and P uptake of wheat was better after legume crops than after wheat in P deficient soils even though adequate N was supplied (Horst et al., 2001; Nuruzzaman et al., 2005). According to Kamh et al. (1999), P efficient legume crops have positive effects on the growth and P uptake of a subsequent maize crop. In addition, Hocking and Randall (2001) demonstrated that white lupin (*Lupinus albus* L.) can increase the use of residual P to sorghum (*Sorghum bicolor* L.) and wheat (*Triticum aestivum L.)* by the exudation considerable amounts of carboxylates into soil. However, this mechanism has been challenged by Jones et al. (2003) and Nuruzzaman et al. (2005a,b) because they found that carboxylates are rapidly decomposed in soil by soil microorganisms (Jones et al., 2003; Nuruzzaman et al., 2005a,b).

The enhanced growth of crops, particularly cereals, grown after legumes has been explained by the following reasons. First, legume crops may solubilize soil P in excess of their own requirements by secreting roots exudates, e.g. protons, acid phosphatases, into soil (Kamh et al., 2002). The excess amount of P could be carried over and utilized by other less P efficient crops grown in rotation or inter-cropped in the cropping system (Gardner and Boundy, 1983; Horst and Waschkies, 1987). Furthermore, P release during the mineralization of legume residues can be another explanation for the elevated crop growth and P acquisition after legumes (Horst et al., 2001; Kamh et al., 2002; Nuruzzaman et al., 2005a,b). After decomposition of the legume residues, P previously taken up by the legume becomes available to the following crop (Oberson, 2011). The main advantage of crop residues is the addition of organic matter to soil (Rasse et al., 2000) which can influence P availability mainly through a range of processes including (i) P release from the residue, (ii) dissolution/desorption of P during residue decomposition (Bolan et al., 1994; Hue et al., 1994; Iyamuremye and Dick, 1996), (iii) microbial immobilization of Pi (Chen et al., 2000), and (iv) the accumulation of Po fractions (Horst et al., 2001). Among them, Po fractions build up seems to have special importance for the maintenance of soil P availability (Seeling and Zasoski, 1993; Oberson et al., 1999). This is because the slower decomposition and release of P from organic matter prevents rapid fixation of Pi (Friessen et al., 1997), and better matches the P requirement of the subsequently grown crops.

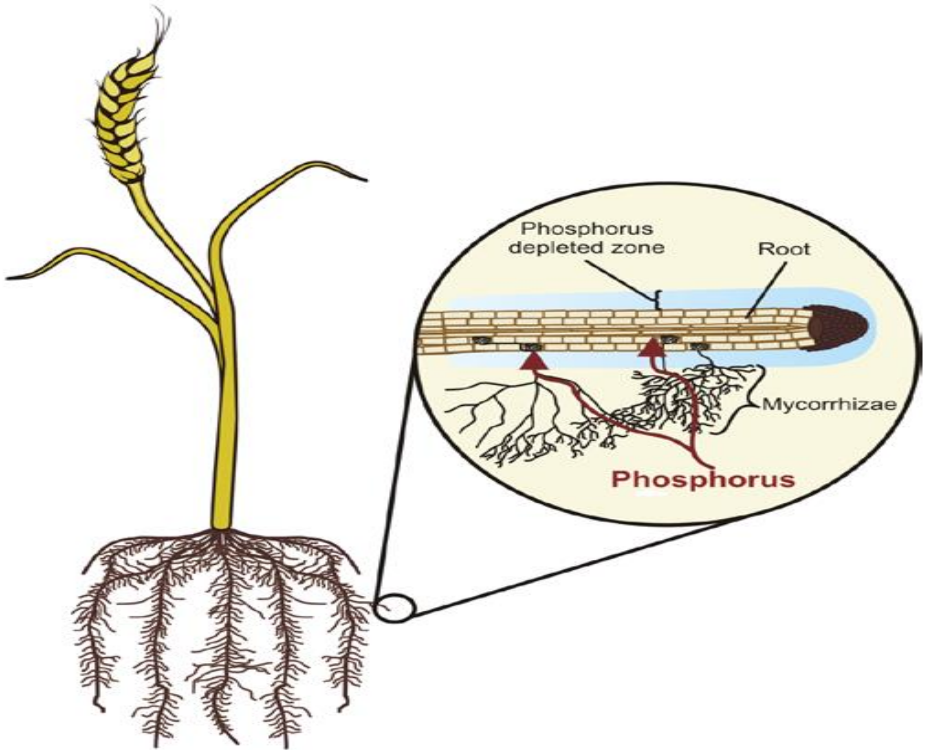

Fig 2.6. Typical mycorrhized plant showing extraradical hyphae of AM fungi (From Roy-Bolduc and Hijri, 2011).

Residue decomposition and the release of P contained in organic materials are mediated by soil organisms as well as by residue chemical compositions such as the concentration of N and P, soluble polyphenol and lignin concentrations (Vanlauwe et al., 2008) and environmental conditions such as moisture and temperature (Lavelle and Spain, 2002). In addition, the carbon : phosphorus (C:P) and N:P ratios of plant materials also influence the mineralization and immobilization of added residue. Crop residues with total P concentrations between 2-3 g kg^{-1}, C:P between 156:1 to 252:1 and N:P between 7:1 to 14:1 were reported to result in a net P release, whereas materials with higher ratios resulted in transient net P immobilization (Brady and Weil, 2002).

3. YIELD AND PHOSPHORUS UPTAKE OF FORAGE LEGUMES AND ANNUAL CROPS OVER TWO YEARS IN FOUR CHERNOZEMIC SOILS OF SASKATCHEWAN

3.1 Preface

The ability of legumes to mobilize soil phosphorus (P) for uptake by the legume and the crops that follow has been well documented globally using various grain legume species, but the role of forage legumes in P mobilization when included in crop rotations has gained less attention, especially in prairie soils. Perennial and annual forage legumes are important components of agro-ecosystem as they are primary sources of forages to supply protein and fiber for livestock rations. In addition, forage legumes contribute biologically fixed nitrogen (N) and sustain the soil by reducing erosion and increasing soil organic matter (SOM) levels. Thus, it is valuable to assess the P mobilization and uptake by forage legumes included in crop rotations in comparison to annual crops under Saskatchewan soil-climatic conditions. A four-year field experiment was conducted at four experimental sites representing different soil climatic zones in Saskatchewan. This chapter presents the results from first two years of research work (2010 and 2011) in which short-term perennial forage legumes (alfalfa and red clover) are compared to annual crops in terms of yield, crop P uptake and removal over a two year period.

3.2 Abstract

Legumes are reported to have a superior ability to mobilize and take up sparingly soluble P from soils compared to non-legume crops. Although many studies have examined the effect of forage legumes on soil N fertility, the P mobilization and P uptake by forage legumes when included in a crop rotation has not been adequately evaluated in the prairies. An experiment was conducted in four soil zones of Saskatchewan: Dark Brown soil zone (Saskatoon), thin Black soil zone (Lanigan), Brown soil zone (Swift Current), and Gray soil zone (Melfort) over two growing seasons. The objective of this study was to compare the yield and P uptake by the rotational crops alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), barley (*Hordeum vulgare* L.), pea (*Pisum sativum* L.) and flax (*Linum usitatissimum* L.) over two years of hay and cereal production. The investigated crop sequences were 1) two years of alfalfa (*Medicago sativa* L.); 2) two years of red clover (*Trifolium pratense* L.); 3) barley (*Hordeum vulgare* L.) in year 1

followed by pea (*Pisum sativum* L.) and 4) barley (*Hordeum vulgare* L.) in year 1 followed by flax (*Linum usitatissimum* L.). Crop biomass yield, plant P concentration as well as crop P uptake and removal were determined for each crop in each year. In 2010 (the first year of the rotation), barley produced higher amounts of plant biomass compared to alfalfa and red clover at Saskatoon, Swift Current and Melfort sites ($P < 0.10$), whereas in 2011 (the second year of the rotation), the biomass production of alfalfa and red clover was much higher than the annual crops (pea and flax) at all four sites ($P < 0.10$). In 2010, among rotation crops, barley took up 2-3 times more P than alfalfa and red clover ($P < 0.10$) at Saskatoon, Swift Current and Melfort sites. No differences were found between alfalfa and red clover P uptake at all sites ($P > 0.10$). When comparing the sites, greatest P uptake was found in barley rotations at Melfort in 2010. In the subsequent year, the P uptake by alfalfa and red clover was significantly greater than in 2010 at all locations. When the rotational crops were compared, alfalfa and red clover took up approximately 3 times more P compared with peas and flax ($P < 0.10$) at Saskatoon, Lanigan and Swift Current sites. Alfalfa P uptake was greater than red clover at Lanigan and Swift Current ($P < 0.10$), but the P uptake (grain+straw) of peas and flax was similar ($P > 0.10$) at these sites. Overall, after two years, alfalfa and red clover removed more P from the soil in the hay harvest compared to P removed in grain in the barley-pea and barley-flax rotations at all sites.

3.3 Introduction

Phosphorus (P) is a necessary macronutrient for plant growth, crop production and quality (Md Alamgir et al., 2012). Low availability of soil P is a major nutritional constraint for primary crop production in many agricultural soils around the world (Chen et al., 2008; Wang et al., 2011). Although soil P deficiency can be corrected or even improved by continued application of P fertilizer (Condron, 2004), crop P fertilizer use efficiency is fairly low in the first year. Crops take up only about 10-30 % of the applied P (Holford, 1997; Syers et al., 2008; Sattari et al., 2012), and a considerable portion of the unutilized P is mainly fixed or precipitated into less labile P forms (Bolland, 1993; Holford, 1997; Richardson et al., 2001; Bünemann et al., 2006). One strategy to maximize the use of accumulated soil P reserves is through growing crops in rotation which can access different components of the soil P reserve (Stutter et al., 2012), thus improving the overall P bioavailability and utilization.

Crop species vary in their ability to take up P from soils according to differences in their root morphology and root hair density, the amounts and types of root exudates released, the symbioses that they form with arbuscular mycorrhizal fungi, and their ability to change rhizosphere pH (Hocking et al., 1997; Nuruzzaman et al., 2005b). Among crop species, legumes are reported to have a remarkable capacity to mobilize and take up sparingly soluble P from soils compared to non-legume crops (Hocking et al., 2000; Nuruzzaman et al., 2005a, b; Hassan et al., 2012). Thus, incorporating these P mobilizing crop species into a cropping system may also be employed as an alternative way to access insoluble native soil P reserves (Ae et al., 1990; Horst et al., 2001). The P mobilization and uptake have been studied frequently in many parts of the world using various grain legume species such as white lupin (*Lupinus albus* L.) and faba bean (*Vicia faba* L.) mainly under laboratory conditions (Kamh et al., 1999; Nuruzzaman et al., 2005a, 2006; Vu et al., 2010; Rose et al., 2010; Hassan et al., 2012; Wang et al., 2012). However, very few studies have examined the P mobilization and uptake by forage legumes included in short-term crop rotations grown under field conditions. In the prairies, the N fixation of forage legumes and their effects on N availability in soil after several years of production is reasonably well documented (Bullied et al., 2002; Malhi et al., 2002), whereas the ability of perennial forage legumes such as alfalfa and red clover to mobilize soil P for plant uptake when included in crop rotations has received less attention.

Forage legumes are essential components of the agro-ecosystem (Morel et al., 2012) due to their important role in dairy and meat production as a primary source of forage to supply protein, fibre and energy for livestock rations (Buxton and Mertens, 1995; Morel et al., 2012). In addition, these crops are also grown for land conservation and enhanced soil fertility when incorporated into the cropping system after continuous cropping (Sheaffer and Seguin, 2003). Alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) are predominant forage crops grown on a large scale worldwide (Taylor and Quesenberry, 1996; Morel et al., 2012), as well as in Western Canada because of their superior productivity and wide adaptation to various soils and environmental conditions when compared to other legumes (Entiz et al., 2002; Sheaffer and Seguin, 2003). Inclusion of these crops in rotation for two years may increase the P availability and crop P uptake. The objective of this study was to compare the yield and P uptake of alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) grown for two years in comparison to barley (*Hordeum vulgare* L.) - pea (*Pisum sativum* L.) and barley - flax (*Linum usitatissimum* L.) in four soil-climatic zones of Saskatchewan.

3.4 Materials and Methods

3.4.1 Site description

The study was conducted at four sites: 1) the Agriculture and Agri-Food Canada Research Farm (AAFC-Scott) at Saskatoon (52°04′N, 108°08′W); 2) the Western Beef Development Centre's (WBDC) Termuende Research Ranch at Lanigan (51°51′N, 105°02′W); 3) the Semiarid Prairie Agricultural Research Centre (SPARC) at Swift Current (50°16′N 107°44′W) and 4) the Melfort Research Farm (MRF) at Melfort (52°08′N, 104 °06′W) Saskatchewan, Canada from 2010 to 2011. The soils at each site are classified according to the Canadian System of Soil Classification. The soil at Saskatoon the site is classified as an Orthic Dark Brown Chernozem, Shellbrook-Hamlin association on a nearly level topography of very fine sandy loam to loam texture (Saskatchewan Soil Survey, 1999). The soil at Lanigan is classified as an Orthic Black Chernozem, Meota-Hamlin association of loamy sand to very fine sandy loam texture, on a very gently sloping topography (Saskatchewan Soil Survey, 1992). The soil at Swift Current is classified as Orthic Brown Chernozem, Swinton association of a silt-loam texture on a gently sloping topography (Saskatchewan Soil Survey, 1990). The soil at Melfort is classified as Orthic Black Chernozem, Tisdale-Eldersley association on a gently sloping topography. The soil textures vary from silty clay loam to silty clay (Saskatchewan Soil Survey, 1987). Soil properties at the sites and climate and ecoregion characterization for the different locations are presented in Table 3.1 and Table 3.2. The monthly precipitation and mean temperature for the experimental years 2010 and 2011 as well as the long-term (30 years from 1971 to 2000) average are presented in Table 3.3 (Environment Canada, 2011, 2012).

Table 3.1. Selected physiochemical properties of soils at the four experimental sites.

Locations	pH ($1:2H_2O$)	EC† ($dS\ m^{-1}$)	OC† (%)	Particle size distribution (%)			Soil texture‡
				Sand	Silt	Clay	
Saskatoon	7.2	0.299	1.9	15.0	39.7	45.2	Silty clay
Lanigan	7.7	0.327	2.4	29.6	50.9	19.5	Silty loam
Swift Current	7.0	0.166	1.6	31.3	47.7	30.9	Clay loam
Melfort	7.9	0.210	4.5	16.4	42.3	41.3	Silty clay

†EC is electrical conductivity; OC is organic carbon content

‡Soil texture was classified according to the Canadian Soil Texture Triangle.

Table 3.2. The climate conditions and environmental characteristics at the four experimental sites.

Sites	Location	Climate	Ecoregion
AAFC-Scott, Saskatoon	52°40′N, 108°08′W	Semi-arid	Moist Mixed Grassland
WBDC, Lanigan	51°51′N, 105°02′W	Semi-arid	Aspen Parkland ecoregion
SPARC, Swift Current	50°16′N, 107°44′W	Semi-arid	Mixed Grassland
MRF, Melfort	52°08′N, 104°06′W	Sub-humid	Aspen Parkland ecoregion

3.4.2 Experimental design and treatments

The two-year field experiment, which was initiated in 2010 at the four experimental sites, was set up as a randomized complete block design with four replicates. Four crop rotations were included as four agronomic treatments in this experiment; the crop rotation treatments are described in Table 3.4. The first year (2010), two forage legumes: alfalfa (2065 M alfalfa) and red clover (Belle Red clover) and two separate set plots of two row barley (CDC Copeland) were included into the crop rotation as rotation crops for the first year. Second year (2011) crops included in the rotation were the pre-established (seeded in 2010) forage legumes alfalfa and red clover, and following the barley in 2010 an annual legume pea (Golden), and oil seed flax (CDC Bethune) were seeded in 2011. Therefore, two different perennial forage legumes (alfalfa and red clover) were compared with cereal crop barley (in the first year) followed by either annual legume (peas) or oil seed flax (in the second year) on their effects on yield, P uptake and removal.

Table 3.3. Monthly mean temperature (°C) and precipitation (mm) from April to October, 2010, 2011 and 30 years mean temperature (from 1971 to 2000) and precipitation at the four study sites in Saskatchewan.

Locations	Year		Apr.	May	June	July	Aug.	Sep.	Oct.†
Saskatoon	2010	Temp. (°C)‡	6.4	9.6	15.3	17.6	16.1	10.5	6.5
	2011	Temp. (°C)	3.1	10.9	15.5	18.4	17.2	14.7	6.5
	30years	Ave. temp. (°C)‡	4.7	11.8	16.0	18.3	17.6	11.5	.
	2010	Precip. (mm)‡	72.6	128.5	169.0	46.0	43.7	87.9	12.2
	2011	Precip. (mm)	0.6	17.5	94.4	68.6	16.5	6.0	.
	30years	Ave. precip.‡	24.2	43.6	60.5	57.3	35.4	30.6	16.9
Lanigan	2010	Temp. (°C)	5.7	9.0	15.7	17.8	16.3	10.3	6.4
	2011	Temp. (°C)	2.4	10.2	14.8	17.8	17.2	13.5	6.4
	30years	Ave. temp. (°C)	4.0	11.3	15.9	18.1	17.3	11.3	.
	2010	Precip. (mm)	64.1	139.0	71.4	80.8	51.6	10.2	25.0
	2011	Precip. (mm)	12.3	20.8	54.2	114.6	54.2	11.5	.
	30years	Ave. precip. (mm)	30.3	53.5	83.9	66.1	53.0	42.6	28.0
Swift Current	2010	Temp. (°C)	6.1	7.8	15.4	17.0	16.8	10.7	7.9
	2011	Temp. (°C)	2.5	9.5	14.3	18.2	18.2	15.1	7.4
	30years	Ave. temp. (°C)	4.9	11.1	15.6	18.1	17.9	11.8	.
	2010	Precip. (mm)	33.7	93.6	121.5	71.5	85.0	99.7	8.4
	2011	Precip. (mm)	25.4	56.9	117.3	68.0	30.4	10.6	.
	30years	Ave. precip. (mm)	22.3	49.5	66.0	52.0	39.9	30.2	16.2
Melfort	2010	Temp. (°C)	5.8	9.1	15.3	17.4	16.0	9.5	6.0
	2011	Temp. (°C)	1.5	10.1	15.4	17.6	17.0	13.8	5.7
	30years	Ave. temp. (°C)	2.5	10.8	15.7	17.4	16.4	10.5	.
	2010	Precip. (mm)	140.6	66.6	113.2	63.6	56.8	92.0	18.4
	2011	Precip. (mm)	8.1	10.5	103.5	73.3	10.7	1.1	.
	30years	Ave. precip. (mm)	24.5	45.6	65.8	75.7	56.8	39.9	24.7

† The 30 years average temperature and 2011 precipitation for October are not available.
‡ Temp. (°C) is temperature; Ave. temp. (°C) is average temperature; Precip. (mm) is precipitation; Ave. precip. (mm) is average precipitation.

In 2010, alfalfa, red clover and barley were seeded in the first week of June with a Fabro plot drill equipped with Atom Jet openers on 20 cm row spacing at three sites except Lanigan, where crops were seeded with the PAMI plot drill equipped with Flexicoil Stealth openers. The plot sizes varied among experimental sites according to the land available: at Swift Current 48 m^2 (6m × 8m), at Saskatoon 37.5 m^2 (6.25 m × 6 m), at Melfort 51.1 m^2 (7 m × 7.3 m) and at Lanigan 36 m^2 (6m × 6 m). The barley was seeded at the rate of 84 kg ha^{-1} and alfalfa and red clover were seeded at the rate of 9 kg ha^{-1} at three sites except at Swift Current, where seeding rate of forage legumes was 4.5 kg ha^{-1} as recommended for drier regions. Nitrogen fertilizer was applied at the recommended rate of 50 kg N ha^{-1} as 46-0-0 with the barley at Swift Current, Saskatoon, Lanigan sites. At Melfort site, N fertilizer was broadcast applied at the rate of 70 kg N ha^{-1} as a 34-0-0 prior to seeding. Fertilizer P was side banded at the recommended rate of 15 kg P_20_5 ha^{-1} as 11-52-0 at all sites for all crops in 2010 only. Alfalfa and red clover hay was harvested by flail plot harvester at all sites and hay yield was recorded. At crop maturity, barley grain yield was determined by harvesting 10 m^2 areas from each plot using a Wintersteiger plot combine; total above ground biomass was hand-harvested from two 1 m^2 areas of the per plot, at three sites: Saskatoon, Swift Current and Melfort. At Lanigan in 2010, barley grain did not mature enough to harvest by the end of September due to undesirable weather conditions. There was a forecast of frost; therefore, biomass (straw+grain) was harvested as a green feed at this site. After the harvest, biomass samples were air dried and weighed.

In 2011, pre-established perennial forage legumes alfalfa and red clover were grown continually, while the annual legume peas and oil-seed flax were seeded at the recommended rate for each location. Peas were inoculated at the recommended rates for each site prior to seeding but not fertilized. Flax was seeded with 70 kg ha^{-1} N fertilizer as 46-0-0. During the growing season, weeds in pea and flax plots were controlled with recommended type and rate of herbicide at each site. Hay was harvested two to three times according to the growing conditions at each site. Pea and flax biomass samples were taken manually, air dried and the biomass (grain+straw) was measured. Pea and flax grains were combined and grain yields were recorded.

Table 3.4. Description of crop rotation treatments.

Rotation	2010	2011
1	Alfalfa	Alfalfa
2	Red clover	Red clover
3	Barley1	Pea
4	Barley2	Flax

3.4.3 Plant analysis

Plant grain and straw samples for 2010 and 2011 were collected, air-dried and ground for P analysis. A 0.25 g sub-sample of ground grain from each replicate was digested using a H_2SO_4- H_2O_2 digestion method (Thomas et al., 1976). Then, the P concentration in the digested solution was determined colorimetrically using a Technicon Autoanalyzer II (Technicon Industrial Systems, 1973). Plant P uptake (grain and straw) (kg ha^{-1}) was calculated multiplying the grain and straw P concentration by grain and straw yield, respectively.

3.4.4 Soil analysis

Soil samples from all sites were collected in the spring of 2010 prior to seeding. At each experimental site, three soil cores were taken from each plot by using a core soil sampler (diameter 4.5 cm and length 100 cm) at two depths: 0-30 cm and 30-60 cm, air-dried, ground and dry sieved to <2 mm. These samples were then analyzed for initial soil characteristics including soil texture, organic matter content, pH and electrical conductivity (EC). After doing a week of pre-treatment, soil texture was determined using a laser scattering particle size distribution analyzer (HORIBA© LTD., 2007). Four composite samples from each experimental site were used for soil texture analysis. The composite samples were prepared by combining a surface soil (0-30 cm) sample from each plot at each site. Soil organic carbon content was analyzed using the Leco C632 carbon combustion analyzer (LECO© Corporation, 2007) following the protocols of Wang and Anderson (1998) (Leco Instruments Limited, Mississauga, Ontario, L5T 2H7). In this method, organic carbon released through Leco combustion at 840 °C for 120 s or less is determined as carbon dioxide through infrared spectroscopy (Wang and Anderson, 1998). Soil pH and EC were measured with a glass electrode using 1:2 soil : water suspensions (Rhoades, 1982).

3.4.5 Statistical analysis

Mean values of crop biomass, P concentrations, P uptake and removal were compared among treatments by one-way analysis of variance tests (ANOVA). The data were analyzed as a RCBD design using Proc. Mixed Procedure of SAS (Version 9.3; SAS Institute., Cary, NC). The treatment (crop types) was the fixed effect and replications in each site were considered as block effect. Means were compared among treatments using Tukey's multi-comparison tests. Before the ANOVA, data were checked for the normality and equality of variances using the UNIVARIATE procedure; however, no transformation was needed. Significance was declared at $P < 0.10$. The coefficient of variation for replicate analyses of soil and plant samples was ≤ 5%. To ensure accuracy, all routine analyses included a standard reference soil or plant material of known established concentration every 40 samples.

3.5 Results

3.5.1 Crop yields

3.5.1.1 Legume hay yield

In 2010, the first year of the forage establishment, alfalfa and red clover hay yields were low, ranging from 800 kg ha^{-1} to 5000 kg ha^{-1} (Fig 3.1A). When the hay production of two forage legumes was compared, alfalfa hay yield was greater than red clover ($P < 0.10$) at Lanigan and Swift Current, but for Saskatoon and Melfort, there were no differences in the hay yield production of alfalfa and red clover ($P > 0.10$) (Fig 3.1A). Among different sites, hay yield of alfalfa is highest at Lanigan, whereas the lowest alfalfa and red clover hay yield was in Swift Current. In 2011, one year after the forage establishment, alfalfa and red clover produced excellent hay yields at all sites, ranging from 8000 kg ha^{-1} to 16000 kg ha^{-1} (Fig 3.1B). The hay yields of alfalfa and red clover were three times higher relative to the first year (year 2010). Among alfalfa and red clover, alfalfa hay yield was higher than red clover hay yield at three sites ($P < 0.10$) except for Lanigan, where there were no differences between hay production of alfalfa and red clover ($P > 0.10$) (Fig 3.1B). Among different sites, the highest hay yield was harvested in Saskatoon, and the lowest hay yield was recorded at Swift Current.

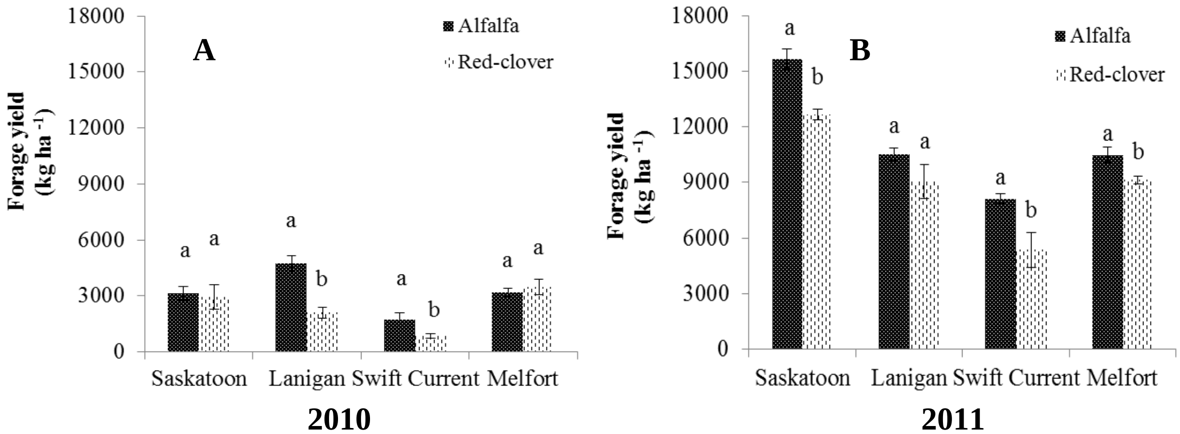

Fig 3.1. Forage yield of alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) grown at four locations in 2010 (A) and 2011 (B) in Saskatchewan, Canada. Error bars represent standard errors. For a given site, treatment means followed by a different letter are significantly different ($P < 0.10$).

3.5.1.2 Crop biomass and grain yields

In 2010 (the first year of the rotation), the barley crop produced greater amounts of plant biomass compared to alfalfa and red clover at Saskatoon, Swift Current and Melfort locations ($P < 0.10$) (Table 3.5). At the Lanigan site, biomass production of alfalfa was numerically higher than barley, but there was no significant differences among the rotation crops ($P = 0.09$). Among four sites, plant biomass production was highest at Melfort and lowest at Swift Current. In 2011 (the second year of the rotation), the biomass production of alfalfa and red clover was much higher than annual crops at all sites ($P < 0.10$) (Table 3.5). At Saskatoon, alfalfa biomass was highest followed by red clover and peas, and flax produced the lowest biomass ($P = 0.011$). At Lanigan, alfalfa and red clover biomass was higher than pea and flax biomass ($P = 0.0002$) but no differences were detected between alfalfa and red clover biomass production, and peas and flax biomass production ($P > 0.10$). At Swift Current, alfalfa biomass was highest followed by red clover, flax and peas ($P < 0.0001$). Finally at Melfort, there were significant differences in biomass production among the various crops ($P < 0.001$); crop biomass production order was alfalfa > red clover > flax > pea. Among the different sites, the highest crop biomass production during the second year of the crop rotation was in Saskatoon, which was different from the first year in which the highest crop biomass was produced at Melfort site. The lowest crop biomass production was found again at the Swift Current site.

In 2010, the barley grain yield was greater at Melfort than other sites (Fig 3.2A). In Lanigan, however, barley was harvested as green feed, so no grain yield was recorded (Fig 3.2A). The pea and flax (grain and seed) yield in 2011 was quite variable (Fig 3.2B). At Saskatoon and Melfort, pea and flax produced better yield compared to Lanigan and Swift Current. At Melfort, flax yield was much higher than pea yield ($P < 0.10$).

Table 3.5. Forage yield of alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) and biomass of barley (*Hordeum vulgare* L.), pea (*Pisum sativum* L.) and flax (*Linum usitatissimum* L.) grown at four locations for two years in Saskatchewan, Canada.

Year	Sites	Biomass†				*P* value
		Alfalfa	Red clover	Barley1	Barley2	
		-------------- (kg ha^{-1}) ---------------				
2010	Saskatoon	3146^{b}‡	2961^{b}	8236^{a}	8031^{a}	0.000
	Lanigan	4726^{a}	2104^{b}	3945^{ab}	4041^{ab}	0.097
	Swift Current	1726^{b}	848^{b}	7101^{a}	7735^{a}	<0.001
	Melfort	3200^{b}	3476^{b}	8820^{a}	8746^{a}	<0.001
		Alfalfa	Red clover	Peas	Flax	
		-------------- (kg ha^{-1}) ---------------				
2011	Saskatoon	15645^{a}	12643^{ab}	8182^{bc}	6131^{c}	0.011
	Lanigan	10527^{a}	9030^{a}	3945^{b}	4042^{b}	0.002
	Swift Current	8117^{a}	5358^{b}	2314^{c}	2716^{c}	<0.001
	Melfort	10467^{a}	9137^{b}	3732^{d}	7029^{c}	<0.001

† Biomass is the total harvested biomass (roots not included).
‡ Means with the different superscript letter in the same row are significantly different ($P < 0.10$).
§ Standard errors are provided in the appendix.

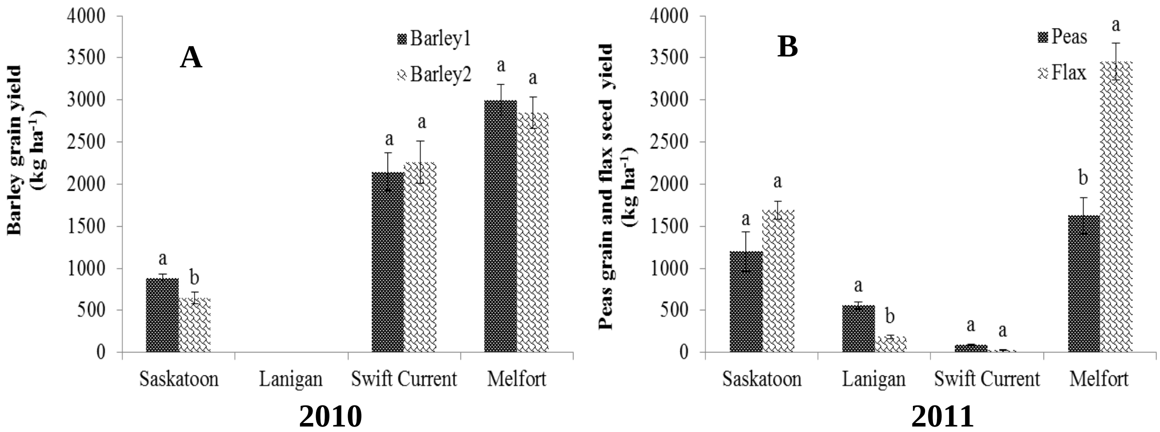

Fig 3.2. Grain yield of barley (*Hordeum vulgare* L.) and peas (*Pisum sativum* L.), and seed yield of flax (*Linum usitatissimum* L.) grown at four locations in 2010 (A) and 2011 (B) in Saskatchewan, Canada. Error bars represent standard errors. For a given site, treatment means with the different letter are significantly different (P < 0.10).

3.5.2 Plant P concentrations

3.5.2.1 Phosphorus concentrations in forage legumes over two years

In 2010, the P concentrations in alfalfa and red clover shoots were similar at Saskatoon and Melfort (P > 0.10), but at Lanigan and Swift Current, P concentration in red clover was higher than alfalfa (P < 0.10) (Fig 3.3A). In 2011, there were no differences between alfalfa and red clover P concentration at Saskatoon, Lanigan and Swift Current sites (P > 0.10) (Fig 3.3B). At Melfort, however, red clover P concentration was greater that alfalfa P concentration (P < 0.10) (Fig 3.3B). When the different sites were compared, the P concentrations in alfalfa and red clover were relatively higher at Lanigan compared to other locations both in the first and second year of crop rotation (2010 and 2011). Higher plant P concentrations are consistent with the high available P content of the Lanigan soil compared to the other three soils (see Chapter 4).

3.5.2.1 Phosphorus concentrations in annual crops over two years

In 2010 and 2011, as expected, barley grain P concentration was higher than straw P concentration (Fig 3.4A, B). Among crops, grain P concentration in flax exceeded grain P concentration in pea at Lanigan. However, at Swift Current and Melfort sites, pea grain P concentration was greater (P < 0.10) (Fig 3.3 C, D). At the Saskatoon site, pea and flax grain P concentrations were similar (P > 0.10). No differences were found in the straw P concentrations among peas and flax at the sites except for Swift Current, where pea straw P concentration was slightly higher than flax straw P concentration. Generally, the barley, peas and flax P concentrations in the grain and the straw were higher in Lanigan site relative to other site, as was observed for the forage legumes.

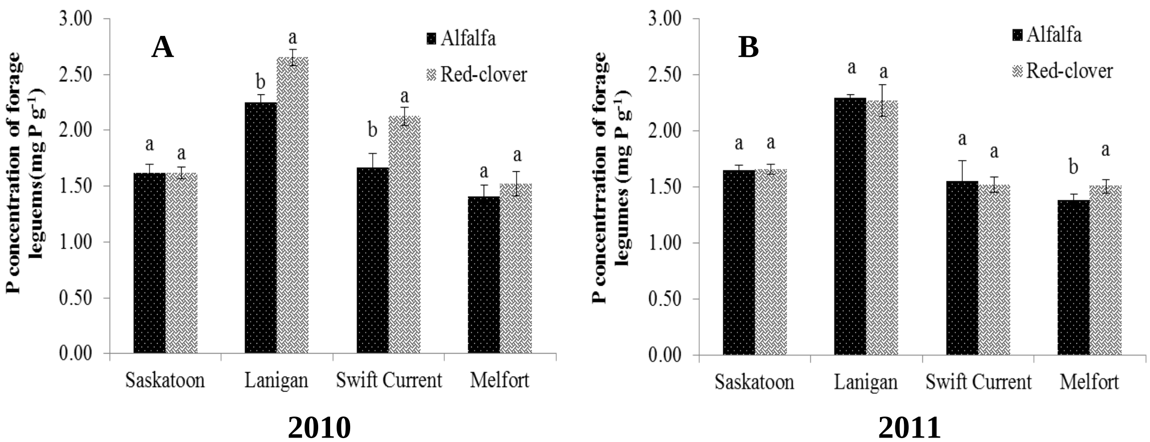

Fig 3.3. The concentration of P in shoot of alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) grown at four sites in 2010 (A) and 2011 (B) in Saskatchewan, Canada. Error bars represent standard errors. For a given site, treatment means followed by the same letter are not significantly different ($P > 0.10$).

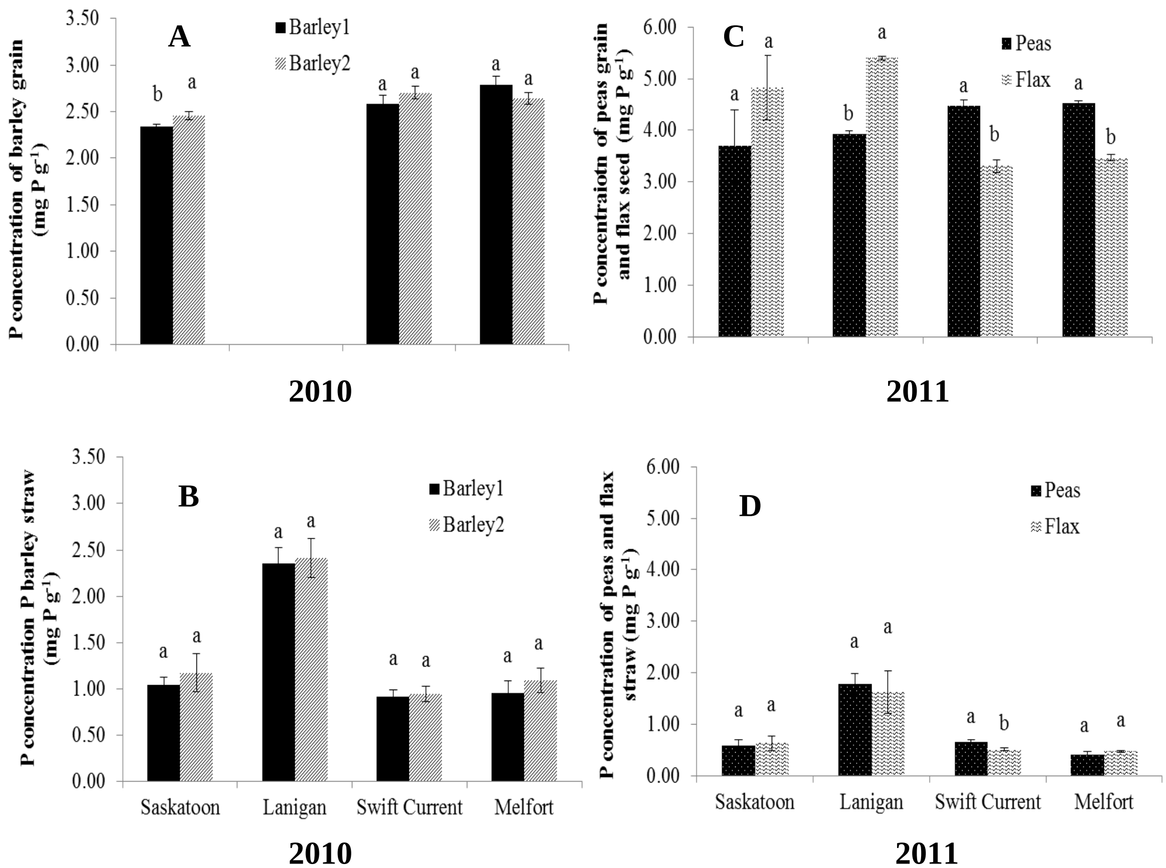

Fig 3.4. The P concentrations of barley (*Hordeum vulgare* L.) peas (*Pisum sativum* L.) and flax (*Linum usitatissimum* L.) grown at four sites in 2010 (A, B) and 2011(C, D) in Saskatchewan, Canada. Error bars represent standard errors. For a given site, treatment means followed by the same letter are not significantly different ($P > 0.10$).

3.5.3 Crop P uptake

3.5.3.1 Phosphorus uptake by forage legumes

The alfalfa and red clover P uptake during the first year of the crop rotation (Fig 3.5A) was low, ranging from 2 kg ha^{-1} to 11 kg ha^{-1}. There were no differences between P uptake of alfalfa and red clover at the Saskatoon, Swift Current and Melfort sites ($P > 0.10$). At Lanigan, however, alfalfa took up approximately twice as much P relative to red clover ($P < 0.10$). When forage legume P uptake among different sites was compared, alfalfa took up more P at Lanigan site, when compared to other sites. In the second year of the crop rotation (year 2011), the P uptake by alfalfa and red clover was increased significantly, approximately 2-4 times, compared to their P uptake in 2010 (Fig 3.5B). Alfalfa P uptake was consistently higher than red clover P uptake at the Saskatoon, Lanigan and Swift Current sites ($P < 0.10$), but this was not observed at Melfort. At this site, the P uptake by alfalfa and red clover was similar ($P > 0.10$). The crop P uptake was highest at Saskatoon followed by Lanigan and Melfort; with the lowest P uptake found at the Swift Current site.

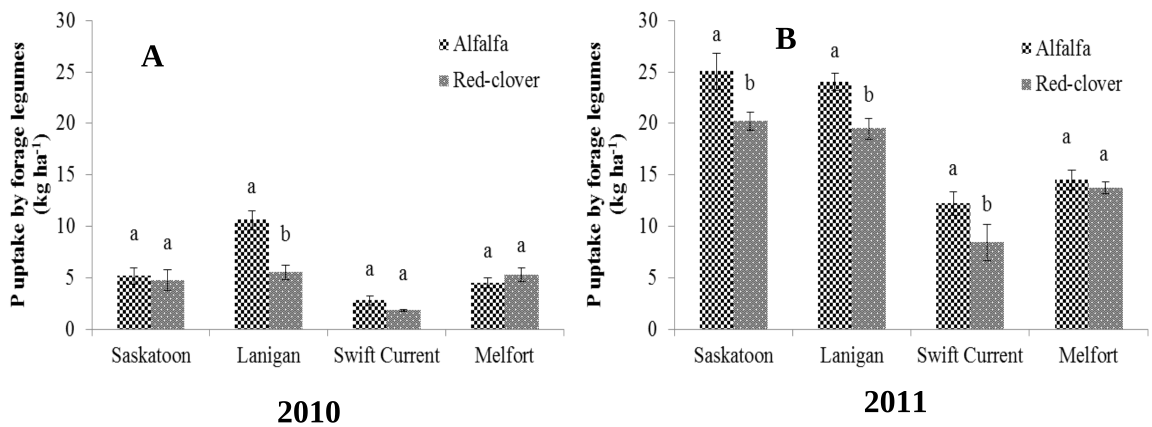

Fig 3.5. Shoot P uptake by alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) grown at four locations in 2010 (A) and 2011 (B) in Saskatchewan, Canada. Error bars represent standard errors. For a given site, treatment means with the different letter are significantly different ($P < 0.10$).

3.5.3.2 Phosphorus uptake by annual crops grown over two years

In 2010, the P uptake in barley grain was slightly higher, ranging from 6 kg ha^{-1} to 8 kg ha^{-1}, than the P uptake in barley straw (4 kg ha^{-1} to 6 kg ha^{-1}) (Fig 3.6A,B) at Swift Current and Melfort. However, the reverse was observed at Saskatoon. Barley grain was not harvested from the Lanigan site in 2010, as it was harvested as green feed. The next year (2011), the grain P uptake by pea and flax was higher than the straw P uptake of these crops at Saskatoon and Melfort (Fig 3.6 C, D). At Lanigan and Swift Current, interestingly, pea and flax straw contained more P than the grain proportion of the crops. At Saskatoon and Melfort, flax seed took up more P compared with the pea grain, but at Lanigan and Swift Current, P uptake associated with pea grain was higher than flax seed ($P < 0.10$). When the straw P uptake of peas and flax was compared, no differences were found at three sites, except for Melfort ($P > 0.10$), where flax straw P uptake was higher than pea straw P uptake ($P < 0.10$).

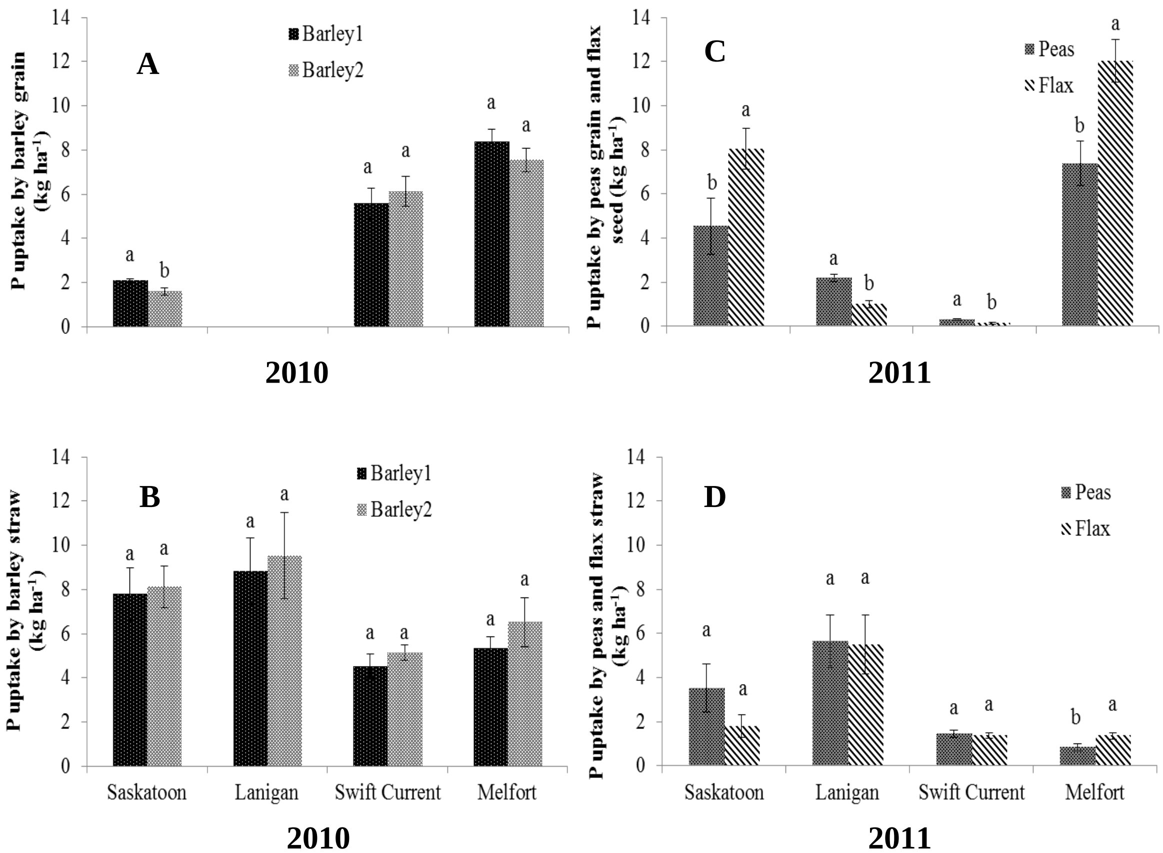

Fig 3.6. P uptake of barley (*Hordeum vulgare* L.), peas (*Pisum sativum* L.) and flax (*Linum usitatissimum* L.) grown at four locations in 2010 (A, B) and 2011(C, D) in Saskatchewan, Canada. Error bars represent standard errors. For a given site, treatment means followed by the same letter are not significantly different ($P > 0.10$).

3.5.3.3 Total P uptake by crops in rotation over two years

Total P uptake (grain+straw) of various rotation crops over the two years (2010 and 2011) at the four locations are shown in Table 3.6. In 2010, the total P uptake by barley was 2-3 times higher than alfalfa and red clover P uptake ($P < 0.10$) at Saskatoon, Swift Current and Melfort. Surprisingly, at the Lanigan site, no differences were found between total P uptake among the rotation crops ($P > 0.10$). In 2011, the P uptake by alfalfa and red clover were significantly greater than in the 2010 establishment year at all locations (Table 3.6). In 2011, alfalfa and red clover took up much greater amounts of P compared with peas and flax ($P < 0.10$) at Saskatoon, Lanigan and Swift Current sites. At Melfort, the amounts of P taken up by alfalfa, red clover and peas were similar and the amounts were significantly higher than P taken up by flax.

Table 3.6. The P uptake by alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) and total P uptake (grain+straw) by barley (*Hordeum vulgare* L.), peas (*Pisum sativum* L.) and flax (*Linum usitatissimum* L.) grown at four locations for two years in Saskatchewan, Canada.

Year	Sites	Total P uptake				*P* value
		Alfalfa	Red clover	Barley1	Barley2	
		-------------- (kg ha^{-1}) ---------------				
2010	Saskatoon	5.14[b]†	4.74[b]	10.77[a]	10.45[a]	0.0009
	Lanigan	10.61[a]	5.53[a]	8.83[a]	9.54[a]	0.1098
	Swift Current	2 .80[b]	1.83[b]	12.07[a]	13.46[a]	<0.0001
	Melfort	4.52[b]	5.28[b]	16.54[a]	17.22[a]	<0.0001
		Alfalfa	Red clover	Peas	Flax	
		-------------- (kg ha^{-1}) ---------------				
2011	Saskatoon	25.04[a]	20.24[a]	8.80[b]	10.88[b]	<0.0001
	Lanigan	24.02[a]	19.49[b]	8.79[c]	6.81[c]	<0.0001
	Swift Current	12.20[a]	8.45[b]	1.82[c]	1.53[c]	<0.0001
	Melfort	14.50[a]	13.76[a]	8.22[b]	13.40[a]	0.0021

† Means followed by a different superscript letter in the same row are significantly different (P < 0.10).

‡ Standard errors are provided in the appendix.

3.5.4 Crop P removal

In 2010, P removal via harvest in the cereal barley crop was greater than alfalfa and red clover P hay removal at Swift Current and Melfort (P = 0.006) (Table 3.7). The harvest P removal by alfalfa and red clover was higher than P removal by barley at Saskatoon (P < 0.10). Alfalfa, red clover and barley P removals were not significantly different at Lanigan (P = 0.11). During the second year (2011), a large amount of soil P was exported from the soil by alfalfa and red clover hay harvest relative to P removal by harvest of pea grain and flax seed (P < 0.10) (Table 3.7) in Saskatoon, Lanigan and Swift Current sites. At Melfort in 2010 due to a high grain yield, barley crops removed more P than alfalfa and red clover (P < 0.10).

The cumulative P removal over two years of crop growth was substantially greater for perennial forage crops in comparison to barley-peas and barley-flax at Saskatoon, Swift Current and Lanigan sites (P < 0.10) (Table 3.7). At Melfort site, after two years of crop rotation, P removals by all rotations crops at this site were comparable (P > 0.10). After the two-years, alfalfa removed a greater amount of P than did red clover, barley-peas and barley-flax at Lanigan and Swift Current (P < 0.10).

Table 3.7. The P removals through harvest by alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), barley (*Hordeum vulgare* L.), peas (*Pisum sativum* L.) and flax (*Linum usitatissimum* L.) grown at four locations for two years in Saskatchewan, Canada.

Year	Sites	Crop P removal†				*P* value
		Alfalfa	Red clover	Barley1	Barley2	
		-------------- (kg ha^{-1}) ---------------				
2010	Saskatoon	5.1^{a}‡	4.7^{a}	2.1^{b}	1.6^{b}	0.006
	Lanigan	10.6^{a}	5.5^{a}	8.8^{a}	9.5^{a}	0.110
	Swift Current	2.8^{b}	1.8^{b}	5.8^{a}	5.9^{a}	0.001
	Melfort	4.5^{b}	5.3^{b}	8.4^{a}	7.5^{a}	0.002
		Alfalfa	Red clover	Peas	Flax	
		-------------- (kg ha^{-1}) ---------------				
2011	Saskatoon	25.0^{a}	20.2^{b}	4.5^{c}	8.0^{c}	<0.0001
	Lanigan	24.0^{a}	19.5^{b}	2.2^{c}	1.0^{c}	<0.0001
	Swift Current	12.2^{a}	8.4^{b}	0.3^{c}	0.1^{c}	<0.0001
	Melfort	14.5^{a}	13.8^{a}	7.4^{b}	12.0^{a}	0.0007
		A-A§	RC-RC	B-P	B-FL	
		-------------- (kg ha^{-1}) ---------------				
2010+2011	Saskatoon	30.2^{a}	25.0^{a}	6.6^{b}	9.6^{b}	<0.0001
	Lanigan	34.6^{a}	25.0^{b}	11.0^{c}	10.5^{c}	<0.0001
	Swift Current	15.0^{a}	10.3^{b}	6.1^{c}	5.8^{c}	0.0003
	Melfort	19.0^{a}	19.0^{a}	15.8^{a}	19.6^{a}	0.1576

† Crop P removal is alfalfa and red clover total above ground biomass P uptake; barley, peas and flax grain and seed P uptake.
‡ Means with the different superscript letter in the same row are significantly different (P < 0.10).
§ A-A is alfalfa-alfalfa; RC-RC is red clover-red clover; B-P is barley-pea; B-FL is barley-flax.
¶ Standard errors are provided in the appendix.

3.6 Discussion

3.6.1 Crop yields

3.6.1.1 Hay yields

The hay yield of alfalfa and red clover in 2010 were much lower than the hay yield of these crops in 2011 due to the growth features of these perennial forage crops. Alfalfa and red clover are small seeded crops that have a slow growing seedling in the first year of the establishment, but these crops can produce the highest hay yield the year after seeding and decline thereafter (Undersander et al., 1990). The alfalfa hay yield was quite high at Lanigan relative to other sites in 2010 (Fig 3.1A), probably due to the favorable precipitation and greater soil nutritional status of the site (see Chapter 4). Unlike other experimental sites, the Lanigan site had a previous history of manure application prior to the establishment of this particular experiment, which improves the soil fertility and creates better nutritional status for alfalfa growth. Much lower alfalfa and red clover hay yield that occurred in Swift Current than other sites could be explained by the lower seeding rate and slower forage establishment in the Brown Soil zone. According to previous studies, the drier growing conditions and lower soil organic matter content are noted to be responsible for poor forage production at this location (Campbell and Zentner, 1993). In 2010, there was enough moisture at this site, but the seeding rate was two times lower than other sites. There was not enough established plants to use soil moisture this year. Another possible explanation for the poor forage production at Swift Current in 2010 could be the poor forage establishment because of the wet soil condition. Thus, the forage production at this site in 2010 was quite low relative to other sites. In 2011, the highest hay production switched from Lanigan to Saskatoon; the reason could be the greater nutrient export through alfalfa harvest in 2010 at Lanigan. After the greater nutrient removal by alfalfa hay in 2010, there were fewer nutrients available for the second year alfalfa growth in Lanigan. It has been reported that under ideal growing conditions, alfalfa has the highest yield potential followed by red clover among all perennial forage legumes (Malhi et al., 2002; Sheaffer and Seguin, 2003). Results at Lanigan and Swift Current in 2010 and at Saskatoon, Swift Current and Melfort in 2011 support this (Fig 3.1 A, B). However, sometimes no differences were detected between hay yields of alfalfa and red clover; reflecting that the hay production of alfalfa and red clover is determined by numerous factors such as soil characteristics, climate conditions and crop features.

3.6.1.2 Crop biomass and grain yields

In 2010, barley produced more biomass than alfalfa and red clover at Saskatoon, Swift Current and Melfort, but the following year, biomass production of forage legumes greatly exceeded the biomass production of annual crops at all four sites. This result is anticipated for annual crop production compared to perennial forage legumes over two years. Crops develop a canopy that provides the factory for optimum photosynthesis to occur over a period of time after planting (Horrocks and Vallentine, 1999). In perennial forage legumes such as alfalfa, clover and smooth bromegrass, development of this canopy is much slower in the first year than annual crops (Horrocks and Vallentine, 1999). As a result, perennial forage species take longer to become established (Horrocks and Vallentine, 1999). In this experiment, in the first year of the forage establishment alfalfa and red clover grew slower and produced less biomass compared to annual crops (Undersander et al., 1990). The following year, the pre-established forage legumes started earlier and grew faster than the newly planted annual crops, subsequently these crops produced far more biomass compared to annual crops (Horrocks and Vallentine, 1999). The grain yield of barley was highest in Melfort, followed by Swift Current, whereas the lowest barley grain yield was harvested from the Saskatoon site (Fig 3.2A). The low barley grain yield in Saskatoon was due to the wet growing conditions (higher than normal precipitation) at this site in 2010 (Table 3.3). In 2011, the grain and seed yield of peas and flax was higher in Melfort and Saskatoon, but quite low in Lanigan and Swift Current. The low pea grain yield and flax seed yield at Lanigan and Swift Current could be explained by the wet seeding condition in the spring and wildlife (deer) predation during the crop growing season at Lanigan and Swift Current respectively (Project report, 2012).

3.6.2 Plant P concentration

Plant analysis in 2010 and 2011 revealed that the P concentration in alfalfa and red clover was higher in 2010, when compared to P concentration of these crops in 2011. This can be attributed to the lower biomass production and less dilution of P in alfalfa and red clover in 2010 compared to 2011. In 2011, these crops utilized more P to produce greater amounts of plant biomass. In 2010 and 2011, red clover P concentration exceeded alfalfa P concentration at Lanigan, Swift Current and Melfort. This can be explained by the following reasons. Firstly, the higher alfalfa biomass production at Lanigan and Swift Current stimulated photosynthesis and plant growth such that the P concentration was diluted in the plant tissue. In addition, red clover has less demand and higher P uptake capability compared to alfalfa due to its more extensive surface rooting system (Undersander et al., 1990). Therefore, it is more likely that this crop could accumulate higher amounts of P in the plant tissue.

At harvest, cereals typically contain around 70 % of their total P in grains, with very little remaining in the straw (Rose et al., 2013). Similar results were found in the current study. The barley grain P concentration was 3 fold higher than the straw P concentration at Saskatoon, Swift Current and Melfort. At Lanigan, straw P concentration was higher than grain P concentration. Typically, during the vegetative growth phase most plant P is contained in the shoot and greater proportions of shoot P are then translocated into the grain at harvest (Rose and Wissuwa, 2012; Rose et al., 2013). The P contained in the shoot was mostly transferred into the grain at Saskatoon, Swift Current and Melfort by harvest. However, at Lanigan, less of the P contained in the barley shoot transferred into the grain likely because the barley was harvested prematurely at this site. Consequently, the barley straw contained greater amounts of P at this site, when compared to other sites. The seed P concentration in flax was higher than peas grain P concentration in Saskatoon and Lanigan, but at Swift Current and Melfort, the pea grain P concentration was greater, which indicates that P concentration in grain is determined not only by intrinsic plant characteristics, but also by different growing conditions.

3.6.3 Plant P uptake and removal

3.6.3.1 Phosphorus uptake of forage legumes and annual crops

Alfalfa and red clover P uptake was lower during the first year of the crop rotation but it increased rapidly during the second year due to the greater biomass production by these crops (Horrocks and Vallentine, 1999). The rapid growth and biomass production by perennial forage legumes in the second year could stimulate plant P uptake in order to meet the increasing P demand (Poorter and Nagel, 2000). Typically, grain P uptake is greater than straw P uptake in many crops because grain contains more P than straw (Rose et al., 2013), but in our study in

Saskatoon the opposite occurred in 2010, where the barley straw took up about 4 times more P relative to the barley grain (Fig 3.6 A, B). The reason could be the reduced grain production of barley in 2010 due to wet growing condition. The lower P uptake of peas and flax grain compared with their straw in Lanigan and Swift Current might also be related to low grain production.

3.6.3.2 Crop total P uptake and removal

When the total P uptake of rotation crops was compared (Table 3.6), annual crops took up more P than forage legumes during the first year of the crop rotation, but in the subsequent year the perennial forage legumes P uptake generally greatly exceeded the annual crop P uptake. This is largely due to the differences in biomass (grain+straw) production of rotation crops in 2010 and 2011. Plants need more nutrients in order to produce greater biomass, so the greater biomass production stimulates the plant P uptake (Poorter and Nagel, 2000). In Lanigan, no differences were detected between P uptake of forage legumes and barley in 2010. It was speculated that the adverse weather condition at Lanigan in 2010 resulted in the reduced barley production and less P uptake.

The crop P uptake patterns at four sites over two years consistently followed the typical crop biomass production patterns. However, the greater inherent ability of legumes to access soil P for uptake also cannot be neglected. According to previous studies (Kamh et al., 1999, 2002; Nuruzzaman et al., 2005a, 2006; Rose et al., 2010; Wang et al., 2011 and Hassan et al., 2012a) legumes have superior ability in P mobilization and uptake when compared to other less P efficient crops. In many studies, legume P mobilizing abilities were revealed by greater legume P uptake and growth as well as higher P uptake and superior yield by crops grown after legumes (Braum and Helmke, 1995; Hocking et al., 2000; Kamh et al., 1999; Vu et al., 2010). In our study, greater P mobilization and uptake by legumes is anticipated to contribute to the greater biomass production of these crops even though it is not easy to differentiate if the higher plant yield and P uptake of legumes are from the plants intrinsic P uptake ability or only from the different biomass production potentials due to the various soil and climate conditions, or from both factors. The differences in crop P uptake among sites presumably resulted from variable soil and climate conditions specific to each site because the various soil-climatic zones in Saskatchewan have an impact on crop production and nutrient uptake (Miller et al., 2002).

After two years of crop rotation, alfalfa and red clover took up and removed much greater amounts of P from the soil in comparison to barley-pea and barley-flax at three of the four sites because the hay harvest removes most above ground material. The rotation crop type can therefore significantly influence soil P depletion because crops differ in their yield potential and in the amount of P removed in the harvested portion (Grant et al., 2002). It is well documented that the above ground biomass of forage legumes contain large amounts of P, and can contribute to high P export from the field when all the above ground biomass was harvested (Mengel and Steffens, 1985; Taylor and Quesenberry, 1996).

3.7 Conclusion

In the first year of the crop rotation, barley produced relatively higher amounts of biomass compared to alfalfa and red clover at all sites. During the second year of the crop rotation, forage legume biomass production largely exceeded the pea and flax biomass production at all sites. Hay yield of alfalfa and red clover was similar in the first year of the crop rotation at three sites, but the second year alfalfa hay yield was higher than red clover at all sites except for Lanigan. Over the two years of crop rotation period, greater biomass was produced in Saskatoon, Melfort and Lanigan while the lowest biomass was produced at the Swift Current site. The P uptake over the two years varied among different rotation crops. Two years of alfalfa and red clover took up greater amounts of P relative to barley-pea and barley-flax at Saskatoon, Lanigan and Swift Current sites, due to substantial biomass production, especially in the second year. Crop P removal was greater in the two-year alfalfa and red clover rotations than in the barley-pea and barley-flax rotations after two years. In this research, crop biomass production, P uptake and removal was not only affected by different rotation crops, but was also influenced by various soil and climatic factors and their variation across growing regions of Saskatchewan in 2010 and 2011.

In this research, it is difficult to determine if the greater P uptake by forage legumes originates simply due to greater biomass production and plant P demand, or reflects an additional contribution from the ability of the legumes to mobilize soil P through unique root/soil biological attributes, or from both. Thus, future research needs to be conducted focusing on root morphology, rhizosphere biological and chemical characteristics, and root exudates from perennial forage legumes, perennial grass species and annual crops in order to better define the nature of the intrinsic ability of forage legumes in P mobilization and uptake in comparison to annual crops.

4. Changes in Forms and Amounts of Phosphorus in Four Chernozemic Soils as Influenced by Two Years of Forage Legume Versus Annual Crops in Rotation

4.1 Preface

The effects of forage legumes such as alfalfa and red clover in rotation on improving soil nitrogen (N) fertility after several years has been well documented, but the ability of these crops to increase soil phosphorus (P) availability when included in crop rotations has received less attention, especially in the prairies. Previous studies have examined the effect of grain legumes on soil P fractions and P mobilizing mechanisms, and other studies have evaluated P transformations as affected by long-term crop rotations, soil and fertilizer management practices. However, no studies have investigated the changes in P availability and distinctive P fractions after a short-term (two-year) period of forage legumes in rotation in comparison to annual crop rotations. Including legumes into a cropping system not only positively affects many soil properties and increases soil N supply, but it can also potentially have a positive impact on soil P availability. In the previous chapter (chapter 3), the yield and P uptake by alfalfa and red clover; and annual crops (barley, pea and flax) in the first two years of a four year rotation were assessed. In this chapter (chapter4), the soil P availability and the changes in P fractions as assessed by chemical methods, as well as soil microbial community structure after two years of forage legume versus annual crops is investigated. The information concerning P availability and changes in P fractions after two years of different crops, emphasizing the effect of perennial forage legumes versus annual crops is helpful in our understanding regarding P mobilizing ability and the overall effects of short-term forage legumes versus annual crops in rotation on soil P fertility. The knowledge produced is useful in establishing beneficial agricultural practices such as adoption of crop rotations that can improve the efficiency of P utilization in soil.

4.2 Abstract

Legumes play an important role in agriculture and natural ecosystems (Alamgir et al., 2012). Including legumes into a cropping system has a positive impact on soil physical properties, soil N supply and soil P availability (Braum and Helmke, 1995; Hocking et al., 2000). Although a series of studies have examined the effect of long-term crop rotations, soil and fertilizer management practices on soil P fractions, no studies have examined the changes in soil P availability and distinctive P fractions as affected by a short-term (two-year) inclusion of forage legumes in a rotation in comparison to annual crops. The objectives of this study were to assess changes in soil test extractable P (modified Kelowna P), soil P supply rate (resin membrane sorbed P) and distinctive labile and stable inorganic P (Pi) and organic P (Po) pools after two years of alfalfa (*Medicago sativa* L.); two years of red clover (*Trifolium pratense* L.); barley (*Hordeum vulgare* L.) followed by pea (*Pisum sativum* L.); and barley followed by flax (*Linum usitatissimum* L.) rotations in four Chernozemic soils in Saskatchewan. After two years of the different crop rotations, soil samples were collected in the spring of 2012 from four experimental sites representing the four soil-climatic zones in Saskatchewan. Soil P assessments and microbial community level physiological profiling were applied to the samples. In this study, two-years of forage legume and annual crop rotations did not significantly affect content of soil available P and P supply rate (P > 0.10) at any of the four sites (Saskatoon, Lanigan, Swift Current and Melfort), despite greater P uptake and removal by forage legumes relative to annual crops in 2010 and 2011 growing seasons. The four crop rotations also had similar amounts of labile and stable P contained in the different sequentially extracted chemical fractions. These results suggest that forage legumes included in a rotation even for only two years will maintain soil P availability in the face of greater P removal during the forage phase of the rotation, at least in the short-term. A two-year rotation of forage legumes may be too short to produce measurable significant differences in sequentially extracted soil P fractions. It is considered that including forage legumes in a rotation for two years is a feasible agronomic approach to maintain and improve both soil P and N supply.

4.3 Introduction

Phosphorus is one of the essential nutritional elements for plant growth and crop production due to its important role in energy metabolism and biosynthesis of nucleic acids and cell membranes (Brady and Weil, 2002). Although the amount of total P is high in most soils, the concentration of plant readily available inorganic P (Pi) is low in most agricultural soils. Much of the P in soil is not immediately available for plant uptake due to adsorption and precipitation of P into less soluble and more stable P forms through various chemical reactions in the soil which vary with soil pH (Holford, 1997; Ticconi and Abel, 2004). Thus, P deficiency is the most common nutritional stress in crop production in many N fertilized soils (Holford, 1997; Wang et al., 2011), affecting 42 % of the cultivated land around the world (Liu et al., 1994). A continuous application of P fertilizer is a common practice for correcting P deficiency in many P limited soils. A long-term application of chemical fertilizers has benefits in sustaining higher crop yield; but, in the long-run, complete reliance on chemical fertilizers comes under scrutiny for the following reasons. First, P fertilizer prices are increasing with the increase of P fertilizer use in agricultural production systems (Rose et al., 2010). Second, the crop fertilizer P use efficiency is relatively low. Only about 10 - 30% of applied P is taken up by plants in the first year (Holford, 1997; Syers et al., 2008; Sattari et al., 2012), as a considerable portion of the remaining P is converted to less soluble and available P forms and is accumulated in the soil as plant unavailable residual P (Halvorson and Black, 1985; Bolland, 1993; Holford, 1997; Richardson et al, 2001; Bünemann et al., 2006). Third, overuse of P fertilizers above crop needs increases the risk of environmental problems such as ground water contamination and eutrophication in some environments (Hodgkin and Hamilton, 1993). This has led to a search for both economically feasible and environmentally sustainable strategies for improving crop production that can reduce total reliance on chemical P fertilizer sources in P deficient soils. The identification and incorporation of suitable crop species that are efficient in P mobilization into cropping systems as rotation crops is considered to be one of the promising agronomic approaches to access insoluble native soil P reserves and to capitalize the use of accumulated residual soil P reserves (Ae et al., 1990; Lynch, 1998; Horst et al., 2001; Stutter et al., 2012).

Legumes play an important role in agriculture and natural ecosystems (Alamgir et al., 2012). Including legumes into a cropping system not only positively affects many soil properties and increases soil N supply, but it also has been shown to have a positive impact on soil P availability. Several studies have shown positive effects of legumes on cereal growth in rotation (Richardson, 2001; Kamh et al., 2002; Nuruzzaman et al., 2005a,b), which cannot solely be explained by N inputs through biological N_2 fixation. Legumes are reported to possess a greater ability to mobilize sparingly soluble P from soils (Kamh et al., 1999; Nuruzzaman et al., 2006; Rose et al., 2010; Wang et al., 2011) compared to less P efficient cereal crops (Braum and Helmke, 1995; Hocking et al., 2000). The ability of legumes to make P more readily available from various P pools has been attributed to (i) modification of rhizosphere pH due to root-induced acidification or alkalization (Gahoonia et al., 1992; Morel and Hinsinger, 1999; Hinsinger et al., 2003); (ii) secretion of carboxylic acids such as malate and citrate (Veneklaas et al., 2003); and (iii) exudation of root or microbially derived P solubilizing enzymes such as acid phosphatases and phytases (Richardson, 2001; Nuruzzaman et al., 2006). In addition, legumes are also capable of improving spatial access to soil P through its special morphological root traits and symbiotic associations with arbuscular mycorrhizal fungi (Hedley et al., 1982).

A series of studies has examined the effect of grain legumes on soil P fractions and P mobilizing mechanisms (Nuruzzaman et al., 2006; Hassan et al., 2012a). Other studies have investigated the P transformation in soil after several years of adoption of different crop rotations, fertilizer application strategies and soil management practices (Hedley et al., 1982; Tiessen et al., 1983; Beck and Sanchez, 1994; Motavalli and Miles, 2002). Several studies have evaluated the relative changes in soil P fractions due to long-term (several years) inclusion of perennial forage legumes in crop rotations (Mckenzie et al., 1992; Daroub et al., 2001; Zheng et al., 2001), but no studies have examined the changes in P availability and distinctive P fractions after short-term (two-year) forage legume rotations in comparison to annual crop rotations. Forage legumes such as alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) are considered an essential component of sustainable agro-ecosystems (Doran and Smith, 1991). There are numerous benefits of including forage legumes into cropping systems (Sheaffer and Seguin, 2003) such as improved soil N fertility, organic matter and tilth, production of high quality forage for livestock feed, as well as reduced incidence of weeds, insects, and diseases in soil. In addition, rotational effects of forage legumes on P availability have also been observed (Campbell et al., 1993). Forage legumes are deep rooted and can bring P solubilized at depth in the root rhizosphere to the surface in organic forms where it can be recycled and released through chemical, physical and biological reactions (Campbell et al., 1993). The ability of indigenous soil P as well as residual fertilizer P added in previous years may therefore be enhanced by legumes.

The sequential P extraction is a widely used method to chemically separate different forms of soil P. Quantifying the various Pi and Po pools through the fractionation procedure helps to understand the nature of soil P as affected by various factors, and aid in predictions of the availability of soil P to plants (Vu et al., 2010). The relative distribution of soil Pi and Po may be influenced by many factors such as initial soil chemical characteristics, soil type, and climate and management practices (Motavalli and Miles, 2002). Thus, the sequential P extraction method was used in this study to evaluate the redistribution of P between various soil P fractions associated with having two years of forage legume versus annual crop in four contrasting soil-climatic zones of Saskatchewan. The specific objectives of this study were (i) to assess changes in plant available soil test P (modified Kelowna extractable concentration and resin membrane supply rate), and various chemically extractable labile and stable Pi and Po pools after two years of alfalfa (*Medicago sativa* L.); two years of red clover (*Trifolium pratense* L.); barley (*Hordeum vulgare* L.) followed by pea (*Pisum sativum* L.) and barley followed by flax (*Linum usitatissimum* L.) in four contrasting Saskatchewan soils. An attempt was also made to evaluate the effects of the different crop rotations on soil microbial community metabolic potential using the community level physiological profiling (CLPP) method with Biolog^{TM} Ecoplates.

4.4 Materials and Methods

4.4.1 Site description and experimental design

The site description and experimental design are described in detail in the previous chapter (Chapter 3). Briefly, a two-year field experiment initiated in 2010 was carried out at four sites: 1) the Agriculture and Agri-Food Canada Research Farm (AAFC-Scott) at Saskatoon in the Dark Brown soil zone (52°04′N, 108°08′W); 2) the Western Beef Development Centre’s (WBDC) Termuende Research Ranch at Lanigan (51°51′N, 105°02′W) in the Black soil zone; 3) the Semiarid Prairie Agricultural Research Centre (SPARC) at Swift Current (50°16′N 107°44′W) in the Brown soil zone and 4) the Melfort Research Farm (MRF) at Melfort (52°08′N, 104°06′W) in the Dark Gray soil zone in Saskatchewan, Canada. Soil properties and climatic conditions at each site were described in the previous chapter. Experimental design at each site was a randomized complete block design (RCBD) with four replications. Four crop rotations were included as four agronomic treatments and the crop rotation treatments are described in Table 4.1. In this experiment, after two years of alfalfa, red clover, barley-pea and barley-flax rotations, soils from these rotations were compared by evaluating soil moisture content, soil available P level, distinct chemically speciated soil P pools, and soil microbial community structure.

Table 4.1. Description of crop rotation treatments.

Rotation	2010	2011
1	Alfalfa	Alfalfa
2	Red clover	Red clover
3	Barley1	Pea
4	Barley2	Flax

4.4.2 Soil sampling and analyses

Soil samples were collected in April 2012 from all plots at all experimental sites. At each site, three core samples were taken from each plot at random by using a core soil sampler (diameter 4.5 cm and length 100 cm) and composited according to depth increments sampled: 0-15 cm, 15-30 cm and 30-60 cm. Soil samples were transported to the laboratory and immediately stored in a fridge at 4 °C prior to gravimetric soil moisture measurement. The gravimetric soil moisture content was determined at all depths by weighing 10 g of wet soil and oven drying at 105 °C for 24 h. The percent (%) of soil moisture was then calculated from the difference between the weight of wet soil and oven dried soil. After measuring the moisture content, soil samples were air-dried, ground and passed through a 2 mm sieve and stored at room temperature prior to further analyses.

4.4.3 Extractable soil P and P supply rate

Available soil P from two depths: 0-15 cm and 15-30 cm was extracted with modified Kelowna solution (Qian et al., 1994). In the procedure, three grams of soil were placed in a 250 mL extraction bottle where 30 mL of modified Kelowna solution was added and the mixture was shaken on a rotary shaker for 5 min. The suspension was then filtered through VWR #454 filter paper and stored in vials in the cooler. The P concentration in extracts was then determined colorimetrically using Technicon Autoanalyzer II (Technicon Industrial Systems, 1973) (Qian et al., 1994).

Soil P supply rate was determined at two depths: 0-15 cm and 15-30 cm using a "sandwich" test (Qian and Schoenau, 2010). Briefly, two snap cap plastic vial lids were filled with air-dried soil (<2 mm); deionized water was then added according to the field capacity of the soil. A "sandwich" was made by sealing the two caps of soil together after inserting a strip of cleaned and regenerated anion exchange membrane (8 cm^2) in between. After 24 hours, membrane strips were then removed from the "sandwich" and washed free of adhering soil. The membrane was eluted in 0.5 M HCl for 1 h to desorb the nutrient ions from anion resin membrane into HCl solution. The P ion concentration in the eluents was measured colorimetrically using Technicon Autoanalyzer II (Technicon Industrial Systems, 1973) (Qian and Schoenau, 2010).

4.4.4 Sequential P extraction

Surface soil samples (0-15 cm) were sequentially extracted using the procedure described by Tiessen and Moir (1993). The procedure used in sequential P extraction is summarized in Fig. 4.1. In this procedure, 0.5 g of air-dried soil was first extracted by shaking with 30 ml of deionized water and a strip of anion exchange membrane, followed sequentially by 0.5 M $NaHCO_3$, 0.1 M NaOH and 1M HCl. Each extraction was performed on a rotary shaker for 16 h. After each extraction, the extracting solution was centrifuged for 10 min at 10 000 g at 0 °C and filtered through a 0.45-μm filter. For resin extraction, the resin membrane was removed from the soil suspension and shaken with 20 ml 0.5 M HCl for 16 h on a rotary shaker. After all the extractions were completed, the remaining P in the soil was considered as residual P; it was measured using an acid (H_2SO_4 + H_2O_2) digestion method (Thomas et al., 1967). In all fractions, Pi in extracts was determined colorimetrically using a molybdate-blue method described by Murphy and Riley (1962). Two measurements were made for $NaHCO_3$ and NaOH extractions. One was to measure the total P (Pt) contents in the solution, which was determined by digestion of each extract with potassium persulfate (adjusted pH neutral) for 16 h at 90 °C. The other one was to determine Pi content after acidifying soil extracts to precipitate organic matter. The Po concentration was calculated as the difference between Pt and Pi. In these extractions, labile (plant available) P is considered to be that extracted with resin and bicarbonate (Schoenau et al., 1989); less available or moderately available P such as amorphous and Al and Fe phosphate is removed by the NaOH (O'Halloran and DeJong, 1987); primary mineral P like Ca-Mg-associated P is extracted with 1M HCl (Williams et al., 1971) and very insoluble residual P was assessed by digestion with concentrated H_2SO_4. Labile Po extracted from bicarbonate fraction may be considered to be of recent microbial origin while sodium hydroxide extractable Po is of a more humified, recalcitrant nature (Schoenau et al., 1989).

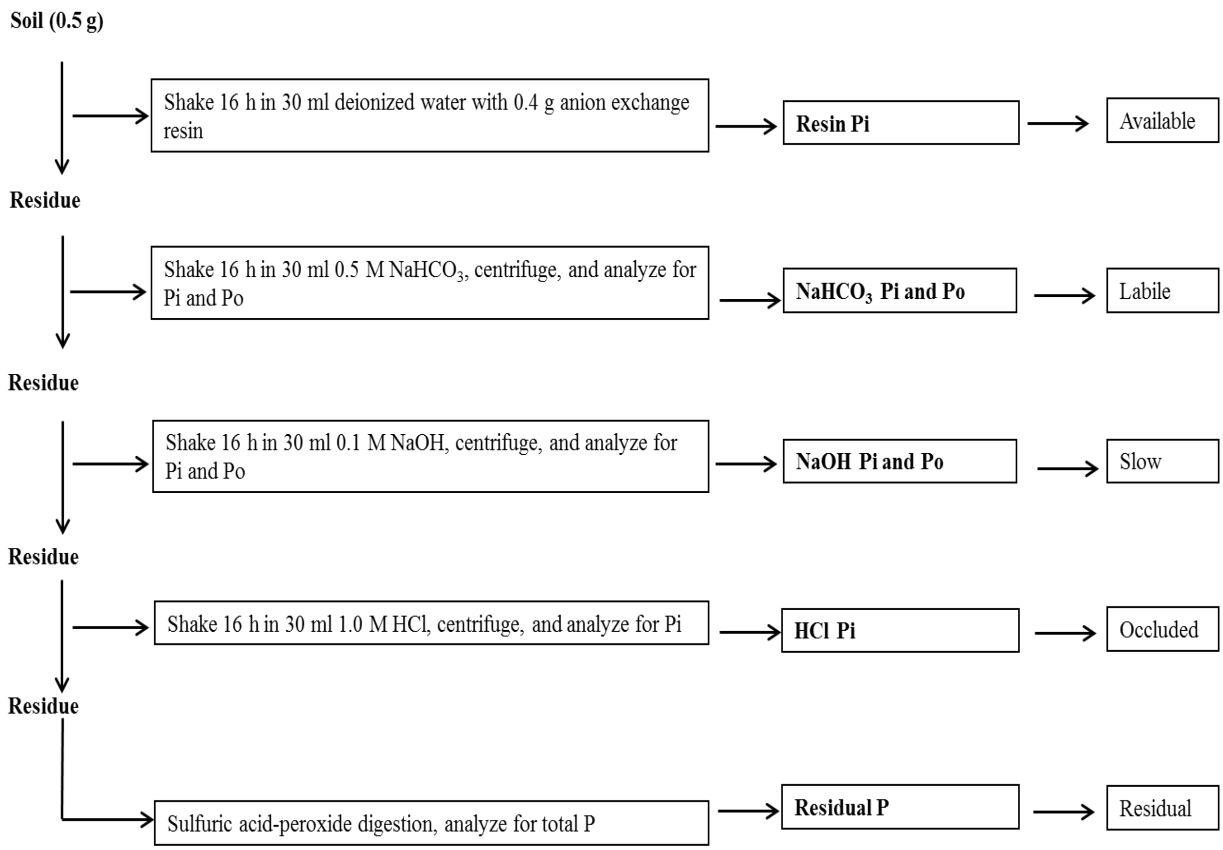

Fig 4.1 Flow chart of sequential phosphorus (P) extraction procedure (Pi is inorganic P, Po is organic P) (Adapted from Schoenau et al., 1989).

4.4.5 Community level physiological profiling (CLPP) using the Biolog EcoPlate™

Community level physiological profiling using the Biolog microtiter EcoPlate™ was used in this experiment to study the functional diversity of whole microbial communities (Garland, 1997). Biolog EcoPlate^{TM} (Biolog, Inc., 49 Harward, CA) consisting of 96 wells, with 31 different carbon substrates and one water blank, replicated three times per plate was used in this experiment. Soils from the Melfort site were chosen for this CLPP analysis because greater microbial activity and microbial diversity was anticipated to arise in soils from this site due to the higher levels of soil organic matter (SOM) content relative to the other sites. Air-dried soil samples were incubated at 70-80% field capacity at room temperature (22 ± 2 °C) for a week and subjected to CLPP analysis using the Biolog EcoPlate™.

Biolog EcoPlate™ inoculation was performed as described by Dunfield and Germida (2003). Briefly, 5 g of wet soil was added to 95 mL of sterile phosphate-buffered saline (PBS) solution and shaken on a rotary shaker for 20 min at 200 rpm (22 °C). Then, 10^{-2}, 10^{-3} and 10^{-4} dilutions were made using sterile PBS. The PBS solution was prepared according to a method described in Molecular Cloning by Sambrook et al. (1989). Briefly, 1L PBS was prepared using 800 ml distilled water followed by the addition of 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, and 0.24 g of KH_2PO_4. Then pH was adjusted to 7.4 with HCl and distilled water was added to a total volume of 1 liter. The 10^{-4} dilution was kept for plate inoculation. This dilution was selected for plate inoculation because it encompasses the range of cell densities predicted to be found in samples originating from agricultural top soils. Each of the micro-plates in the Biolog Ecoplate™ was inoculated with 100 µL of 10^{-4} dilution sample. Inoculated plates were incubated at 28 °C for 7 days. To study reaction patterns, color development was measured by determining the optical density at 590nm (every 24 h over the 7 days incubation period) on a Spectra Max Plus 384 micro-plate reader (Molecular Device, Sunnyvale, CA). The average well color development (AWCD) was calculated as described by Garland and Mills (1991).

4.4.6 Statistical analyses

4.4.6.1 Data analysis for soil moisture and P measurements

Soil moisture content, P fractions, modified Kelowna P and P supply rate for treatments at a site were analyzed using one-way analysis of variance tests (ANOVA). The data were analyzed as a RCBD design using Proc. Mixed Procedure of SAS (Version 9.3; SAS Institute., Cary, NC). The treatment (crop rotations) was the fixed effect and replications in each site were considered as block effect. Means were compared among treatments using Tukey's multi-comparison tests. Before the ANOVA, data were checked for the normality and equality of variances using the UNIVARIATE procedure; however, no transformation was needed. Significance was declared at $P < 0.10$. The coefficient of variation for replicate analyses of soil and plant samples was ≤ 5%. To ensure accuracy, all routine analyses included a standard reference soil or plant material of known established concentration every 40 samples.

4.4.6.2. Biolog EcoPlate™ data analysis

To determine the overall rate of color development (substrate utilization) and identify a standardized point for further functional diversity analysis, average well color development (AWCD) was calculated by subtracting color development in the control well from each substrate well and calculating a mean (Garland and Mills, 1991). When negative absorbance values were recorded, a value of zero was assigned to that well (Harch et al., 1997).

4.5 Results

4.5.1 Soil moisture content

Gravimetric soil moisture content was measured after two years of forage legume and annual crop rotations on samples collected in the spring of 2012 and the results are shown in Table 4.2. Generally, different cropping sequence did not have a significant effect on soil moisture contents (%) measured at three sampling depths: 0-15 cm, 15-30 cm and 30-60 cm ($P > 0.10$) at all four experimental sites, except for surface soil moisture (0-15 cm) content at Melfort. At this site, soil moisture content (0-15 cm) was higher after barley-pea relative to two years of alfalfa ($P < 0.10$) rotation. There was a trend of soil moisture depletion by two years of alfalfa rotation evident at depth in the soil profile (30-60cm) relative to other crop rotations at all four sites, especially at Melfort and Swift Current, although the differences were not statistically significant ($P > 0.10$). When comparing the soil moisture content at different sites, the highest soil moisture content was measured at Melfort at all soil sampling depths. The second highest soil moisture content was found in Saskatoon followed by Lanigan. The lowest amount of soil moisture was measured at the site in Swift Current at all sampling depths (0-15 cm, 15-30 cm and 30-60 cm).

Table 4.2. Gravimetric soil moisture contents (% water by weight) in the soil profile after two years of forage legume and annual crop rotations at four sites in Saskatchewan, Canada.

Sampling depth	Sites	Gravimetric soil moisture content				*P* value‡
		A-A†	RC-RC	B-P	B-FL	
		-------------------- (%) ---------------------				
0-15 cm	Saskatoon	27.2	23.3	28.9	28.1	0.399
	Lanigan	27.2	26.1	27.5	24.8	0.695
	Swift Current	20.3	21.7	19.9	20.9	0.842
	Melfort	32.9[b]§	34.7[ab]	35.4[a]	34.3[ab]	0.094
15-30 cm	Saskatoon	25.0	23.8	24.6	28.6	0.488
	Lanigan	21.4	17.0	21.1	19.0	0.257
	Swift Current	17.9	20.6	19.2	18.9	0.891
	Melfort	29.2	27.8	27.3	28.2	0.836
30-60 cm	Saskatoon	19.6	20.9	22.1	20.3	0.492
	Lanigan	17.4	17.4	18.7	16.0	0.820
	Swift Current	15.1	17.0	18.3	16.2	0.900
	Melfort	24.2	23.4	31.9	25.7	0.233

† A-A is alfalfa-alfalfa; RC-RC is red clover-red clover; B-P is barley-pea; B-FL is barley-flax rotation.
‡ Letters are assigned to treatments that have significant differences among them ($P < 0.10$).
§ Means with a different superscript letter in the same row are significantly different ($P < 0.10$).
¶ Standard errors are provided in the appendix.

4.5.2 Soil extractable P content and P supply rate

The amounts of soil test extractable (modified Kelowna) P measured at two depths (0-15 cm and 15-30 cm) were similar among all treatments; no significant differences were found between different crop rotations at all locations ($P > 0.10$) (Fig 4.2). When the amounts of surface soil (0-15 cm) extractable P at different sites were compared, the highest level of soil extractable P was found at Lanigan, whereas the other sites have similar, lower plant available P content after growing two years of forage legumes and annual crops. The amount of soil extractable P measured deeper in the soil profile (15-30 cm) was slightly higher at Lanigan and Swift Current sites in comparison to Saskatoon and Melfort. The differences in extractable P among various sites were more pronounced in the surface soil layer (0-15 cm) than subsurface

(15-30 cm) at different sites. As expected, available P was lower in sub-surface soil (15-30 cm) than the surface soil (0-15 cm).

In this experiment, the trend of soil P supply rate (resin membrane) measured in the surface soil (0-15cm) was quite similar to the trend observed in the soil extractable P concentrations (Fig 4.3). As well, little difference was found in the pattern of soil P supply rate and extractable P content in the subsurface soil (15-30cm). The soil P supply rate at both sampling depths (0-15 cm and 15-30 cm) was similar among different crop rotations at all four locations ($P > 0.10$). Among different sites, the highest P supply rate in the surface soil (0-15 cm) was observed at Lanigan; the P supply rate at the 15-30 cm soil depth was slightly higher at Swift Current compared with the other sites.

4.5.3 Sequentially extracted soil P fractions

Two years of forage legume and annual crop rotations did not significantly affect the distribution of soil P between various fractions at all four locations ($P > 0.10$) (Table 4.3), with two exceptions. The exceptions occurred with the residual P fraction at Lanigan and the $NaHCO_3$-Po fraction at Melfort ($P < 0.10$) (Table 4.3). The concentration of various P pools, however, varied among locations (Fig. 4.4). At the Saskatoon site, there was no significant difference in labile (resin Pi, $NaHCO_3$-Pi and -Po), moderately labile (NaOH-Po and -Pi) and stable (HCl-extractable P and residual P) P fractions after growing two years of alfalfa, red clover, barley-pea and barley-flax ($P > 0.10$) (Table 4.3). The soil at this site, irrespective of the grown crop type, was characterized by larger stable P pools, medium content of moderately available P pools and smaller labile P pools (Fig. 4.4A). Specifically, at this site, the size of HCl-extractable P and residual P fractions were greater, representing 47% and 28% of total P respectively, while the magnitude of NaOH-Po and Pi fractions were in the middle, accounting for 13% and 6% of total P. Finally, the resin Pi, $NaHCO_3$-Pi and -Po fractions were the smaller P pools, each accounting only for 2%, 3% and 1 % of the total soil P pool.

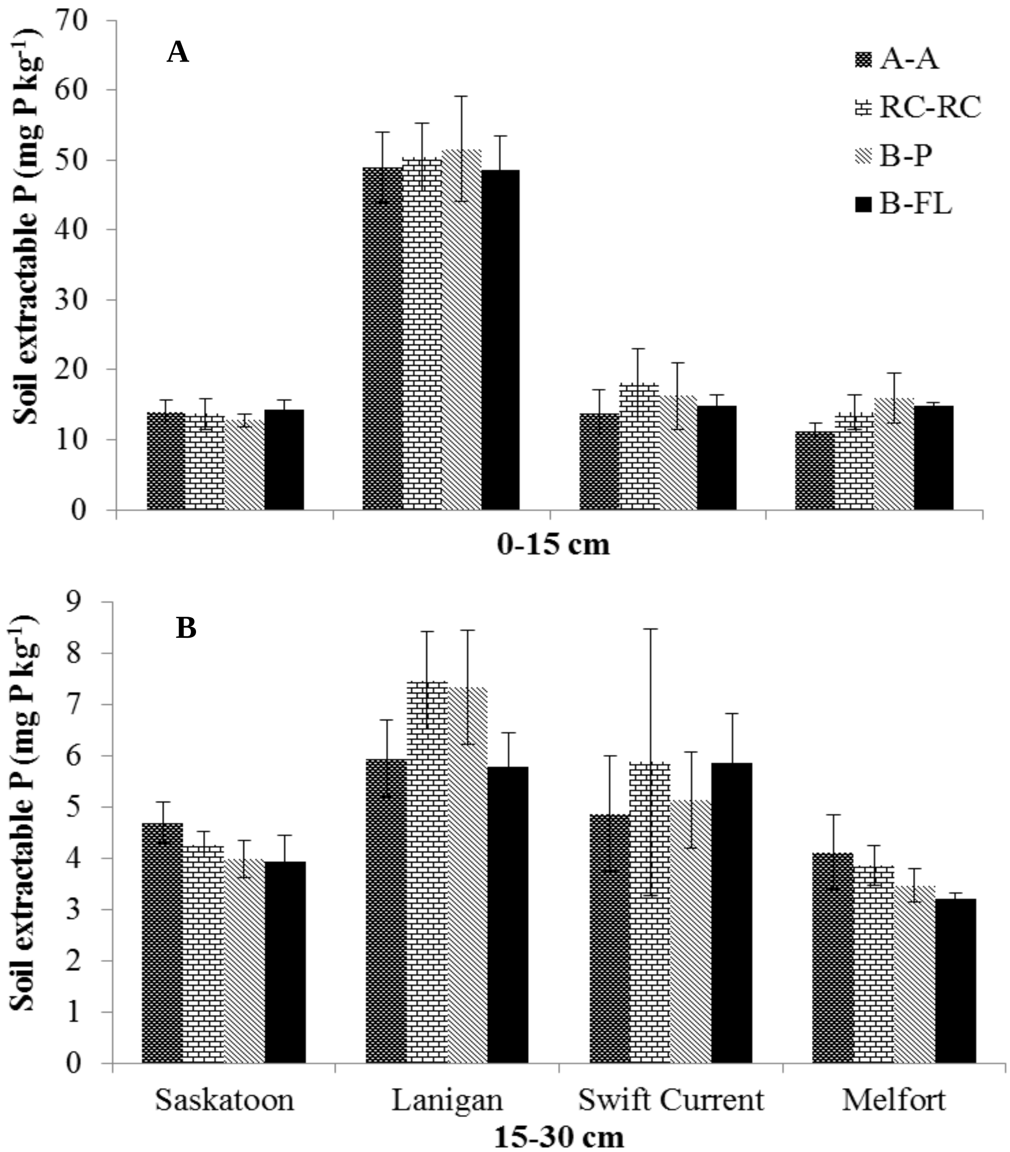

Fig 4.2. The concentration of soil extractable (modified Kelowna) P at two depths: 0-15 cm (A) and 15-30 cm (B) after two years of alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), barley (*Hordeum vulgare* L.) – pea (*Pisum sativum* L.) and barley (*Hordeum vulgare* L.) – flax (*Linum usitatissimum* L.) rotations at four sites in Saskatchewan, Canada. (A-A=alfalfa-alfalfa; RC-RC=red clover-red clover; B-P=barley-pea; B-FL=barley-flax). Error bars represent standard errors.

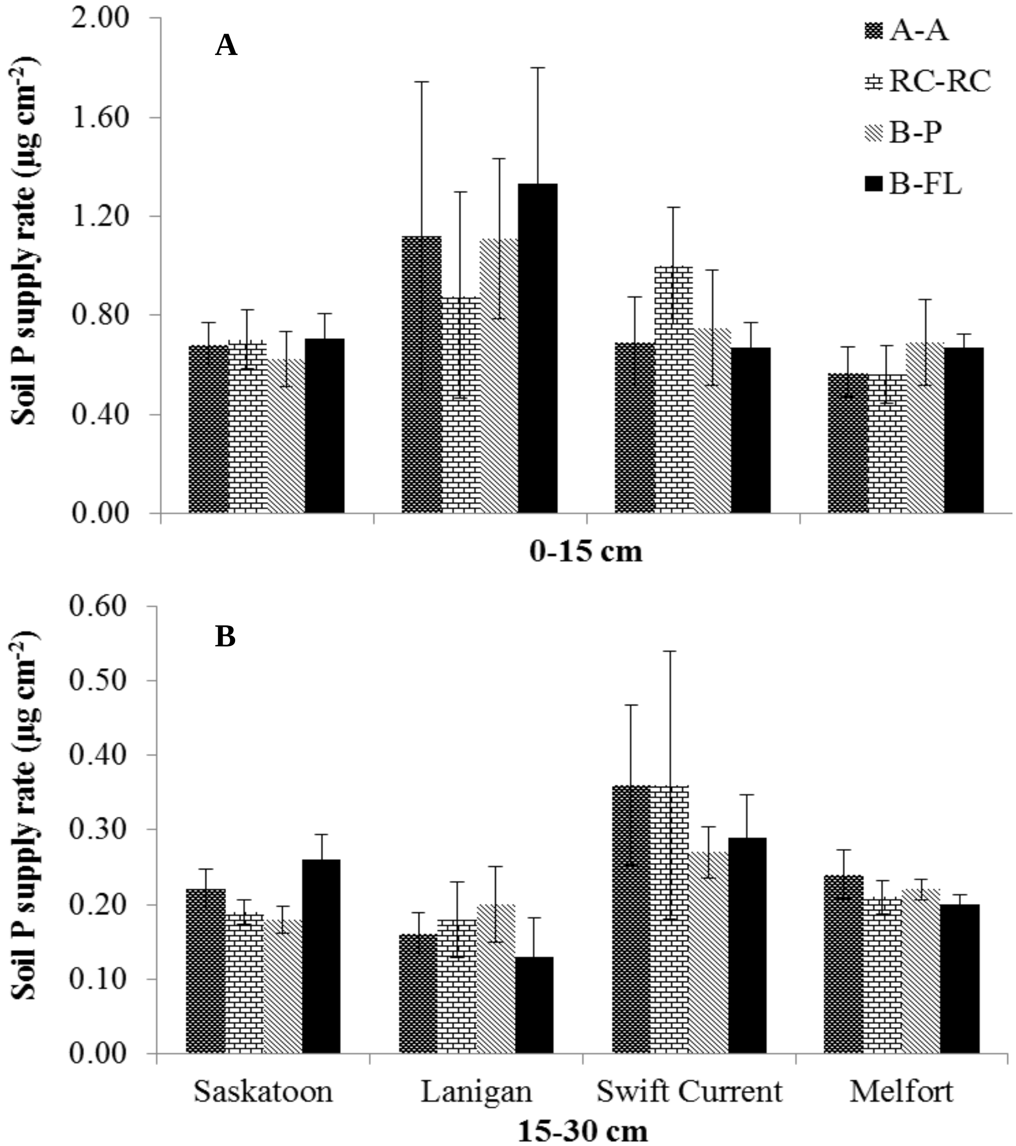

Fig 4.3. Soil P supply rate at two depths: 0-15 cm (A) and 15-30 cm (B) measured using resin membrane after two years of alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), barley (*Hordeum vulgare* L.) – pea (*Pisum sativum* L.) and barley (*Hordeum vulgare* L.) – flax (*Linum usitatissimum* L.) rotations at four sites in Saskatchewan, Canada. (A-A=alfalfa-alfalfa; RC-RC=red clover-red clover; B-P=barley-pea; B-FL=barley-flax). Error bars represent standard errors.

At the Lanigan site, different crop rotations significantly affected the content of P in the residual P fraction (*P* < 0.10) (Table 4.3). Two years of red clover rotation resulted in the highest amount of residual P in comparison to annual crop rotations of barley-pea and barley-flax (*P* < 0.10). Two years of alfalfa produced the second highest amount of residual P among all crop rotations (*P* < 0.10). Other P fractions (both stable and labile) were not influenced by two years of different crop rotations (*P* > 0.10). The largest soil P pool at this site was HCl-extractable P fraction, which consisted of 29% of total P, followed by NaOH-Po, residual P and NaOH-Pi pools, each comprising 21%, 19% and 15% of total soil P (Fig. 4.4B). Relatively higher amounts of plant available soil P pools ($NaHCO_3$-Pi and resin Pi fractions) were found at this site, each containing 10% and 5% of the total P (Fig. 4.4B) when compared with other sites. The smallest P pool measured was the $NaHCO_3$-Po, which only accounted for 1% of total P at this site (Fig. 4.4B).

At Swift Current, crop rotations did not have a significant impact on the concentrations of various soil P fractions (*P* > 0.10) (Table 4.3). The size of HCl-extractable P fraction was the largest among all P fractions representing 42% of total P (Fig. 4.4C); the second largest P pool measured was the residual P fraction, which contained 26% of the total P (Fig. 4.4C). The NaOH-Po and Pi pools were the third largest P pool, each consisting of 15% and 9% of total P respectively (Fig. 4.4C). The smallest P pools at this site were the available P fractions of $NaHCO_3$-Pi, -Po and resin P, contributing 3%, 2% and 3% of total P respectively (Fig. 4.4C).

At the Melfort site, the amount of $NaHCO_3$-Po was significantly increased in the barley-flax rotation when compared to other crop rotations (*P* < 0.10); but no differences were detected between two years of alfalfa, red clover and barley-pea rotations (*P* > 0.10) (Table 4.3). Unlike other sites, the largest P pool was found to be the residual P fraction at this site, accounting for 32% of total P (Fig. 4.4D). The NaOH-Po, -Pi and HCl-extractable P fractions accounted for 30%, 11 % and 19% of total P (Fig. 4.4D). The smallest P pools were again found to be the $NaHCO_3$-Pi and Po and resin P fractions respectively comprising 3%, 2% and 3% of total P (Fig. 4.4D).

The relative sizes of labile (resin P, $NaHCO_3$-Pi and -Po), moderately labile (NaOH-Pi and -Po) and stable (HCl-P and residual-P) P pools at four sites in Saskatoon, Lanigan, Swift Current and Melfort were found to occur in ratios of 1:3:12; 1:2:3; 1:3:9 and 1:6:7 respectively. In general, the residual P and HCl-extractable P pools were the major P pools; the NaOH-Pi and Po pools were the second largest pools and the $NaHCO_3$-Pi, -Po and resin P fractions were the smaller P pools at all four locations.

Table 4.3. Distribution of inorganic phosphorus (Pi) and organic phosphorus (Po) fractions (mg P kg^{-1}) in soils after two years of forage legume and annual crop rotations at four sites in Saskatchewan, Canada.

Sites	P fractions	A-A†	RC-RC	B-P	B-FL	*P* value‡
		------------- (mg P kg^{-1})----------------				
Saskatoon	Resin-Pi	23.1	17.4	19.1	22.7	0.707
	$NaHCO_3$-Pi	36.2	32.7	36.0	32.5	0.820
	$NaHCO_3$-Po	19.8	18.6	17.1	17.9	0.828
	NaOH-Pi	85.1	88.5	72.0	74.7	0.562
	NaOH-Po	179.5	149.5	132.5	160.3	0.278
	HCl-Pt	566.5	552.6	629.9	562.5	0.276
	Residual-Pt	358.9	345.2	349.9	342.8	0.922
Lanigan	Resin-Pi	67.8	71.6	69.7	78.7	0.824
	$NaHCO_3$-Pi	153.0	175.0	176.1	151.4	0.792
	$NaHCO_3$-Po	13.6	32.2	20.1	18.0	0.361
	NaOH-Pi	238.7	209.6	257.7	214.7	0.674
	NaOH-Po	299.3	370.7	284.1	363.3	0.738
	HCl-Pt	475.5	472.4	412.9	418.1	0.618
	Residual-Pt	309.7[ab]§	333.2[a]	271.7[c]	279.0[bc]	0.043

† A-A is alfalfa-alfalfa; RC-RC is red clover-red clover; B-P is barley-pea and B-FL is barley-flax.

‡ Letters are assigned to P fractions that have significant differences among treatments ($P < 0.10$).

§ Means with a different superscript letter in the same row are significantly different ($P < 0.10$).

¶ Standard errors are provided in the appendix.

Table 4.3. continued.

Sites	P fractions	A-A†	RC-RC	B-P	B-FL	*P* value‡
		------------- (mg P kg^{-1})----------------				
Swift Current	Resin-Pi	25.7	41.3	35.6	35.0	0.606
	$NaHCO_3$-Pi	36.2	38.9	32.7	36.4	0.940
	$NaHCO_3$-Po	18.3	20.4	13.8	31.8	0.375
	NaOH-Pi	88.9	107.9	99.3	114.6	0.740
	NaOH-Po	153.4	185.8	136.1	188.4	0.317
	HCl-Pt	474.3	520.7	492.2	421.4	0.373
	Residual-Pt	267.6	305.9	264.6	308.8	0.639
Melfort	Resin-Pi	39.6	39.6	44.7	41.8	0.942
	$NaHCO_3$-Pi	47.0	61.9	60.0	58.6	0.438
	$NaHCO_3$-Po	22.2^{b}§	22.8^{b}	22.9^{b}	35.2^{a}	0.088
	NaOH-Pi	175.3	188.6	188.8	186.0	0.950
	NaOH-Po	523.2	423.4	441.9	553.8	0.320
	HCl-Pt	298.4	325.5	337.0	310.2	0.496
	Residual-Pt	509.9	496.0	569.5	561.4	0.377

† A-A is alfalfa-alfalfa; RC-RC is red clover-red clover; B-P is barley-pea and B-FL is barley-flax.

‡ Letters are assigned to P fractions that have significant differences among treatments ($P < 0.10$).

§ Means with a different superscript letter in the same row are significantly different ($P < 0.10$).

¶ Standard errors are provided in the appendix.

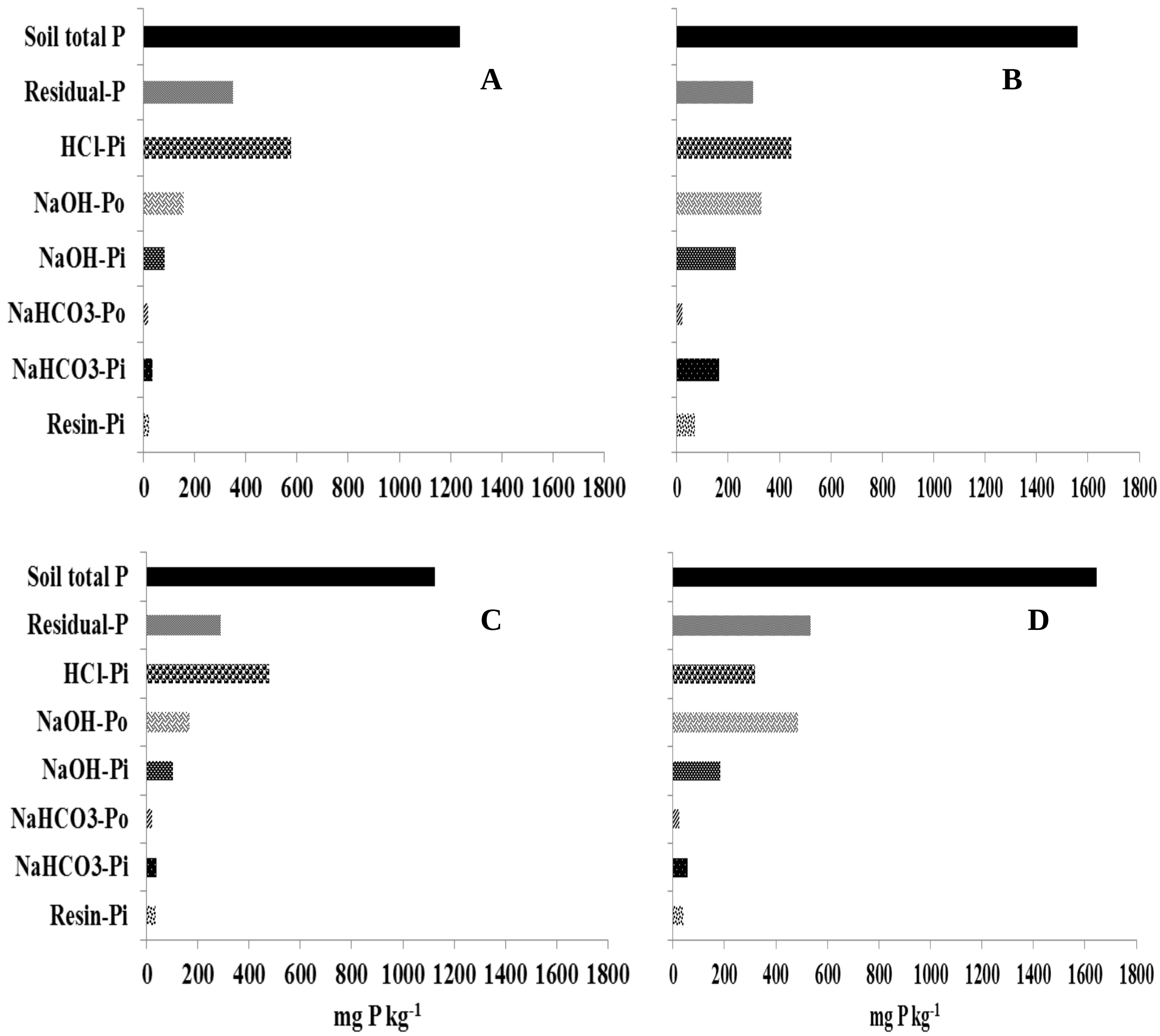

Fig. 4.4 Inorganic P (Pi) and organic P (Po) pools (mg P kg^{-1}) measured in the spring of 2012 (after two years of forage legume and annual crop rotations) at Saskatoon (A), Lanigan (B), Swift Current (C) and Melfort (D) in Saskatchewan, Canada.

4.5.4 Soil microbial community structure

Soil microbial community structure was assessed using the Biolog EcoPlate™ and the results are shown in the appendix. The average well color development (AWCD) calculated for each biolog plate was too low to allow for adequate separation among treatments in this study, in contrast to previous studies using different soils (Weber and Legge. 2009; Frąc et al., 2012; Hannah, 2013). Despite repeating the assessment twice, no color development patterns were found over the 7 days of the incubation period, leading to the conclusion that the Biolog EcoPlate™ was not capable of revealing any microbial community differences in these soils.

4.6 Discussion

4.6.1 Soil moisture content

In this study, no significant differences were found in gravimetric soil moisture content influenced by different crop rotations (0-15 cm, 15-30 cm and 30-60 cm) at all four sites ($P >$ 0.10) (Table 4.2), with one exception. The exception was the significantly lower surface soil (0-15cm) moisture content measured after two years of alfalfa rotation at Melfort site in comparison to the other crop rotations (Table 4.2). Also, at this site, there was a trend of soil moisture depletion in the deeper soil layers (30-60 cm) of the two-years of alfalfa rotation. The lower soil profile moisture content at Melfort after two years of alfalfa is explained by the deep rooting system and prolonged use of soil moisture over the growing season by this perennial crop. According to previous studies, forage legumes result in greater soil moisture consumption and depletion by the end of the season compared to annual small grain crops (Frye et al., 1988; Astatke et al., 1995; Huggins et al., 2001).

At the other three sites, the soil moisture content at three sampling depths (0-15 cm, 15-30 cm and 30-60 cm) was similar after two years of different crop rotations. This may reflect a reduced effect of perennial forage due to the short-term (for two years) period for growth. It may also be possible that the greater soil moisture depletion of forage legumes at depth was not completely accounted for due to the sampling depth to only 60 cm utilized in this experiment. Significant surface soil moisture recharge through rains in the fall of 2011 and snowmelt in spring of 2012 at these sites could also mask differences in soil moisture depletion in the previous two years. Numerous studies have shown lower soil moisture content after a perennial legume like alfalfa compared to annual small grain crops (Astatke et al., 1995) when sampling deeper in the soil profiles (0-200 cm). Some studies have found no difference in soil moisture availability at the depth of 0-50cm after growing forage legumes versus annual crops, but significantly lower amounts of available soil water was measured at deeper soil depths (50-100 cm) (Astatke et al., 1995). In addition, according to our grain yield results in 2012, there was a depression in wheat grain yield grown after two years of forage legumes (see Chapter 5) at Swift Current, where crop growth was largely limited by soil moisture content in 2012. The yield depression of wheat following two years of alfalfa could be an indication of greater soil moisture depletion by growing alfalfa. Thus, more research needs to be conducted regarding the feasibility of growing forage legumes in short-term rotation in severely moisture limiting conditions.

4.6.2 Extractable P content and soil P supply rate

Comparing available P levels in the soil after two years of different crop rotations (alfalfa-alfalfa, red clover-red clover, barley-pea, barley-flax), the available P content and P supply rates were all similar ($P > 0.10$); this indicating that having two years of alfalfa, red clover, barley-pea or barley-flax did not significantly affect the amounts of soil test extractable available P or soil P supply rate. This is despite higher P uptake and crop P removal in the forage legume treatments in previous years (2010 and 2011). As described in the previous chapter (see Chapter 3), forage legumes utilized and removed greater amounts of soil P due to the longer growing season and higher dry matter production and removal in haying; as a result, a lower amount of soil P left in the soil for the succeeding crop P use would be anticipated. However, the amounts of plant available P were very similar among all treatments. These results suggest that forage legumes in rotation can maintain soil P fertility in the face of greater P removal during the forage phase of the rotation, at least in the short-term. The ability of alfalfa and red clover in sustaining soil available P in the short-term even with the greater P removal by these crops when hayed could be partially explained by the turnover of the extensive root system of these perennial forage legumes (Daroub et al., 2001). According to previous studies, a considerable amount of P was added to the soil through alfalfa root biomass turnover (Lory et al., 1992; Blumenthal and Russele, 1996; Daroub et al., 2001). Daroub et al. (2001) estimated that alfalfa root could contribute 11.44 kg P ha^{-1} per year when the alfalfa root residue contains 0.2% P content. Phosphate fertilizer recommendations for barley and spring wheat based on the Kelowna soil test method are presented in Appendix (Table. A2). The general soil test rating for P is also shown in Appendix (Table. A3).

When comparing the available P levels of different sites, the Lanigan site has the highest extractable P and P supply rate in the surface soil (Fig 4.2, 4.3) due to the previous history of manure application at this site. The other three sites have similar low extractable P contents and P supply rates. The relatively low available P status of the Saskatoon, Swift Current and Melfort sites indicate that these sites are moderately P deficient at the beginning of the wheat and canola phase of the rotations in 2012 and 2013 (results from the wheat and canola crops in the following years will be presented in the next chapter).

4.6.3 Soil P fractionation

In this study, with a couple of exceptions, including a short period (two years) of forage legume versus annual crop in rotation did not have significant impact on the distribution of soil P fractions (Table 4.3). These results could be explained by the short rotation period (only two years) used in this study. A two-year rotation of forage legumes may be too short to produce measurable significant differences in sequentially extracted soil P fractions. In a field study conducted at the Michigan, the United States of America, Daroub et al. (2001) also found no differences in soil organic P fractions among four annual cropping systems and an alfalfa rotation and they attributed the similarities between different cropping systems to the short period (7 years) of crop rotations. Horst et al. (2001) showed in their study that a positive rotational effect of P-mobilizing crops is mainly due to the transfer of readily available P via crop residues. According to Alamgir et al. (2012), soil P can be mobilized during legume residue decomposition as legume residues contain more P, have lower C:P ratios than cereal crops (Nuruzzaman et al., 2005a) and favoring net P mobilization. In our study, alfalfa and red clover were harvested as hay; only the root biomass was left in the soil. Less legume residue return (only root biomass) to the soil presumably is another explanation for the similar amounts of P fractions measured after two years of different crop rotations.

In this experiment, there are some discrepancies between the results and previous studies which showed that legumes resulted in higher levels of labile or moderately labile P fractions and lower levels of stable P fractions (Zheng et al., 2001; Motavalli and Miles, 2002 Nuruzzaman et al., 2005a; Hassan et al., 2012a,b; Wang et al., 2012). However, most of this past research involved several years of legume growth in the evaluation or else they were pot studies that were conducted in a greenhouse or under controlled experimental conditions (Zheng et al., 2001; Motavalli and Miles, 2002; Nuruzzaman et al., 2005; Hassan et al., 2012a; Wang et al., 2012) where concentration of roots per unit volume of soil is greater than in the field. In our experiment, the changes in P fractions were studied under field experimental conditions and the crop rotation period was only two years. These factors might be responsible for the discrepancy in our findings.

Similar concentrations of labile P fractions in the soil (resin P, $NaHCO_3$-Pi, and -Po) were measured after two years of forage legume and annual crop rotations (Table 4.3) at the sites, despite the greater P uptake and removal of P in forage legume hay harvested in 2010 and 2011. The reason for this similar amount of labile P fractions could be due to the extensive root systems of alfalfa and red clover (Daroub et al., 2001). According to previous studies, alfalfa added greater amounts of biomass to the soil through its root system (Lory et al., 1992; Blumenthal and Russelle, 1996). Daroub et al. (2001) evaluated the P distribution after 7 years of alfalfa rotation and found an increase in available P content. According to Daroub et al., (2001), the total P contribution of alfalfa root per year would be 68.6 kg P ha^{-1} in six years if alfalfa contained 0.25% of P in the root residue. In this study, forage legumes did not deplete, but in fact maintained soil available P when included in crop rotations, which suggests that forage legumes in rotation can sustain soil P fertility in the face of greater P removal during the forage phase of the rotation, at least in the short-term.

At the Lanigan site, it is noteworthy that two years of forage legume (alfalfa-alfalfa, and red clover-red clover) resulted in higher concentration of soil P in the residual fraction (Table 4.3). This fraction, obtained by strong sulfuric acid/peroxide digest is believed to be very recalcitrant, comprised of insoluble P minerals of very low plant availability. It can also contain humus which has a complex degradable nature (Hedley et al., 1982; Tiessen and Moir, 1993). The reason for this effect is not known but could reflect more intense microbial activity in the alfalfa rotation. The only other significant effect was found in higher $NaHCO_3$-Po content in the barley-flax rotation at the Melfort site (Table 4.3). The reason for this might be the contribution of crop residue to the organic P extracted with $NaHCO_3$. During this rotation, flax crop residue did not have enough time to mineralize completely into Pi fractions due to its recalcitrant nature, as a result higher amounts of $NaHCO_3$-Po fraction from transitional microbial products accumulated. Another contributing factor is that flax is a crop that supports a high degree of mycorrhizal infection of the roots (Thingstrup et al., 1998). Although AM fungal populations were not measured in the current study, high P uptake and concentration in shoots and roots of flax promoted by AM fungal colonization of the flax roots may result in accumulation of labile microbially derived organic P extractable by sodium bicarbonate in the soil.

The differences in the size of the soil P fractions among sites (Fig 4.4A, B, C, D) are likely related to the variations in inherent soil characteristics such as content of primary mineral P in the parent material, weathering, soil pH and organic matter content, and long-term management practices like fertilizer addition, crop residue management and soil management practices as well as the climatic conditions of each site. Soil P availability and the relative distribution of soil Pi and Po may be influenced by many factors such as initial soil chemical characteristics, soil type, and climate and management practices such as fertilizer and manure amendment (Blake et al., 2000; Krishna, 2002; Motavalli and Miles, 2002; Bünemann et al., 2004).

4.6.3 Soil microbial community structure

The soils from this study did not reveal any color development that could be indicative of microbial community structure differences. The possible reason for lack of color development could be due to the limited microbial activity with the use of air-dried soil instead of fresh soil. Thus, this study of the soil microbial community structure using Biolog Ecoplates™ was inconclusive due to failure of the technique to achieve the expected performance in these soils.

4.7 Conclusion

After two years of forage legume or annual crop, gravimetric soil moisture contents in the soil profile to 60 cm depth were not significantly affected by crop rotation at the four sites in Saskatchewan. Similar concentrations of extractable, available P and P supply rate were measured among the four rotations at all four locations, despite the greater P uptake and P removal by forage legumes compared to annual crops in 2010 and 2011. Two years of forage legume versus annual crop generally did not have a significant impact on the distribution of soil P between various Pi and Po pools. Consistent with the soil P test assessments, the labile P fractions were similar among different crop rotations. These results suggest that forage legumes can maintain soil P fertility despite the greater P removal by these crops through greater biomass harvest. Two years of crop rotation period might be too short to produce significant changes in the various chemically extracted P fractions. Thus, including forage legumes in a crop rotation for two years is considered as a beneficial agronomic practice that can preserve soil P fertility at least in the short-term. Further studies may consider the effects of several cycles of this rotation over a number of years. This study evaluated the effect of crop rotations using bulk soil samples. It may be useful to also do the P fractionation using rhizosphere soil, if such soil could be separated out for measurement easily and consistently. Sequential P fractionation was used in this study and many other studies to evaluate the effect of cropping systems and fertilizer application on soil P fractions and to determine the static P content, which makes it difficult to elucidate the actual P transformation mechanisms in detail. Using both the sequential extraction method and the ^{32}P tracer technique may be a more rewarding approach to follow the P transformations between the different P pools in soil over the short-term.

5. YIELD AND UPTAKE OF PHOSPHORUS BY WHEAT AND CANOLA GROWN AFTER TWO YEARS OF FORAGE LEGUME AND ANNUAL CROPS

5.1 Preface

The positive effects of legumes on the growth and nutrient uptake of following crops, especially cereals in subsequent years have been well documented in many regions around the world. Previous studies reported that certain legume species, particularly white lupin (*Lupinus albus* L.), chickpea (*Cicer arietinum* L.), field pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.), are not only capable of mobilizing available soil phosphorus (P) for their own requirement, but they are also capable of enhancing the growth and P uptake of following crops. Although effects of these grain legumes on the growth and uptake of soil P by a subsequent crop have been intensively investigated; no studies have examined the effect of a short duration (2 years) of forage legume on yield and P uptake by following cereal and oil-seed crops. This study assesses the yield and uptake of P in grain and straw of wheat *(Triticum aestivum* L.) and canola (*Brassica napus* L.) grown in rotation following two years of different crops at four soil-climatic zones in Saskatchewan. In chapter 3 of this thesis, the yield and P uptake by alfalfa and red clover versus annual crops (barley, pea, and flax) in the first two years of a four year rotation was covered. In chapter 4, the soil P availability and changes in P fractions after two years of forage legume versus annual crops was reported. In this chapter (chapter 5), (i) the effect of the preceding two years of forage legume and annual crops on the yield and P uptake by wheat and canola crops grown in the last two years of the four year rotations and (ii) the impact of four-year crop rotations on soil P dynamics and P balance are examined.

5.2 Abstract

Annual legumes have been shown to enhance the growth and P uptake of following crops, but there is lack of information on the effect of perennial forage legumes included in rotation for a short duration such as two years on yield and P uptake of following crops like wheat (*Triticum aestivum* L.) and canola (*Brassica napus* L.). A field study was conducted in four soil zones within Saskatchewan: Dark Brown soil zone (Saskatoon), thin Black soil zone (Lanigan), Brown soil zone (Swift Current) and Dark Gray soil zone (Melfort) (i) to assess the

effect of the previous cropping on yield and P uptake of following wheat and canola grown in the two subsequent years and (ii) to evaluate the effect of the different crop rotations over the four years on soil P dynamics and P balance. In this study, four different crop sequences (alfalfa-alfalfa, red clover-red clover, barley-pea and barley-flax) employed over two years were compared for their effect on the yield and P uptake of wheat followed by canola over the next two years. Wheat grain yield was positively affected by forage legume rotations at three sites, but at the Swift Current site, it was negatively affected by two years of alfalfa ($P < 0.10$). Wheat P uptake was improved significantly by two years of red clover at Lanigan, Swift Current and Melfort ($P < 0.10$), but at Saskatoon it was not affected by any crop rotations ($P > 0.10$). Grain yield of canola grown after the wheat was also significantly enhanced by forage legume rotations, especially after two years of red clover rotation at Saskatoon and Lanigan and after two years of alfalfa rotation at Melfort ($P < 0.10$). At Swift Current, crop rotations did not have a significant effect on canola grain yield ($P > 0.10$). There were no significant differences in canola P uptake following different crop rotations at all four locations ($P > 0.10$). Wheat and canola grain yield improvements by forage legumes at different locations are mainly attributed to improved soil nitrogen (N) supply and enhanced soil physical and biological characteristics.

According to the soil P test results, modified Kelowna extractable P and soil P supply rate measured after wheat and canola harvest were not affected by different crop rotations at all sites ($P > 0.10$) despite the greater P removal by forage legumes during the first two years of the four year crop rotation period at all sites and the enhanced P removal by wheat and canola crops following forage legume rotations at Lanigan and Melfort. This indicates that forage legumes are able to maintain soil P fertility in the face of greater P removal by crops in rotation, at least in the short-term. Four years of continuous cropping with a very low addition of fertilizer P resulted in a significant reduction of soil P fertility at all locations. Thus, to preserve soil P fertility and to maintain a high crop yield and quality in agricultural production, it is important to apply P according to the crop P demand and P removal characteristics of the rotation used.

5.3 Introduction

Legumes are becoming increasingly important in cropping systems due to their beneficial effects on soil nutrient availability (Graham and Vance, 2003; Hassan et al., 2012a,b). The positive effects of legumes on the growth and nutrient uptake of following crops, especially cereals in subsequent years have been well documented in many regions around the world (Kamh et al., 1999; Horst et al., 2001 Hocking and Randall, 2001; Hens and Hocking, 2004; Nuruzzaman et al., 2005a, b; Hassan et al., 2012a). For example, in a rotation study conducted in Western Australia, faba bean (*Vicia faba* L.) increased cereal growth and P uptake in low available P soils (Nuruzzaman et al., 2005a). Legumes also showed beneficial effects on the growth and yield of following cereal crops in subsequent years in western Canada (Wright, 1990; Stevenson and Kessel, 1996). Although the positive effects of legumes on the following crops are generally attributed to (i) an improved N status of soils through the symbiotic N_2 fixation of legumes (Peoples et al., 1995), (ii) breaking soil borne diseases, and (iii) improving soil physical characteristics (Imai, 1991), there is some evidence that enhanced P solubility is another factor that is responsible for the beneficial effects of legumes on subsequent annual crops (Nuruzzaman et al., 2005a). Several studies have demonstrated that the growth and P uptake of wheat (*Triticum aestivum* L.) was better after legume crops than after wheat in P deficient soils even though adequate N was supplied (Horst et al., 2001; Nuruzzaman et al., 2005a).

Many research results showed that, some legumes, particularly white lupin (*Lupinus albus* L.), chickpea (*Cicer arietinum* L.), field pea *(Pisum sativum* L.) and faba bean (*Vicia faba* L.), are not only capable of mobilizing available soil P for their own requirement, but they are also capable of enhancing the growth and P uptake of following crops such as wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) (Hens and Hocking, 2004; Nuruzzaman et al., 2005b). According to Kamh et al. (1999), P efficient legume crops have positive effects on the growth and P uptake of a subsequent maize crop (*Zea mays* L.). In addition, Hocking and Randall (2001) demonstrated that white lupin (*Lupinus albus* L.) can increase the availability of residual P to sorghum (*Sorghum bicolor* L.) and wheat (*Triticum aestivum* L.) by releasing considerable amounts of carboxylates and other organic acids into the soil. In a field trial, Horst et al. (2001) also observed a positive rotational effect of P-efficient leguminous crops on the less P-efficient cereal crops.

The enhanced growth of crops grown after legumes has been explained through various effects. First, the legume crop may solubilize soil P in excess of their own requirements and the excess amounts of P could be carried over and utilized by other less P efficient crops grown in rotation in the cropping system (Gardner and Boundy, 1983; Horst and Waschkies, 1987; Kamh et al., 2002). Furthermore, P release during the mineralization of legume residues can be another explanation for the elevated crop growth and P acquisition after legumes (Horst et al., 2001; Kamh et al., 2002; Nuruzzaman et al., 2005a,b; Oberson, 2011). The main advantage of crop residues is the addition of organic matter to the soil (Rasse et al., 2000) which can influence P availability mainly through a range of processes including (i) P release from the residue, (ii) dissolution/desorption of P during residue decomposition (Amann and Amberger, 1984; Bolan et al., 1994; Iyamuremye and Dick, 1996), (iii) microbial immobilization of inorganic P (Chen et al., 2000), and (iv) the accumulation of organic P fractions (Horst et al., 2001).

The effect of grain legumes as preceding crops on the growth and P uptake of following crops have been intensively investigated in many countries around the world in pot experiments conducted under controlled environmental conditions or green house conditions (Kamh et al., 1999; Horst et al., 2001; Hocking and Randall, 2001; Hens and Hocking, 2004; Nuruzzaman et al., 2005a,b; Hassan et al., 2012b). However, the effect of perennial forage legumes on yield and P uptake of following crops has received less attention, especially when the forage legumes are in the rotation for only a short duration such as two years. To maximize the beneficial effects of legumes in crop rotation, it is important to understand the ability of various legume crops (both forage and grain legumes) in enhancing the yield and P uptake of different subsequent crops following in rotation under field experimental conditions. The objectives of this study were (i) to assess the effect of two previous years of alfalfa, red clover, barley followed by pea and barley followed by flax on the yield and P uptake by wheat and then canola grown in the next two years and (ii) to evaluate the effect of four-year crop rotations on soil P dynamics and P balance under four soil-climatic zones in Saskatchewan.

5.4 Materials and Methods

5.4.1 Site description

The study was conducted at four sites: 1) the Agriculture and Agri-Food Canada Research Farm (AAFC-Scott) at Saskatoon (52°04′N, 108°08′W); 2) the Western Beef Development Centre's (WBDC) Termuende Research Ranch at Lanigan (51°51′N, 105°02′W);

3) the Semiarid Prairie Agricultural Research Centre (SPARC) at Swift Current (50°16′N 107°44′W) and 4) the Melfort Research Farm (MRF) at Melfort (52°08′N, 104 °06′W) Saskatchewan, Canada from 2012 to 2013. The soil at Saskatoon the site is classified as an Orthic Dark Brown Chernozem, Shellbrook-Hamlin association on a nearly level topography of very fine sandy loam to loam texture (Saskatchewan Soil Survey, 1999). The soil at Lanigan is classified as an Orthic Black Chernozem, Meota-Hamlin association of loamy sand to very fine sandy loam texture, on a very gently sloping topography (Saskatchewan Soil Survey, 1992). The soil at Swift Current is classified as Orthic Brown Chernozem, Swinton association of a silt-loam texture on a gently sloping topography (Saskatchewan Soil Survey, 1990). The soil at Melfort is classified as Orthic Black Chernozem, Tisdale-Eldersley association on a gently sloping topography. The soil textures vary from silty clay loam to silty clay (Saskatchewan Soil Survey, 1987). Soil properties at these sites and climate and ecoregion characterization for the different locations are presented in Table 5.1 and Table 5.2. The monthly precipitation and mean temperature for the experimental years 2012 and 2013 as well as the long-term (30 years from1971 to 2000) average are presented in Table 5.3 (Environment Canada, 2012, 2013).

Table 5.1. Selected physiochemical properties of soils at the four experimental sites.

Locations	pH (1:2H_2O)	EC† (dS m^{-1})	OC† (%)	Particle size distribution (%)			Soil texture‡
				Sand	Silt	Clay	
Saskatoon	7.2	0.299	1.9	15.0	39.7	45.2	Silty clay
Lanigan	7.7	0.327	2.4	29.6	50.9	19.5	Silty loam
Swift Current	7.0	0.166	1.6	31.3	47.7	30.9	Clay loam
Melfort	7.9	0.210	4.5	16.4	42.3	41.3	Silty clay

† EC is electrical conductivity; OC is organic matter content.
‡ Soil texture was classified according to the Canadian Soil Texture Triangle.

Table 5.2. The climate conditions and environmental characteristics at the four experimental sites.

Sites	Location	Climate	Ecoregion
AAFC-Scott, Saskatoon	52°40′N, 108°08′W	Semi-arid	Moist Mixed Grassland
WBDC, Lanigan	51°51′N, 105°02′W	Semi-arid	Aspen Parkland ecoregion
SPARC, Swift Current	50°16′N, 107°44′W	Semi-arid	Mixed Grassland
MRF, Melfort	52°08′N, 104°06′W	Sub-humid	Aspen Parkland ecoregion

Table 5.3. Monthly mean temperature (°C) and precipitation (mm) from April to October in 2012, 2013 and 30 years mean temperature and precipitation (from 1971 to 2000) at the four study sites in Saskatchewan.

Locations	Year		Apr.	May	June	July	Aug.	Sep.	Oct†
Saskatoon	2012	Temp. (°C)‡	4.4	10.1	15.8	19.7	17.3	13.0	1.7
	2013	Temp. (°C)	-2.3	13.0	15.5	17.4	18.9	15.2	3.3
	30years	Ave. temp. (°C) ‡	4.7	11.8	16.0	18.3	17.6	11.5	.
	2012	Precip. (mm) ‡	27.3	108.3	121.1	80.9	48.5	8.0	.
	2013	Precip. (mm)	6.2	15.2	115.9	35.2	14.7	14.9	.
	30years	Ave. precip. ‡	24.2	43.6	60.5	57.3	35.4	30.6	16.9
Lanigan	2012	Temp. (°C)	5.0	10.1	16.2	19.8	16.8	11.7	1.3
	2013	Temp. (°C)	-3.9	12.3	15.6	16.6	17.5	13.8	2.8
	30years	Ave. temp. (°C)	4.0	11.3	15.9	18.1	17.3	11.3	.
	2012	Precip. (mm)	24.3	86.1	89.4	72.0	7.4	0.0	.
	2013	Precip. (mm)	3.4	36.8	107.8	47.2	12.9	50.7	.
	30years	Ave. precip. (mm)	30.3	53.5	83.9	66.1	53.0	42.6	28.0
Swift Current	2012	Temp. (°C)	5.5	9.9	16.8	20.0	19.3	14.2	2.9
	2013	Temp. (°C)	-0.9	12.6	15.5	16.8	19.2	15.2	4.0
	30years	Ave. temp. (°C)	4.9	11.1	15.6	18.1	17.9	11.8	.
	2012	Precip. (mm)	42.0	101.9	113.4	22.0	10.9	6.8	.
	2013	Precip. (mm)	11.8	11.2	103.0	50.4	13.5	42.8	.
	30years	Ave. precip. (mm)	22.3	49.5	66.0	52.0	39.9	30.2	16.2
Melfort	2012	Temp. (°C)	2.6	9.6	15.2	18.9	17.1	12.4	1.1
	2013	Temp. (°C)	-3.9	12.0	15.4	16.4	17.7	14.4	2.8
	30years	Ave. temp. (°C)	2.5	10.8	15.7	17.4	16.4	10.5	.
	2012	Precip. (mm)	24.7	55.2	112.3	97.8	68.1	12.6	.
	2013	Precip. (mm)	3.0	18.0	96.9	100.0	10.6	17.0	.
	30years	Ave. precip. (mm)	24.5	45.6	65.8	75.7	56.8	39.9	24.7

† 2012 and 2013 precipitation data and 30 years average temperature data for October is not available.
‡ Temp. (°C) is temperature; Ave. temp. (°C) is average temperature; Precip. (mm) is precipitation; Ave. precip. (mm) is average precipitation.

5.4.2 Experimental design and treatments

A two-year field experiment was initiated in 2012 following two years of forage legume and annual crop rotations established in 2010 and 2011 at four locations in Saskatchewan, Canada (see chapter 3). The experimental design at each site was a randomized complete block design (RCBD) with four replications. In the spring of 2012 wheat and in 2013 canola was uniformly planted on all plots of the four crop rotations that were in place for 2010 and 2011: alfalfa-alfalfa, red clover-red clover, barley-pea and barley-flax. These different crop rotations were utilized as the treatments to evaluate the residual effects on following wheat and canola crops grown in 2012 and 2013 respectively. Crop rotation treatments are described in Table 5.4.

Table 5.4. Description of crop rotation treatments.

Rotation	2012	2013
1	Wheat	Canola
2	Wheat	Canola
3	Wheat	Canola
4	Wheat (N rate)	Canola (N rate)

At the end of April 2012 (year three), a pre-seed burn off of weeds was accomplished with a 0.4 L ha^{-1} of Roundup (RT glyphosate) on all plots at all four locations. An extra application was done on the alfalfa plots at the rate of 0.8 L ha^{-1}. To calculate the N fertilizer replacement value as required for another study[1], rotation four (barley-flax) plots at all locations were sub-divided into five subplots and N rate treatments were imposed on the sub-plots. The N rates were equivalent to 0, 40, 80, 120 and 160 kg N ha^{-1}. For the research in this thesis, the 0 N subplots were the plots that were sampled and analyzed for soil and plant yield and P content. No P fertilizers were applied on any of the plots of any of the rotations. Wheat (Unity VB spring wheat) was seeded during the first week of June with a Fabro plot drill equipped with AtomJet knifeopeners using a 22-cm row spacing at a recommended rate for each location. In-crop weed-control at Swift Current was achieved by spraying a tank mix of Horizon NG (Clodinafop-propagyl 60 g L^{-1}) and Buctril M (Bromoxynil 280 g L^{-1} and MCPA 280 g L^{-1}) at 0.19 L ha^{-1} on July 06, whereas in Saskatoon, in-crop weeds were controlled by spraying 0.32 L ha^{-1} of Bifenthrin or Tundra (Bromoxynil 87.5 g L^{-1}, Fenoxaprop-P-ethyl 46 g L^{-1} and Pyrasulfotole

15.5 g L^{-1}) on June 25 and July 05. At Lanigan, weed control was achieved by the application of Liquid Ammonium Sulphate or Infinity (Pyrasulfotole 37.5 g L^{-1} and Bromoxynil 210 g L^{-1}) at 0.8 L ha^{-1} on June 19. Finally, at Melfort, the weeds were controlled by an application of Buctril M (Bromoxynil 280 g L^{-1} and MCPA 280 g L^{-1}) at 0.4 L ha^{-1} on June 07. At crop maturity, wheat biomass yield was determined by hand harvesting $1m^2$ above ground biomass samples in each plot. Biomass samples were dried at 30 °C and weighed to determine biomass yield and then threshed. The straw and grain were separated, dried and ground for the estimation of P concentration. Wheat grain yield was also determined in the middle of September by harvesting 10 m^2 areas from each plot using a Wintersteiger plot combine at three locations except for Lanigan, where wheat grain yield was harvested with a REM plot combine.

In April of 2013, pre-seed weed control was accomplished with a 0.4 L ha^{-1} of Roundup (RT glyphosate) on all plots at all four locations. Rotation four (barley-flax) subplots were repeated again in 2013 (same treatment structure as 2012) and received one of five rates of N fertilizer (equivalent to 0, 40, 80, 120, and 160 kg N ha^{-1}). No N fertilizer was applied on other crop rotations except for rotation four (barley-flax) and as in 2012, in 2013 the 0 N subplot of this rotation was used for soil and plant sampling for this thesis research. Also, as in 2012, in 2013 no P fertilizers were applied on any crop rotations at all locations. A 100 kg ha^{-1} K_2SO_4 (equivalent to 20 kg S ha^{-1}) fertilizer was applied on all plots across the whole site at every site to ensure that S deficiency did not limit canola growth. Canola (*Brassica napus* cv. LL130) was seeded in late May with a Fabro plot drill equipped with AtomJet knifer opener with a 22 cm row spacing at a recommended rate at each location. In-crop weed control was achieved by applying Liberty herbicide at a recommended rate for each location. At a typical swathing stage (40% pod color change), canola biomass was sampled by taking 0.5 m^2 samples from each plot. Biomass samples were dried and weighed to determine biomass yield, and then threshed. The straw and grain were separated, dried and ground to estimate the canola straw and grain P concentration. Canola grain yield was also determined in the middle of September by harvesting 10 m^2 areas from each plot using a Wintersteiger plot combine at three locations except for Lanigan, where wheat grain yield was harvested with a REM plot combine.

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[1]- Calculating N fertilizer replacement value was not an objective of this particular study. It was calculated to achieve the objective of another project utilizing the same sites.

5.4.3 Soil sampling and analyses

Soil samples were taken at three different times: in the fall (October) of 2012, in the spring (April) of 2013 and in the fall (October) of 2013 respectively from all plots at all experimental sites. At each site, three core samples were taken from each plot at random using a core soil sampler (4.5 cm diameter and 100 cm length) and composited according to sampled depth increments: 0-15 cm, 15-30 cm and 30-60 cm. Soil samples were then transported to the laboratory and immediately stored in a fridge at 4 °C prior to gravimetric soil moisture measurement. The gravimetric soil moisture content was determined at all depths by weighing 10 g of wet soil and oven drying at 105 °C for 24 h. The percent (%) of soil moisture was then calculated from the difference between the weight of wet soil and oven dried soil. After measuring the moisture content, soil samples were air-dried, ground and passed through a 2 mm sieve and stored at room temperature prior to doing further analyses.

5.4.3.1 Initial soil characteristics

Initial characteristics of soils including soil texture, organic matter content, pH and EC were determined in a sub-sample of processed soil collected from all plots at all locations in the spring of 2010. After a week of pre-treatment, soil texture was determined by a laser scattering particle size distribution analyzer (HORIBA© LTD., 2007). Four composite samples from each experimental site were used for soil texture analysis. The composite samples were prepared by combining a surface soil (0-30 cm) sample from each plot at each site. Soil organic carbon content was analyzed using the Leco C632 carbon combustion analyzer (LECO© Corporation, 2007) following the protocols of Wang and Anderson (1998) (Leco Instruments Limited, Mississauga, Ontario, L5T 2H7). In this method, organic carbon released through combustion at 840 °C for 120 s or less is determined as carbon dioxide through infrared spectroscopy (Wang and Anderson, 1998). Soil pH and electrical conductivity were measured with a glass electrode using 1:2 soil : water suspensions (Rhoades, 1982).

5.4.3.2 Extractable soil P and P supply rate

Available soil P from two depths: 0-15 cm and 15-30 cm was extracted with modified Kelowna solution (Qian et al., 1994). In the procedure, three grams of soil were placed in a 250 mL extraction bottle where 30 mL of modified Kelowna solution was added and the mixture was shaken on a rotary shaker for 5 min. The suspension was then filtered through VWR #454 filter paper and stored in vials in the cooler. The P concentration in extracts was then determined colorimetrically using Technicon Autoanalyzer II (Technicon Industrial Systems, 1973) (Qian et al., 1994).

Soil P supply rate was determined at two depths: 0-15 cm and 15-30 cm using a “sandwich” test (Qian and Schoenau, 2010). Two snap cap plastic vial lids were filled with air-dried soil (<2 mm); deionized water was then added according to the field capacity of the soil. A “sandwich” was made by sealing the two caps of soil together after inserting a strip of cleaned and regenerated anion exchange membrane (8 cm^2) in between. After 24 hours, the membrane strips were removed from the “sandwich” and washed free of adhering soil. The membrane was then eluted in 0.5 M HCl for 1 h to desorb the nutrient ions from the anion resin membrane into HCl solution. The P ion concentration in the eluents was measured colorimetrically using Technicon Autoanalyzer II (Technicon Industrial Systems, 1973) (Qian and Schoenau, 2010).

5.4.4 Plant analysis

Wheat and canola grain and straw samples were harvested, air-dried and ground for the P analysis. A 0.25 g sub-sample of ground grain and straw from each replicate was digested using a H_2SO_4- H_2O_2 digestion method (Thomas et al., 1976). The P concentration in the digested solution was then determined colorimetrically using a Technicon Autoanalyzer II (Technicon Industrial Systems, 1973). Plant P uptake (grain and straw) (kg ha^{-1}) was calculated multiplying the grain and straw P concentration by grain and straw yield respectively.

5.4.5 Statistical analysis

Crop biomass, P concentrations, P uptake and removal, soil moisture, modified Kelowna P and P supply rate for treatments at a site were analyzed using one-way analysis of variance tests (ANOVA). The data were analyzed as a RCBD design using Proc. Mixed Procedure of SAS (Version 9.3; SAS Institute., Cary, NC). The treatment (crop types) was the fixed effect and replications in each site were considered as block effect. Means were compared among treatments using Tukey’s multi-comparison tests. Before the ANOVA, data were checked for the normality and equality of variances using the UNIVARIATE procedure; however, no transformation was needed. Significance was declared at $P < 0.10$. The coefficient of variation for analysis, soil and plant samples were less replicate than ≤ 5%. All routine analysis included a standard reference soil or plants every 40 samples.

5.5 Results

5.5.1. 2012 Wheat crop

5.5.1.1 Wheat yield

Wheat grain yield was significantly affected by different crop rotations at all four locations ($P < 0.10$) (Table 5.5). At Saskatoon and Melfort, wheat grain yield was greater after forage legume rotations in comparison to an annual legume: barley-pea and non-legume: barley-flax rotations ($P < 0.10$). At Lanigan, wheat produced higher grain yield after two forage legume and an annual legume rotation relative to a non-legume (barley-flax) rotation ($P < 0.10$). At Swift Current, however, there was a yield depression in wheat yield following two years of alfalfa rotation compared to the other crop rotations ($P < 0.10$). When wheat grain yield produced at the different sites was compared, the highest wheat grain yield was harvested at the Melfort site, followed by the Saskatoon and Lanigan sites, while the lowest grain yield was produced at the Swift Current site.

Wheat total biomass yield was also significantly influenced by the rotation system at three locations ($P < 0.10$) except for Lanigan, where wheat total biomass was not affected by different crop rotations ($P > 0.10$) (Table 5.5). At Saskatoon, the highest wheat total biomass was measured after a non-legume rotation: barley-flax, whereas the lowest wheat biomass was measured after two years of red clover rotation ($P < 0.10$). At Melfort, wheat total biomass was greater following two forage legume rotations and barley-flax rotation compared to wheat total biomass after barley-pea rotation ($P < 0.10$). Finally, at Swift Current, wheat total biomass was lower after two years of alfalfa rotation than wheat biomass following other crop rotations ($P < 0.10$). Among the different sites, the highest wheat total biomass was produced at Saskatoon while the lowest biomass was occurred at Swift Current. The wheat total biomass production at Lanigan and Melfort was intermediate.

Table 5.5. Effect of two years of forage legume (alfalfa-alfalfa; red clover-red clover) and annual crop (barley-pea; barley-flax) rotation on grain and biomass yield of the following wheat crop at four sites in Saskatchewan, Canada.

	Sites	Biomass yield				*P* value‡
		A-A-W†	RC-RC-W	B-P-W	B-FL-W	
		------------- (kg ha^{-1}) --------------				
Wheat grain¶	Saskatoon	2529[ab]§	2792[a]	1982[bc]	1740[c]	0.008
	Lanigan	2283[a]	2535[a]	2515[a]	1736[b]	0.001
	Swift Current	1304[b]	1856[a]	1739[ab]	2073[a]	0.025
	Melfort	3623[a]	3377[ab]	3204[b]	2501[c]	<0.001
Total biomass¶	Saskatoon	9792[ab]	9239[b]	9754[ab]	9882[a]	0.082
	Lanigan	8922	8868	8640	6643	0.157
	Swift Current	1239[b]	2274[a]	1840[a]	2200[a]	0.001
	Melfort	8617[a]	7966[a]	6821[b]	7910[a]	0.004

† A-A-W is alfalfa-alfalfa-wheat; RC-RC-W is red clover-red clover-wheat; B-P-W is barley-pea-wheat; B-FL-W is barley-flax-wheat.
‡ Letters are assigned to treatments that have significant differences among them ($P < 0.10$).
§ Means with the different superscript letter in the same row are significantly different ($P < 0.10$).
¶ Data for the grain biomass and total biomass for rotation four (barley-flax) was taken from the zero N rate sub-plots. Wheat total biomass is the harvested above ground biomass.
# Standard errors are provided in the appendix.

5.5.1.2. Wheat P concentration

The P concentration in wheat straw and grain was not significantly affected by different crop rotations at all locations ($P > 0.10$), except at the Swift Current, where wheat grain P concentration was lowest after an annual non-legume rotation relative to other crop rotations ($P < 0.10$) (Table 5.6). When the straw P concentration among the different sites was compared, the Melfort site had the highest straw P concentration followed by Saskatoon and Lanigan sites, whereas the lowest P concentration was found at the Swift Current site. Among four sites, the Melfort and Lanigan sites had the greater grain P concentrations compared to the Saskatoon and Swift Current sites.

Table 5.6. Effect of two years of forage legume (alfalfa-alfalfa; red clover-red clover) and annual crop (barley-pea; barley-flax) rotation on the straw and grain P concentrations of a following wheat crop at four sites in Saskatchewan, Canada.

	Sites	P concentration				*P* value‡
		A-A-W†	RC-RC-W	B-P-W	B-FL-W	
		--------------------	(mg P g^{-1})	--------------------		
Wheat straw	Saskatoon	0.90	0.67	0.94	1.09	0.320
	Lanigan	0.73	0.74	0.60	0.78	0.239
	Swift Current	0.29	0.29	0.25	0.15	0.500
	Melfort	1.06	1.12	1.21	1.09	0.465
Wheat grain	Saskatoon	3.76	2.93	3.77	4.16	0.160
	Lanigan	4.13	4.34	4.15	4.43	0.484
	Swift Current	3.45[a]§	3.72[a]	3.37[a]	2.62[b]	0.022
	Melfort	4.44	4.54	4.57	4.47	0.987

† A-A-W is alfalfa-alfalfa-wheat; RC-RC-W is red clover-red clover-wheat; B-P-W is barley-pea-wheat; B-FL-W is barley-flax-wheat.
‡ Letters are assigned to treatments that have significant differences among them (P < 0.10).
§ Means with a different superscript letter in the same row are significantly different (P < 0.10).
¶ Standard errors are provided in the appendix.

5.5.1.3 Wheat P uptake

Wheat straw P uptake was not significantly influenced by crop rotations at Saskatoon, Lanigan and Melfort ($P > 0.10$). At Swift Current, wheat straw P uptake was greatest following two years of red clover but it was lowest after two years of alfalfa and barley-pea rotations ($P < 0.10$) (Table 5.7). Wheat straw uptake at at this site was very low due to the low straw yield and low straw P concentration measured. At two locations: Lanigan and Melfort, wheat grain P uptake was significantly different among crop rotations ($P < 0.10$), but at the other two locations crop rotation did not significantly affect wheat grain P uptake ($P > 0.10$). At Lanigan, wheat grain P uptake was highest after two years of red clover and barley-pea rotations but it was lowest after barley-flax rotation ($P < 0.10$). At Melfort, wheat grain P uptake was greatest after two years of alfalfa and red clover rotations and lowest after barley-flax rotation ($P < 0.10$). Wheat total above ground P uptake (grain plus straw) was significantly affected by rotation system at three sites except for Saskatoon. At Lanigan, Swift Current and Melfort, the highest total P uptake by wheat was found in wheat grown after two years of red clover rotation ($P <$

0.10) and the lowest total P uptake was measured after barley-flax rotation at Lanigan and Melfort sites ($P < 0.10$). At Swift Current, unlike other sites, the lowest total P uptake of wheat was found after two years of alfalfa rotation relative to other crop rotations ($P < 0.10$).

Table 5.7. Effect of two years of forage legume (alfalfa-alfalfa; red clover-red clover) and annual crop (barley-pea; barley-flax) rotation on P uptake of a following wheat crop at four sites in Saskatchewan, Canada.

P uptake	Sites	P uptake				*P* value‡
		A-A-W†	RC-RC-W	B-P-W	B-FL-W	
		------------- (kg ha^{-1}) -------------				
Wheat straw	Saskatoon	6.5	4.3	7.6	9.0	0.179
	Lanigan	5.0	4.7	3.7	3.8	0.503
	Swift Current	0.01^{b}§	0.10^{a}	0.02^{b}	0.04^{ab}	0.046
	Melfort	5.2	5.1	4.4	5.9	0.105
Wheat grain	Saskatoon	9.5	8.0	7.4	7.2	0.406
	Lanigan	9.5^{ab}	11.0^{a}	10.4^{a}	7.7^{b}	0.013
	Swift Current	4.5	6.9	5.8	5.5	0.109
	Melfort	16.1^{a}	15.4^{a}	14.7^{ab}	11.2^{b}	0.033
Wheat total	Saskatoon	16.0	12.4	15.0	16.2	0.389
	Lanigan	14.4^{ab}	15.6^{a}	14.2^{ab}	11.5^{b}	0.056
	Swift Current	3.8^{b}	7.0^{a}	5.9^{ab}	5.4^{ab}	0.028
	Melfort	21.3^{a}	20.5^{a}	19.1^{ab}	17.1^{b}	0.034

† A-A-W is alfalfa-alfalfa-wheat; RC-RC-W is red clover-red clover-wheat; B-P-W is barley-pea-wheat; B-FL-W is barley-flax-wheat.

‡ Letters are assigned to treatments that have significant differences among them ($P < 0.10$).

§ Means with the different superscript letter in the same row are significantly different ($P < 0.10$).

¶ Standard errors are provided in the appendix.

5.5.1.4 Soil moisture content (fall 2012 and spring 2013)

Soil moisture content was measured in the fall of 2012 after wheat harvest and results are shown in Table 5.8. There were some small but statistically significant differences in soil moisture content among the different rotation treatments at Lanigan ($P < 0.10$) but the soil moisture contents measured for other sites were not affected by different crop rotations ($P > 0.10$). At Lanigan, the sub-soil (15-30 cm and 30-60 cm) moisture contents were higher after the two year red clover rotation compared to soil moisture contents measured after a barley-flax (15-30 cm) and barley-pea rotation (30-60 cm) rotation. Soil moisture content at Swift Current could not be assessed in samples from the fall of 2012 because the samples were air-dried immediately after removal from field.

The soil moisture content was measured again in the spring of 2013 prior to canola seeding. There were no significant differences in soil moisture contents among different crop rotations measured from all locations in the spring of 2013 ($P > 0.10$) and results were not included. All 2013 treatment plots had wheat grown the previous year.

Table 5.8. Gravimetric soil moisture contents (% water by weight) measured in fall 2012 in the soil profile after different crop rotations at three sites in Saskatchewan, Canada.

Sampling depth	Sites¶	Gravimetric soil moisture content				*P* value‡
		A-A-W†	RC-RC-W	B-P-W	B-FL-W	
		--------------------	(%)	----------------------		
0-15 cm	Saskatoon	21.2	23.2	21.8	20.3	0.324
	Lanigan	17.8	20.6	11.8	10.1	0.128
	Melfort	32.4	31.2	32.0	30.3	0.546
15-30 cm	Saskatoon	23.9	27.1	24.2	23.9	0.789
	Lanigan	9.7[ab]§	10.9[a]	8.2[ab]	6.3[b]	0.028
	Melfort	28.1	30.4	30.7	28.0	0.250
30-60 cm	Saskatoon	21.8	27.0	30.6	29.1	0.584
	Lanigan	11.3[ab]	17.8[a]	8.8[b]	16.2[ab]	0.064
	Melfort	25.2	27.4	27.5	27.8	0.310

† A-A-W is alfalfa-alfalfa-wheat; RC-RC-W is red clover-red clover-wheat; B-P-W is barley-pea-wheat; B-FL-W is barley-flax-wheat.
‡ Letters are assigned to treatments that have significant differences among them ($P < 0.10$).
§ Means with the different superscript letter in the same row are significantly different ($P < 0.10$).
¶ Soil moisture content at Swift Current site was not measured due to soil moisture loss prior to measurement.
# Standard errors are provided in the appendix.

5.5.1.5 Soil extractable P and P supply rate after wheat growth (fall 2012 and spring 2013)

The modified Kelowna extractable P and anion exchange resin membrane P supply rate were measured in the fall of 2012 after wheat harvest (Figs 5.1, 5.2). There were no significant differences in modified Kelowna extractable P or P supply rate among any of the rotations at the sites ($P > 0.10$) (Fig 5.1, 5.2). Higher wheat yields after forage legume rotations at three sites and the greater wheat P removal following alfalfa rotation at Lanigan and Melfort did not produce significant reductions in extractable available P or P supply rate compared to other treatments, nor did the lower wheat yield and P removal after alfalfa rotation at Swift Current result in significantly higher soil available P. Among different sites, the Lanigan site had the highest modified Kelowna extractable P or P supply rate at 0-15 cm sampling depth relative to other sites.

The modified Kelowna extractable P and P supply rate in the soil were measured again in the spring of 2013 prior to canola seeding; and the results are shown in Fig 5.3 and 5.4. The treatment effects are similar to those obtained for the fall 2012 sampling. In the spring of 2013, there were no significant differences in modified Kelowna extractable P or P supply rate among the rotations at the sites despite the higher yields and crop P removal in the forage legume rotations at Lanigan and Melfort ($P > 0.10$). The Lanigan site continued to have the highest extractable P and P supply rates in the surface (0-15cm) depth compared to other sites.

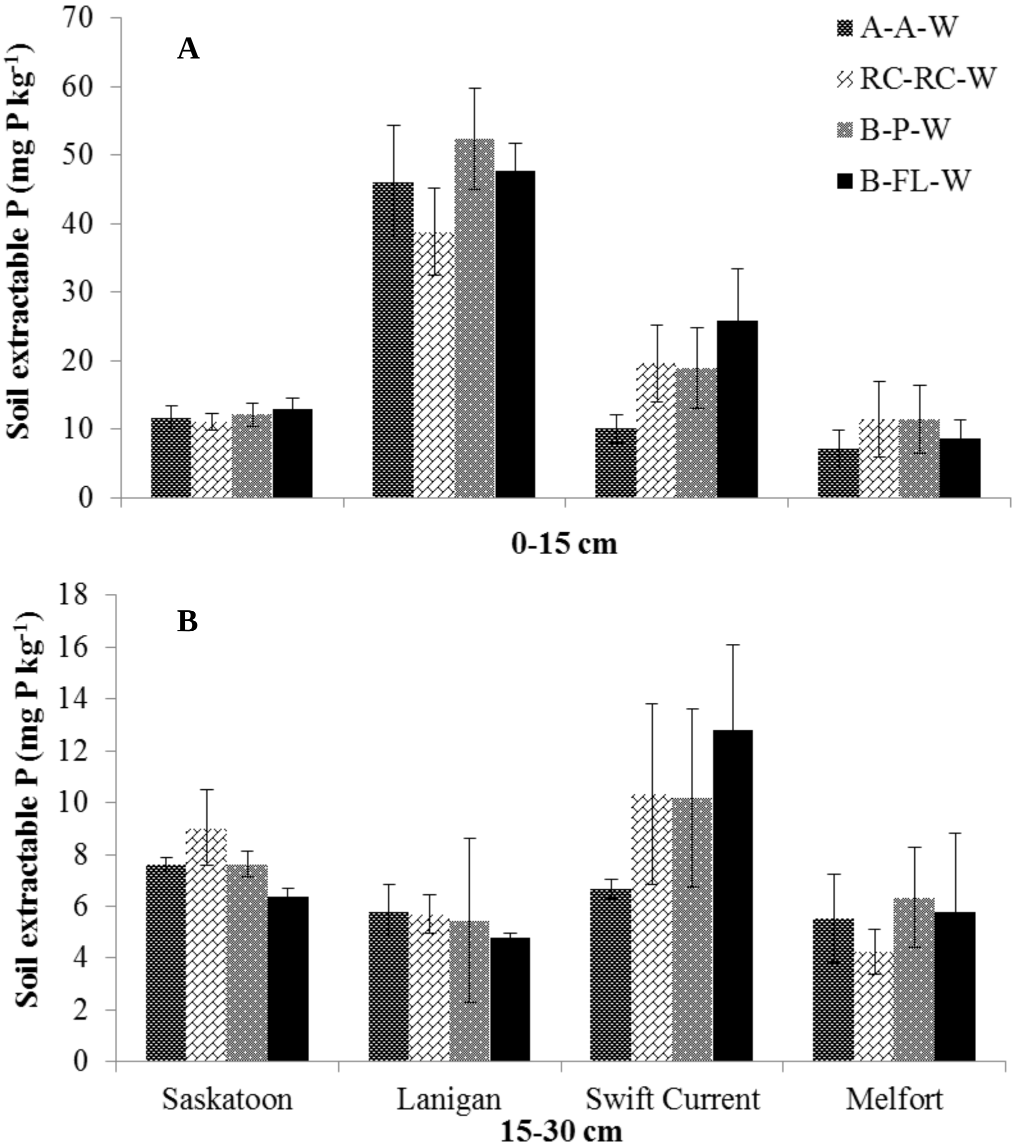

Fig. 5.1. The concentrations of soil extractable P meassured at two depths: 0-30 cm (A) and 30-60 cm (B) in the fall of 2012 in the four different crop rotations at the four sites in Saskatchewan, Canada. (A-A-W=alfalfa-alfalfa-wheat; RC-RC-W=red clover-red clover-wheat; B-P-W=barley-pea-wheat; B-FL-W=barley-flax-wheat). Error bars represent standard errors.

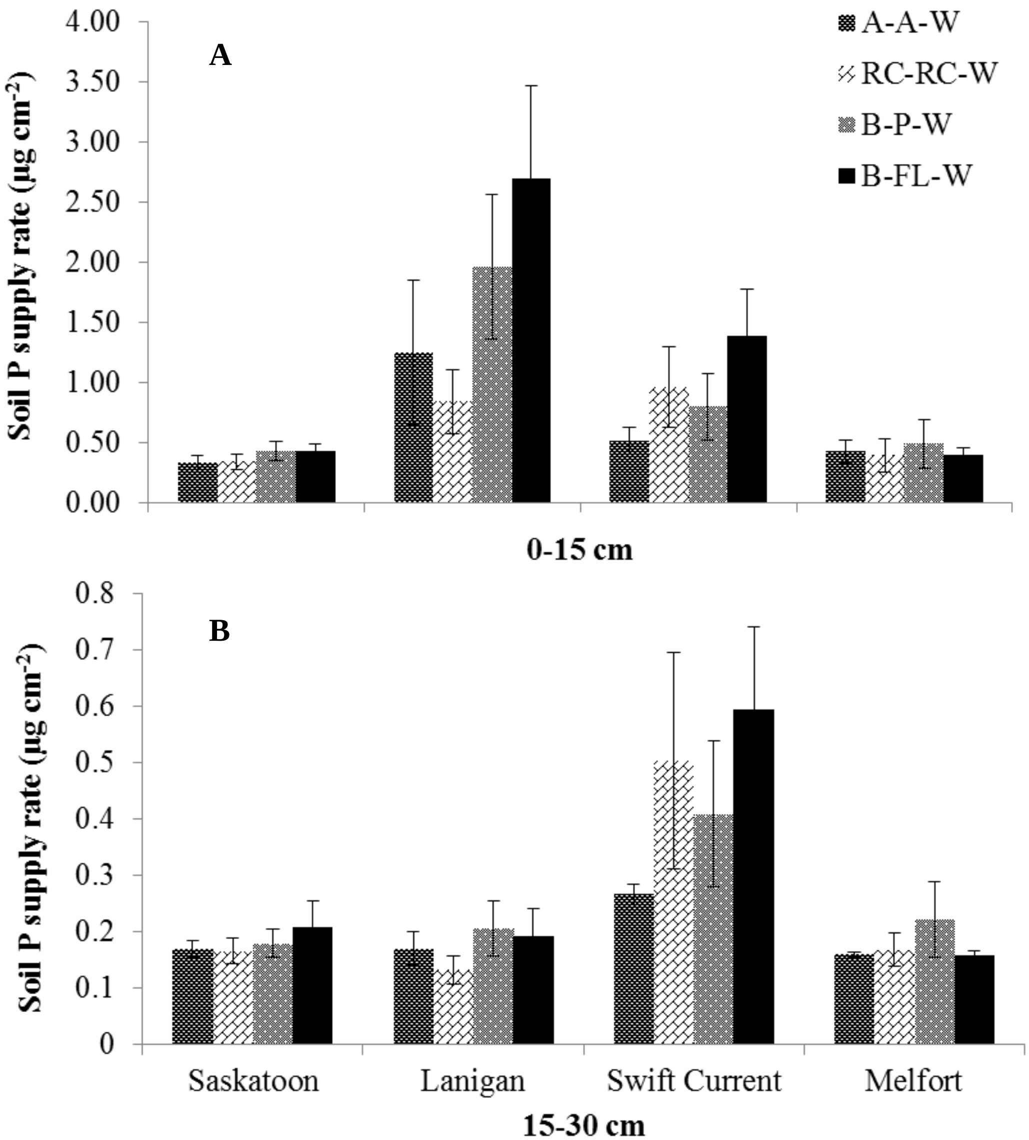

Fig. 5.2. Soil P supply rates measured at two depths: 0-30 cm (A) and 30-60 cm (B) in the fall of 2012 in the four different crop rotations at the four sites in Saskatchewan, Canada. (A-A-W=alfalfa-alfalfa-wheat; RC-RC-W=red clover-red clover-wheat; B-P-W=barley-pea-wheat; B-FL-W=barley-flax-wheat). Error bars represent standard errors.

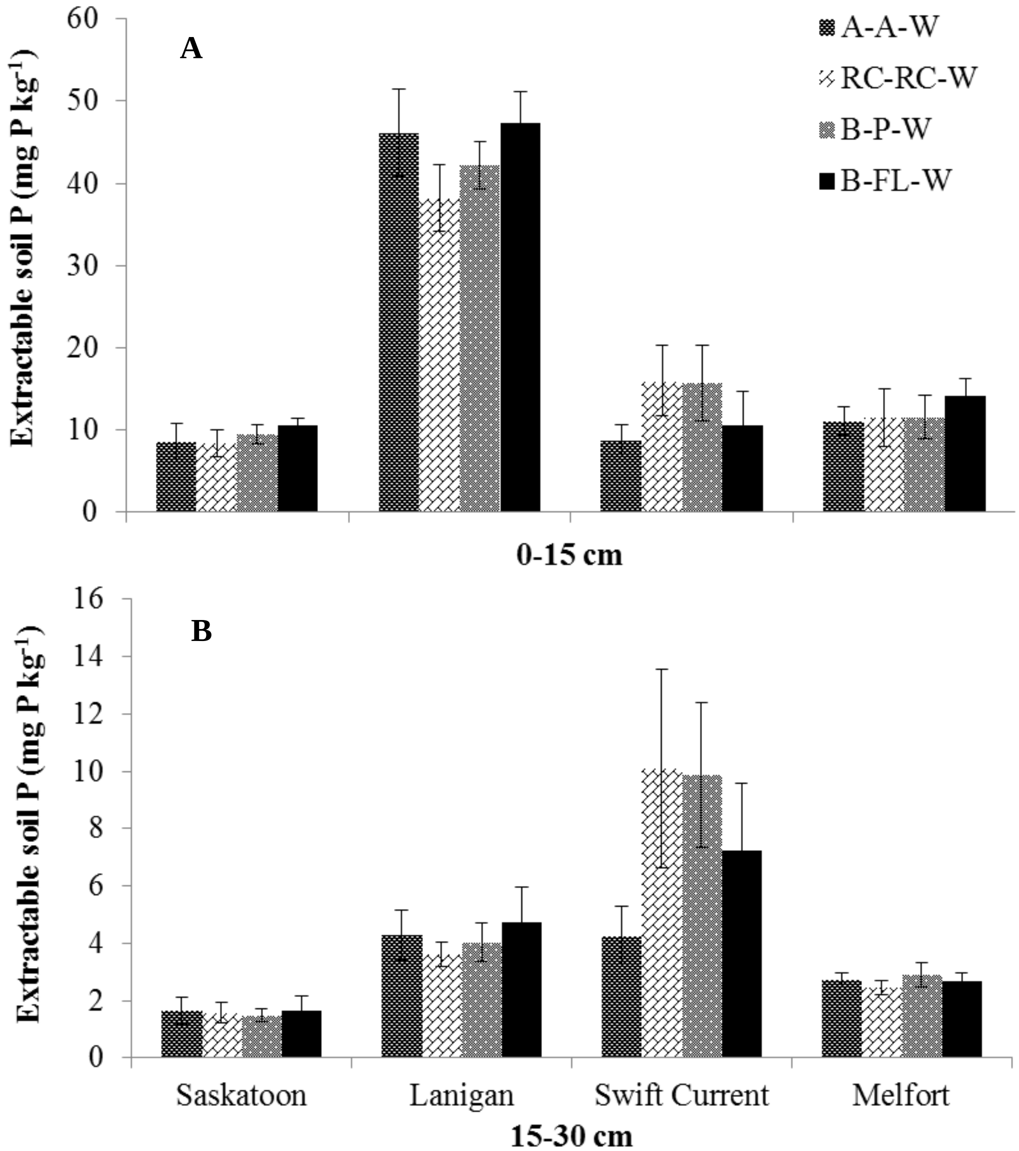

Fig. 5.3. The concentrations of soil extractable P measured at two depth: 0-30 cm (A) and 30-60 cm (B) in the spring 2013 in the crop rotations at four sites in Saskatchewan, Canada. (A-A-W=alfalfa-alfalfa-wheat; RC-RC-W=red clover-red clover-wheat; B-P-W=barley-pea-wheat; B-FL-W=barley-flax-wheat). Error bars represent standard errors.

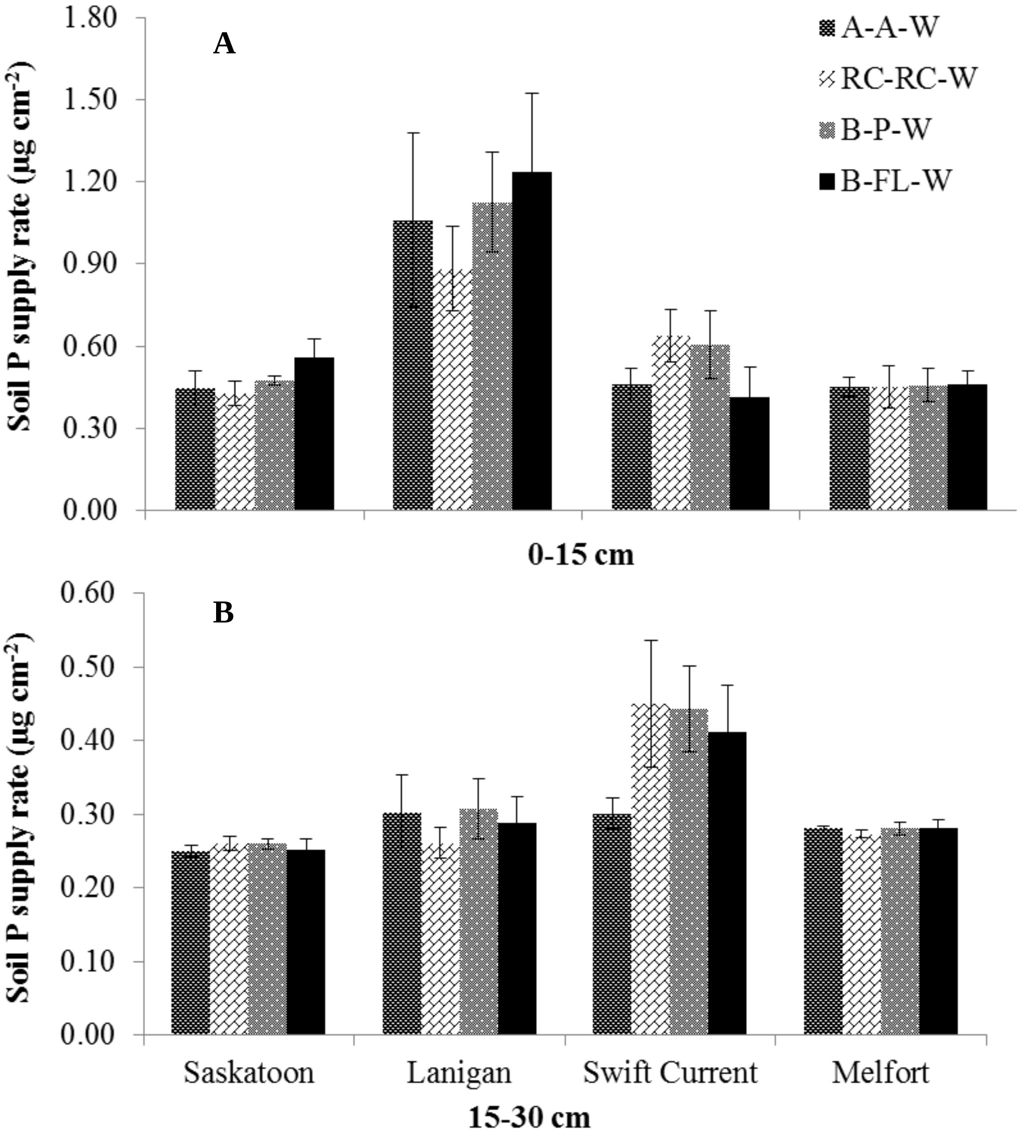

Fig. 5.4. Soil P supply rates measured at two depths: 0-30 cm (A) and 30-60 cm (B) in the spring 2013 in the different crop rotations at four sites in Saskatchewan. (A-A-W=alfalfa-alfalfa-wheat; RC-RC-W=red clover-red clover-wheat; B-P-W=barley-pea-wheat; B-FL-W=barley-flax-wheat). Error bars represent standard errors.

5.5.2 2013 Canola crop

5.5.2.1 Canola yield

Legume rotations in 2010 and 2011 showed a significant positive effect on 2013 canola grain yields at Saskatoon, Lanigan and Melfort ($P < 0.10$) (Table 5.9). Canola grain yield was higher in the red clover based rotation relative to barley-flax rotation at Saskatoon and Lanigan ($P < 0.10$). At Melfort, the yields were relatively higher in the rotation with two years of alfalfa when compared to canola grain yield following barley-pea and barley-flax ($P < 0.10$) rotations ($P < 0.10$). At Swift Current, crop rotations did not have a significant impact on canola grain production ($P > 0.10$). Canola grain yield was not decreased in the second year following the alfalfa, while wheat grown the first year following the following two years of alfalfa was at the Swift Current site.

Crop rotation also had revealed a significant effect on canola total biomass at Saskatoon and Swift Current sites ($P < 0.10$), but at the other two locations there was no significant impact of crop rotations on canola total biomass ($P > 0.10$) (Table 5.9). At Saskatoon, two years of red clover rotation resulted in higher canola total biomass relative to the other crop rotations ($P < 0.10$), whereas at Swift Current, canola total biomass was greater with two years of alfalfa in the rotation than a barley-pea ($P < 0.10$).

Table 5.9. Effect of rotation (alfalfa-alfalfa-wheat; red clover-red clover-wheat, barley-pea-wheat; barley-flax-wheat) on yield of a following canola crop at four sites in Saskatchewan, Canada.

	Sites	Biomass yield				*P* value‡
		A-A-W-C†	RC-RC-W-C	B-P-W-C	B-FL-W-C	
		------------------ ($kg\ ha^{-1}$) ------------------				
Canola grain¶	Saskatoon	1995^{ab}§	2356^{a}	1608^{ab}	1508^{b}	0.065
	Lanigan	1934^{ab}	2352^{a}	1815^{ab}	1509^{b}	0.091
	Swift Current	1301	946	764	1056	0.165
	Melfort	3455^{a}	2791^{ab}	2319^{b}	2109^{b}	0.003
Canola total¶	Saskatoon	5389^{b}	7531^{a}	5176^{b}	5192^{b}	0.007
	Lanigan	6426	6866	6221	5134	0.415
	Swift Current	6960^{a}	4615^{ab}	4040^{b}	4530^{ab}	0.058
	Melfort	2994	3161	2448	2653	0.237

† A-A-W-C is alfalfa-alfalfa-wheat-canola; RC-RC-W-C is red clover-red clover-wheat-canola; B-P-W-C is barley-pea-wheat-canola; B-FL-W-C is barley-flax-wheat-canola.
‡ Letters are assigned to treatments that have significant differences among them ($P < 0.10$)
§ Means with the different superscript letter in the same row are significantly different ($P < 0.10$).
¶ Data for the grain and total biomass for rotation four (barley-flax) was taken from the zero N rate sub-plots. Canola total biomass is the harvested above ground biomass.
# Standard errors are provided in the appendix.

5.5.2.2 Canola P concentration

Canola grain P concentration was significantly affected by crop rotation at Saskatoon, Swift Current and Melfort ($P < 0.10$) but at Lanigan, crop rotations did not have a significant effect on canola grain P concentration ($P > 0.10$) (Table 5.10). At Saskatoon, canola grain P concentration was greater after two years of alfalfa, barley-pea and barley-flax rotations, but it was lower after two years of red-clover ($P < 0.10$) rotation. At Melfort, the greatest canola grain P concentration was measured after barley-pea rotation but the lowest canola grain P concentration occurred after two years of red-clover and alfalfa rotations ($P < 0.10$). At Swift Current, canola grain P concentration was higher following two years of red clover and barley-pea rotations than following two years of alfalfa rotation ($P < 0.10$). Canola straw P concentration was significantly affected by crop rotation only at Swift Current site ($P < 0.10$)

(Table 5.10). At this site, canola straw P concentration was greater following barley-flax rotation than following two years of alfalfa rotation ($P < 0.10$).

Table 5.10. Effect of rotation (alfalfa-alfalfa-wheat; red clover-red clover-wheat, barley-pea-wheat; barley-flax-wheat) on straw and grain P concentrations of a following canola crop at four sites in Saskatchewan, Canada.

	Sites	P concentration				*P* value‡§
		A-A-W-C†	RC-RC-W-C	B-P-W-C	B-FL-W-C	
		-------------------- ($mg\ P\ g^{-1}$) --------------------				
Canola straw	Saskatoon	0.76	0.73	0.85	0.89	0.183
	Lanigan	2.43	0.74	2.19	1.01	0.469
	Swift Current	0.89[b]§	1.15[ab]	1.23[ab]	1.41[a]	0.071
	Melfort	0.40	0.40	0.44	0.48	0.796
Canola grain	Saskatoon	4.84[a]	4.30[b]	5.06[a]	5.26[a]	0.002
	Lanigan	5.36	5.18	5.64	5.44	0.399
	Swift Current	6.34[b]	7.44[a]	7.41[a]	7.26[ab]	0.039
	Melfort	4.96[c]	5.09[bc]	5.61[a]	5.43[ab]	0.086

† A-A-W-C is alfalfa-alfalfa-wheat-canola; RC-RC-W-C is red clover-red clover-wheat-canola; B-P-W-C is barley-pea-wheat-canola; B-FL-W-C is barley-flax-wheat-canola.
‡ Letters are assigned to treatments that have significant differences among them (P < 0.10).
§ Means with the different superscript letter in the same row are significantly different (P < 0.10).
¶ Standard errors are provided in the appendix.

5.5.2.3 Canola P uptake

Generally, crop rotations did not significantly affect canola P uptake (canola straw, grain and total crop P uptake) ($P > 0.10$) (Table 5.11). The only exception was with canola grain P uptake at Melfort, where it was significantly higher following two years of alfalfa rotation than following barley-pea and barley-flax rotations ($P < 0.01$) (Table 5.11).

5.5.2.4 Soil moisture (fall 2013)

Crop rotations had little effect on soil moisture content measured in the fall of 2013 at the end of the rotation, except for the soil moisture content measured in the 30-60 cm sampling depth at Swift Current (Table 5.12). At this site, canola following two years of alfalfa rotation had reduced soil moisture in the 30-60 cm sampling depth compared to other crop rotations at the same sampling depth ($P < 0.10$).

Table 5.11. Effect of rotation (alfalfa-alfalfa-wheat; red clover-red clover-wheat, barley-pea-wheat; barley-flax-wheat) on P uptake by a following canola crop at four sites in Saskatchewan, Canada.

	Sites	P uptake				*P* value‡
		A-A-W-C†	RC-RC-W-C	B-P-W-C	B-FL-W-C	
		-------------------	(kg ha^{-1})	--------------------		
Canola straw	Saskatoon	2.6	3.9	3.0	3.3	0.312
	Lanigan	7.8	3.1	6.8	3.2	0.515
	Swift Current	5.2	4.4	3.9	4.9	0.868
	Melfort	0.2	0.1	0.2	0.1	0.916
Canola grain	Saskatoon	9.7	10.2	8.0	7.8	0.369
	Lanigan	10.4	12.3	10.3	8.2	0.262
	Swift Current	8.0	7.2	5.7	7.6	0.502
	Melfort	17.2^{a}§	14.3^{ab}	12.0^{b}	12.7^{b}	0.045
Canola total	Saskatoon	12.3	14.0	11.1	11.1	0.363
	Lanigan	18.2	15.4	17.1	11.4	0.297
	Swift Current	13.2	11.6	10.2	12.5	0.789
	Melfort	16.1	14.5	12.1	12.9	0.414

† A-A-W-C is alfalfa-alfalfa-wheat-canola; RC-RC-W-C is red clover-red clover-wheat-canola; B-P-W-C is barley-pea-wheat-canola; B-FL-W-C is barley-flax-wheat-canola.

‡ Letters are assigned to treatments that have significant differences among them (P < 0.10).

§ Means with the different superscript letter in the same row are significantly different (P < 0.10).

¶ Standard errors are provided in the appendix.

Table 5.12. Gravimetric soil moisture contents (% water by weight) measured in fall 2013 in the soil profile after different crop rotations at four sites in Saskatchewan, Canada.

Sampling depth	Sites	Gravimetric soil moisture content				*P* value‡
		A-A-W-C†	RC-RC-W-C	B-P-W-C	B-FL-W-C	
		---------------------- (%) ----------------------				
0-15 cm	Saskatoon	15.7	17.8	16.8	16.5	0.391
	Lanigan	9.8	10.7	11.6	11.5	0.681
	Swift Current	18.9	25.6	23.2	20.4	0.388
	Melfort	26.8	26.3	26.6	29.6	0.429
15-30 cm	Saskatoon	21.7	20.2	21.0	19.3	0.300
	Lanigan	8.1	13.6	8.5	11.6	0.516
	Swift Current	20.5	21.3	23.1	22.3	0.924
	Melfort	21.8	22.3	22.8	22.3	0.836
30-60 cm	Saskatoon	21.4	25.6	22.9	26.0	0.737
	Lanigan	10.5	9.7	18.3	9.1	0.402
	Swift Current	12.6[b]§	18.0[a]	17.8[ab]	18.3[a]	0.056
	Melfort	21.7	21.6	23.1	23.2	0.371

† A-A-W-C is alfalfa-alfalfa-wheat-canola; RC-RC-W-C is red clover-red clover-wheat-canola; B-P-W-C is barley-pea-wheat-canola; B-FL-W-C is barley-flax-wheat-canola.
‡ Letters are assigned to treatments that have significant differences among them (P < 0.10).
§ Means with the different superscript letter in the same row are significantly different (P < 0.10).
¶ Standard errors are provided in the appendix.

5.5.2.5 Extractable soil P and P supply rate after canola growth (fall of 2013)

The modified Kelowna extractable P and P supply rate were measured in the fall 2013 after the canola harvest at the end of the four year rotation (Figs 5.5, 5.6). The soil P availability evaluation (modified Kelowna extractable soil P and resin membrane P supply rate) revealed no significant differences in modified Kelowna extractable P or P supply rate among different crop rotations at all four sites ($P > 0.10$) (Fig 5.5, 5.6). Greater grain yield and P removal after two years of forage legume rotations at Saskatoon, Lanigan and Melfort and the higher P uptake after two years of alfalfa at the Melfort site did not significantly alter soil available P as assessed by either modified Kelowna extractable P or the P supply rate. Among different sites, the Lanigan site had the highest modified Kelowna extractable P and P supply rate at both sampling depths (0-15 cm and 15-30 cm) relative to the other sites.

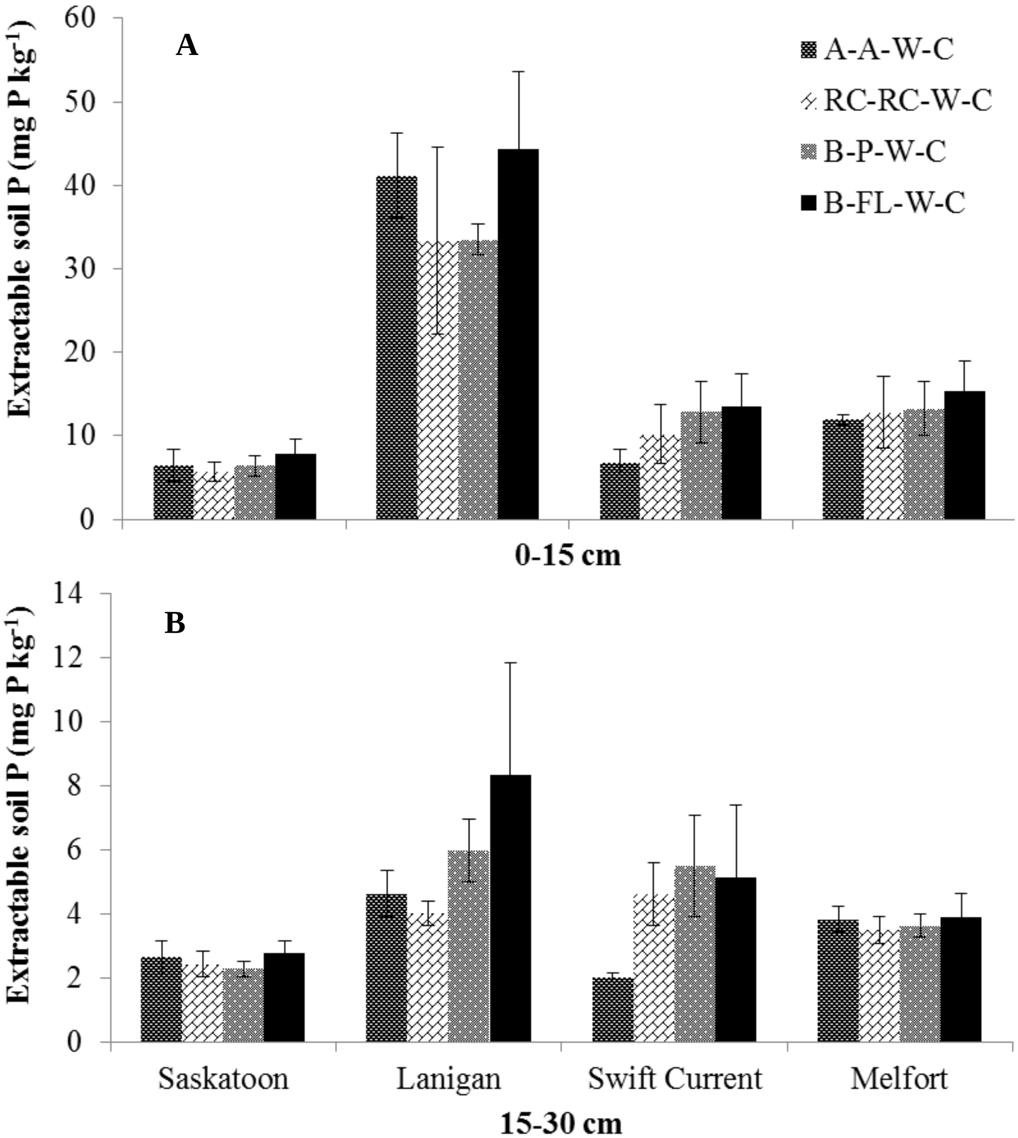

Fig. 5.5. The concentrations of soil extractable P measured in two depths: 0-30 cm (A) and 30-60 cm (B) in fall 2013 in four different crop rotations conducted over 2010-2013 at four sites in Saskatchewan, Canada. (A-A-W-C=alfalfa-alfalfa-wheat-canola; RC-RC-W-C=red clover-red clover-wheat-canola; B-P-W-C=barley-pea-wheat-canola; B-FL-W-C=barley-flax-wheat-canola). Error bars represent standard errors.

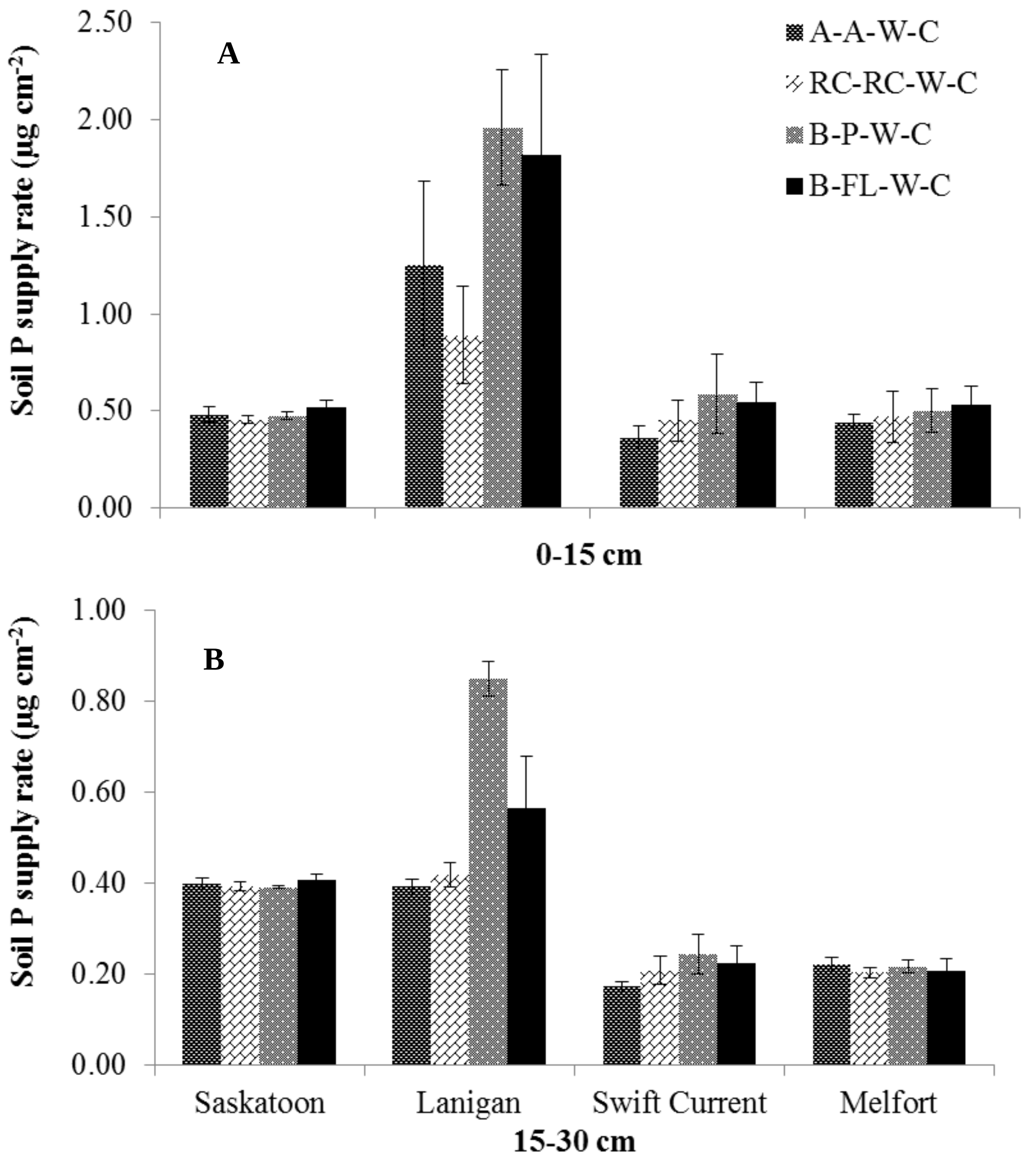

Fig. 5.6. Soil P supply rate measured in two depths: 0-30 cm (A) and 30-60 cm (B) in fall 2013 in four different crop rotations conducted over 2010-2013 at four sites in Saskatchewan, Canada. (A-A-W-C=alfalfa-alfalfa-wheat-canola; RC-RC-W-C=red clover-red clover-wheat-canola; B-P-W-C=barley-pea-wheat-canola; B-FL-W-C=barley-flax-wheat-canola). Error bars represent standard errors.

5.5.3 Crop P removal and P balance over a four-year rotational cycle

After four years of crop rotation, P balance (surplus or deficit) is calculated as the difference between total P added from external sources (fertilizer) during the rotational cycle and harvested P removed from the system in crop biomass (Table 5.13). Four years of cropping with the addition of only a small amount of P fertilizer added to each treatment at the start of the rotational cycle in 2010 (15 kg $P_2 0_5$ ha^{-1}) resulted in a continuous drain on the soil P pool at all locations. Crop rotations significantly affected crop total P removal and P balance at each site (P < 0.10). Among four rotation systems, crops in rotation one (alfalfa-alfalfa-wheat-canola) and rotation two (red clover-red clover-wheat-canola) removed greater P from the system and resulted in a more negative P balance (greater deficit) compared to crops in rotation three (barley-pea-wheat-canola) and rotation four (barley-flax-wheat-canola) at all locations (P < 0.10).

Changes in the modified Kelowna extractable P in the surface soil (0-15 cm) over the two years (2012 and 2013) following the two years of forage legumes versus annual crops are shown in Fig 5.7. Cropping with a very low addition of P fertilizer resulted in the depletion of available P in the top soil (0-15 cm) at the Saskatoon, Lanigan and Swift Current sites (Fig 5.7 A, B, C). In Saskatoon, soil available P decreased rapidly for all crop rotations from the spring of 2012 to the fall of 2013 without P fertilizer addition (Fig 5.7 A). At the Lanigan and Swift Current sites, soil available P also declined but the decline rate was slower when compared to the Saskatoon site (Fig 5.7C and D). At the Melfort site, soil available P diminished quickly from the spring 2012 to fall 2012, then unlike other sites, it increased from fall 2012 to fall 2013 (Fig 5.7B).

Table 5.13. Phosphorus balance from 2010 to 2013 in the four different crop rotations at the four sites in Saskatchewan, Canada.

Site	Treatment	Fertilizer P applied‡	P removed in biomass§	P balance¶
		----------------------	(kg ha^{-1})	------------------------
Saskatoon	A-A-W-C†	6.6	49.4[a]#	-42.8[b]
	RC-RC-W-C	6.6	43.2[a]	-36.6[b]
	B-P-W-C	6.6	22.1[b]	-15.5[a]
	B-FL-W-C	6.6	24.7[b]	-18.1[a]
Lanigan	A-A-W-C	6.6	54.5[a]	-47.9[d]
	RC-RC-W-C	6.6	48.3[b]	-41.7[c]
	B-P-W-C	6.6	22.9[c]	-16.4[b]
	B-FL-W-C	6.6	17.0[d]	-10.4[a]
Swift Current	A-A-W-C	6.6	27.5[a]	-21.0[b]
	RC-RC-W-C	6.6	24.4[ab]	-17.9[ab]
	B-P-W-C	6.6	17.4[b]	-10.9[a]
	B-FL-W-C	6.6	18.0[b]	-11.5[a]
Melfort	A-A-W-C	6.6	52.3[a]	-45.7[b]
	RC-RC-W-C	6.6	48.7[ab]	-42.2[ab]
	B-P-W-C	6.6	43.5[b]	-37.0[a]
	B-FL-W-C	6.6	42.4[b]	-35.8[a]

† A-A-W-C is alfalfa-alfalfa-wheat-canola; RC-RC-W-C is red clover-red clover-wheat-canola; B-P-W-C is barley-pea-wheat-canola; B-FL-W-C is barley-flax-wheat-canola.

‡ The same amount of fertilizer P (15 kg P_20_5 ha^{-1}) was applied to all plots at all four locations during the first year of the crop rotations (spring 2010). Thereafter no fertilizer P was applied to any plots at all locations.

§ The P removal is the harvested biomass uptake. In this experiment, the biomass uptake was all harvested biomass P uptake for forage legumes and the grain P uptake for the annual crops.

¶ P balance was calculated by subtracting the amount of applied P from the crop P removal.

Means with a different superscript letter in the same column are significantly different (P < 0.10).

†† Standard errors are provided in the appendix.

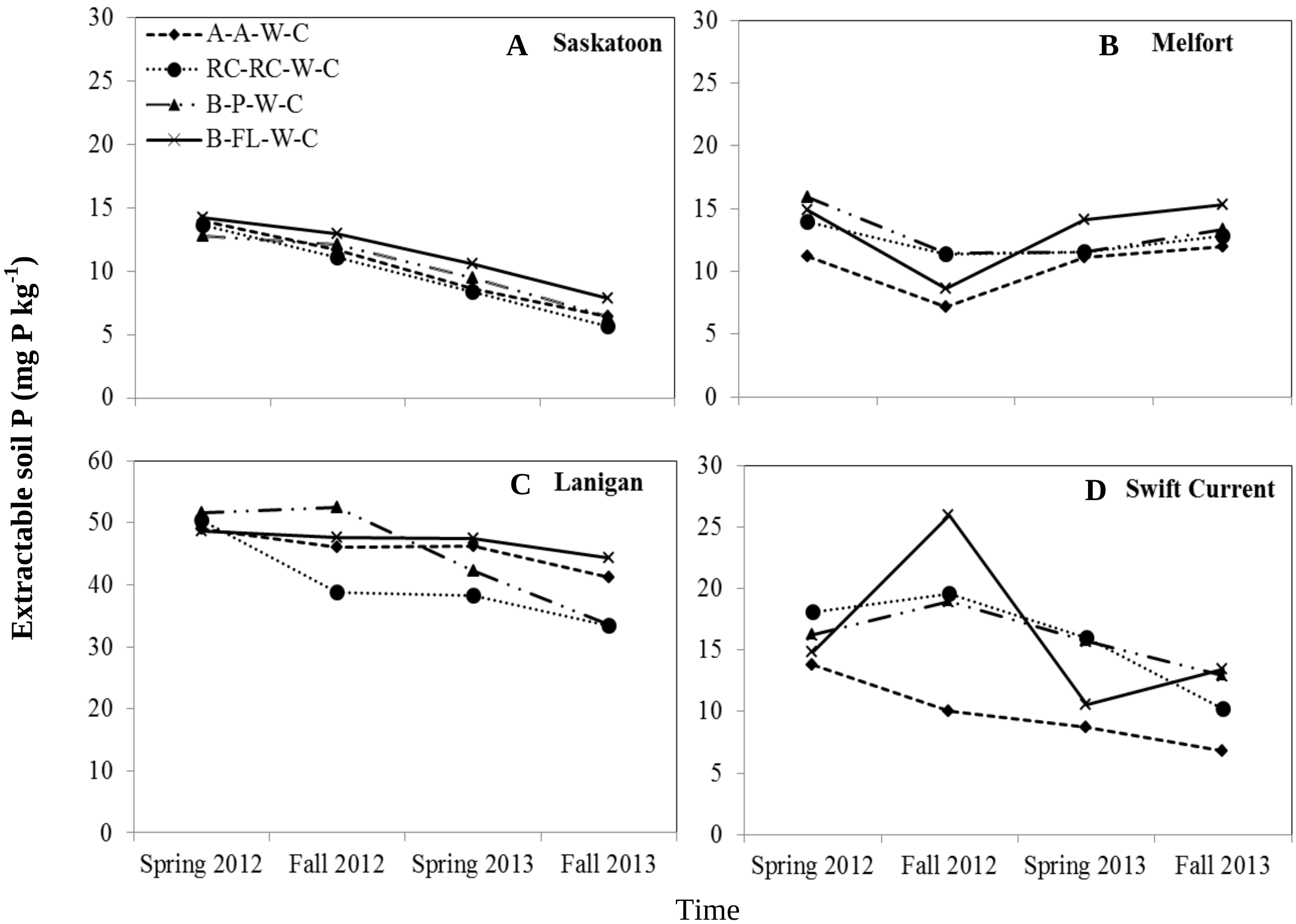

Fig. 5.7. Changes in extractable (modified Kelowna) soil P in the top soil (0-15 cm) over 2012 and 2013 in Saskatoon (A), Melfort (B), Lanigan (C) and Swift Current (D). (A-A-W-C is alfalfa-alfalfa-wheat-canola; RC-RC-W-C is red clover-red clover-wheat-canola; B-P-W-C is barley-pea-wheat-canola; B-FL-W-C is barley-flax-wheat-canola). Standard errors are provided in the appendix.

5.6 Discussion

5.6.1 Wheat and canola yield

In this experiment, wheat grain yields were generally greater following forage legume rotations relative to an annual non-legume rotation (barley-flax) at Saskatoon, Melfort and Lanigan (P < 0.10) (Table 5.5). Increased growth and biomass production of wheat after legumes was also observed in pot experiments conducted in Western Australia and Southern Australia (Nuruzzaman et al., 2005a,b and Hassan et al., 2012b) and in a field experiment conducted in Northern Nigeria (Horst et al., 2001). The increased wheat grain yield after forage legume rotations is likely attributed to an improvement of the soil N supply provided by N_2 fixation (McCown et al., 1988; Evans et al., 1991; Peoples et al., 1995). However, other rotational benefits of legume pre-crops, such as improved soil physical and biological characteristics, and less cereal diseases may also be equally important in enhanced cereal production (McCown et al., 1988; Lattif et al., 1992). It was reported in previous studies that legumes are able to mobilize P in excess of their own requirement and this extra P could be carried through and used by less P-efficient cereal crops that follow in rotation (Kamh et al., 1999; Horst et al., 2001; Nuruzzaman et al., 2005a,b; Hassan et al., 2012b).

In our experiment, improved P nutrition of legume crops (including alfalfa and red clover) might be another contributing factor for positive rotational effects of legumes on following wheat grain yield. This is demonstrated by the enhanced P uptake of wheat following two years of alfalfa and red clover rotation at Melfort and increased P uptake of wheat following two years of alfalfa rotation at Lanigan compared to wheat following a non-legume annual crop rotation (barley-flax). In addition, all the available P measurements (modified Kelowna extractable P and P supply rate) made in this research showed similar amounts of soil available P and P supply rate after different crop rotations despite significantly greater P removal by crops in the two years of forage legume rotations, and sometimes in the following wheat and canola crops as well. If forage legumes only contribute to soil N fertility and soil physical and biological conditions, P deficiency would be a limiting factor for wheat grain production on forage legume rotation plots. However, at three sites, wheat grain yield was higher after forage legume rotations, which indicate that improved soil N fertility, soil physical and biological conditions as well as P nutrition due to forage legume preceding in the rotation are all likely responsible for the positive rotational effect of forage legumes on following wheat grain yield at Saskatoon, Lanigan and Melfort. At Swift Current; however, wheat grain yield was negatively influenced by two years of alfalfa rotation, which could be explained by the greater soil moisture depletion through extensive root systems of alfalfa. It is likely that under limited soil moisture conditions (Brown soil zone at Swift Current), soil moisture depletion by the extensive root systems of alfalfa was responsible for the lower yield observed in the following crops (Astatke et al., 1995; Zenter et al., 2003).

Yield of canola grown after wheat was significantly increased with two years of red clover at Saskatoon and Lanigan and with two years of alfalfa at Melfort when compared to canola grain yield following non-legume: barley-flax rotation. This could be explained by factors like improved soil N fertility through biological N_2 fixation of legumes, breaking soil borne diseases, improving soil physical conditions and enhanced P solubility (Imai, 1991; Peoples et al., 1995; Nuruzzaman et al., 2005b) that persisted longer than just one growing season after the termination of the forage. Among those factors, improved soil N availability by forage legumes could be considered as the main reason for the positive rotational effects of forage legume crops on canola grain yield at Saskatoon, Lanigan and Melfort. At Swift Current, in 2013 there was no depression on canola yield after two years of alfalfa in 2010 and 2011, even though greater soil moisture depletion was evident. This suggests that the main contributing factor for the lack of change in canola grain yield at this site might be due to improved soil conditions following two years of alfalfa rotation.

5.6.2 Wheat and canola P concentrations and P uptake

Wheat straw and grain P concentrations were similar after different crop rotations at all locations ($P > 0.10$). The only exception was the lower wheat grain P concentration following barley-pea rotation at Swift Current ($P < 0.10$), which can be explained by P dilution in wheat grain as a result of the higher grain yield production of wheat following barley-pea rotation. The higher plant biomass production reflects stimulated photosynthesis and plant growth such that the P concentration was diluted in plant tissue.

Crop rotations significantly affected the canola straw and grain P concentrations at different locations ($P < 0.10$). The effect of crop rotations on canola grain P concentration was more pronounced than the canola straw P concentration. The differences between canola grain and straw P concentrations at various sites can be explained by the variations in canola biomass production. At the sites where canola biomass production (especially grain biomass) was higher, P was diluted in plant tissue; therefore P was less concentrated. On the other hand, at the sites where canola biomass production was lower, P was less diluted in the plant tissue due to the slow plant growth and less biomass production. For example, the canola grain P concentration was lower after two years of red clover rotation compared to other crop rotations at Saskatoon, where canola grain yield was higher following the same rotation compared to other rotations. At Swift Current and Melfort, canola grain P concentration was lower after two years of alfalfa rotation compared to other crop rotations due to P dilution through greater canola grain yield production.

5.6.3 Wheat and canola P uptake

The P uptake by wheat was significantly influenced by crop rotation at three sites but not at the Saskatoon site. At Melfort, wheat had the higher P uptake following forage legume rotations than following the non-legume barley-flax rotation. At Lanigan, wheat took up greater P after the red clover rotation compared to the wheat P uptake after barley-flax rotation ($P < 0.10$). The greater P uptake by wheat following forage legume rotations could be due to the generally improved conditions for wheat growth following the alfalfa and red clover rotations such as greater N availability as revealed in enhanced crop N uptake (data not shown) that would also contribute to increased demand for, and uptake of, P. It is evident that despite higher plant removal of P from soil in the forage legume rotations in the first two years (2010 and 2011), the soil can still supply additional amounts of P to match the enhanced N availability from the legume in rotation. At Swift Current, however, wheat P uptake was lowest after two years of alfalfa rotation compared to wheat P uptake following other crop rotations due to the limited crop biomass production resulting from soil moisture depletion. Overall, the wheat P uptake patterns at four sites consistently followed the typical crop biomass production patterns.

Unlike wheat P uptake, canola P uptake was not affected by crop rotations at all four sites. Even though forage legume rotations positively affected the biomass production of canola through enhanced soil conditions and nutrient availability, they did not cause any change in canola P uptake. The lack of differences in canola P uptake among different crop rotations could be explained by the variations in P concentrations in canola plant tissues (grain+straw) and the differences in canola biomass. Plant P uptake was determined by two factors: P concentrations in canola plant tissues (grain+straw) and the canola biomass (grain+straw) yield. There was a dilution in canola P concentration following forage legume rotations. When the P uptake was determined, the greater canola grain and straw yield following forage legume rotations were offset by lower straw and grain P concentrations in canola following the same rotations. As a result, a similar amount of P uptake was calculated for canola crops following the different crop rotations.

5.6.4 Soil moisture content

In this study, soil moisture content measured during each soil sampling period (fall 2012, spring 2013 and fall 2013) was not significantly influenced by different crop rotations with a few exceptions. The exceptions were the significantly lower soil moisture content measured after wheat harvest from the barley-flax rotation at 15-30 cm sampling depth and from the barley-pea rotation measured at 30-60 cm sampling depth at Lanigan in the fall of 2012 and the significantly lower soil moisture content measured at depth (30-60 cm) at the Swift Current site in the fall of 2013. At Swift Current, the lower soil moisture content measured after canola following two years of alfalfa could be explained by alfalfa's deep rooting system and prolonged use of soil moisture during first the two years of crop rotation period. In addition, in 2013 there was a yield increase in canola grain following alfalfa rotation relative to other crop rotations (although differences are not significant), thus it was considered that the enhanced canola growth following alfalfa rotation depleted more water to produce higher grain and total biomass. According to previous studies, forage legumes result in greater soil moisture consumption and depletion by the end of the season compared to annual small grain crops (Astatke et al., 1995; Huggins et al., 2001). Our results under soil moisture limited condition at Swift Current (Brown soil zone) were similar to those previous findings, except when the soil moisture conditions were not as limiting such as at Saskatoon, Lanigan and Melfort. At these three sites, no soil moisture depletion was found from all sampling depths at all sampling periods (fall 2012, spring and fall 2013) due to perennial forage legume rotations. Similar amounts of soil moisture measured at these three sites may reflect a reduced effect of perennial forage due to the short-term (only two years) period for growth. Also, significant surface soil moisture recharge through rains in the fall of 2012 and snowmelt in spring of 2013 at these sites could also contribute to recharge and mask differences in soil moisture depletion in the previous two years.

Under soil moisture limited conditions such as the Brown soil zone at Swift Current, alfalfa depleted soil moisture to a greater extent compared to the other crops. Thus, care must be taken when including perennial forage legumes like alfalfa into a short-term crop rotation for the benefits of soil N and P fertility when the soil moisture is limited. Further studies are warranted to examine the soil moisture depletion by various forage legumes and annual crops grown under different soil-climatic conditions in Saskatchewan.

5.6.5 Extractable soil P and P supply rate

Comparing available P levels in the soil at different sampling periods (fall 2012, spring and fall 2013) after wheat and canola harvest, the available P content and P supply rates measured at each soil sampling time were similar among rotations at all the sites. This is despite the higher P uptake and crop P removal in the forage legume treatments in previous years (2010 and 2011) at all sites and the higher P removal of wheat and canola following forage legume rotations at the Lanigan and Melfort sites. Lack of evidence of soil P depletion in the rotations with two years of forage legumes suggests that these soils can maintain available P in the face of greater P depletion by the legumes themselves as well as enhanced P removal of following annual crops, at least in the short-term.

The ability of alfalfa and red clover in sustaining soil available P in the short-term even with the greater P removal by these crops when hayed and the following cereal crops when the grain biomass was harvested could be explained by the following reasons. First, the recycling of P through the extensive root system of these perennial forage legumes (Daroub et al., 2001). According to previous studies, a considerable amount of P was added to the soil through alfalfa root biomass turnover (Lory et al., 1992; Blumenthal and Russele, 1996; Daroub et al., 2001). In a field experiment conducted in Michigan, Daroub et al. (2001) estimated that alfalfa root could contribute 11.44 kg P ha^{-1} per year when the alfalfa root residue contains 0.2% P content. Alamgir et al. (2012) reported that soil P can be mobilized during legume residue (including root and shoot residue) decomposition as legume residues contain more P, have lower C:P ratios than cereal crops (Nuruzzaman et al., 2005a,b) and favour net P mobilization. The main advantage of legume root residues is the addition of organic matter to the soil (Rasse et al., 2000) which can influence P availability mainly through the accumulation of organic P fractions (Horst et al., 2001). The organic P fractions that build up have special importance for the maintenance of soil P availability (Sharpley, 1985; Seeling and Zasoski, 1993; Oberson et al., 1999) because the slower decomposition and release of P from organic matter prevents rapid fixation of Pi

(Friessen et al., 1997), and better matches the P requirements of the subsequently grown crops. Another explanation for the ability of alfalfa and red-clover to sustain soil available P in the short-term could be due to the improved P acquisition by wheat and canola which resulted from the robust root systems of these crops and modification of soil biological properties by previous forage legume crops (Horst et al., 2001). Wheat and canola grown on forage legume plots might develope healthier root systems which enable them to explore larger soil volume and take up greater amounts of soil P relative to wheat and canola crops grown on non-legume plots. In modification of soil biological properties context, mycorrhizal infection rate might be of particular importance. Even though the mycorhizal infection rate of plants or AM fungi populations were not determined in our experiment, Horst et al. (2001) showed in a field study that mycorhizal infection was significantly enhanced after most legume crops compared to maize after maize. The last possible reason for the soil available P being maintained by alfalfa and red-clover could be the P transformation between different P pools. It is well known that soil labile P pools are quite well buffered via equilibrium with more stable P forms (McKenzie et al., 1992; Nuruzzaman et al., 2005a,b; Vu et al., 2010 a,b), and that it can take a few years of a change in management practice to produce significant changes in the labile pool amounts.

When comparing the available P levels of different sites at different sampling periods (fall 2012, spring and fall 2013), the Lanigan site had the highest extractable P and P supply rate in the surface soil (0-15 cm) (Fig 5.3, 5.4, 5.5 and 5.6A) compared to other sites during each sampling periods due to its history of manure application prior to the establishment of the experiment in 2010.

5.6.6 Crop P removal, soil P dynamics and P balance over a four year rotational cycle

In this experiment, soil available P (modified Kelowna extractable P) declined gradually due to the low P addition and greater crop P removal over the four years of the crop rotation periods at Saskatoon, Lanigan and Swift Current sites. However, at Melfort, it decreased markedly, followed by an increase. The depletion of soil available with low or no P inputs was also revealed in previous studies conducted in India and Missouri (Reddy et al., 1999; Motavalli and Miles et al., 2002). At Melfort, the greater P depletion at the beginning of the crop rotation was mainly due to greater crop P removal. The increased available P level from the spring of

2013 to the fall of 2013 could be due to combination of the following factors. First, a replenishment of available P and other less available soil P fractions, especially from organic P pool which is large in this soil. Secondly, the P addition through crop residue turnover due to the higher available soil moisture content at the site. Thirdly, it could be possible that the Black Chernozemic soil has lower P sorption capacity relative to other soil types, which could decrease Pi fixation and contribute to the increase in the available P level from 2012 to 2013 at this site.

A four-year continuous cropping cycle with a very low P addition reduced soil P fertility resulted in a negative P balance (deficit) on all crop rotation treatments at all four locations. At Saskatoon and Lanigan, soil P deficit was greater in alfalfa-alfalfa-wheat-canola and red clover-red-clover-wheat-canola compared to annual legume and non-legume crop rotations: barley-pea-wheat canola, barley-flax-wheat-canola. This was due to the significantly greater P removal through enhanced biomass production by crops in rotation. During the first two years of crop rotations (especially the second year) forage legumes produced significantly greater biomass relative to annual crops. Also, wheat and canola following forage legume produced significantly higher biomass during the last two years of the crop rotations.

In this study, soil P fertility was depleted every year by the crop biomass harvest without adequate P replenishment through fertilizer addition or manuring. Thus, in agricultural production, it is critical to apply sufficient P to match the crop P removal over time in order to preserve the soil P fertility.

5.7 Conclusion

In this experiment, improved wheat and canola grain yields following two years of alfalfa and red clover are attributed to the well-known impacts on enhanced soil N supply and improved soil physical conditions, but also likely reflect a positive effect on P availability. Maintenance of soil available P levels and meeting crop demand for P uptake in the face of greater removal of P in the rotations containing forage legumes indicates a positive influence of forage legumes in short-term rotation on soil P availability. However, the relative importance of each factor is unknown at this time. In an effort to clarify the relative roles played by P availability and N fixation in the increased biomass production, we are proposing that we provide the legumes with sufficient N fertilizers to control legume N_2 fixing ability. In doing this, we would clarify the relative role of P in increasing crop biomass production. At Swift Current, alfalfa depleted soil moisture to a greater extent compared to other annual crops and resulted in the yield depression of following wheat. Therefore, care must be taken when including perennial forage legumes like alfalfa into a short-term crop rotation for the benefits of soil N and P fertility when the soil moisture is limited. Further studies are warranted to examine the soil moisture depletion by various forage legumes and annual crops grown under different soil-climatic conditions in Saskatchewan.

6. OVERALL SYNTHESIS AND CONCLUSIONS

6.1 Summary of findings

Phosphorus is an essential macronutrient for all living organisms due to its important role in energy metabolism and the biosynthesis of nucleic acid and membranes (Brandy and Weil 2002). In many soils worldwide, P deficiency is a major nutritional constraint because of its low availability in the soil. Growing P efficient legume crops in rotation which can access different components of the soil P reserve can be utilized as an efficient strategy to maximize the use of accumulated soil P reserves. Thus, including two years of alfalfa and red clover into a crop rotation can be a valuable approach in improving soil P availability and the yield and P uptake of following crops in rotation.

The first objective of this research work was to evaluate the impact of including a two-year period of forage legumes (alfalfa and red clover) along with annual crops (barley-pea and barley-flax) on soil P forms, amounts and availability and uptake of P by the crops in rotation. During the first two years of this crop rotation period, two years of alfalfa and red clover took up and removed greater amounts of soil P relative to barley-pea and barley-flax at Saskatoon, Lanigan and Swift Current sites, due to substantial biomass production, especially in the second year. After two years of forage legume and annual crop rotations, similar concentrations of available P and P supply rate were measured after the four rotations at all four locations, despite the greater P uptake and P removal by forage legumes compared to annual crops in 2010 and 2011. Two years of forage legume versus annual crop generally did not have a significant impact on the distribution of soil P between various Pi and Po pools. Grain yields of wheat and canola crops grown following two years of forage legume and annual crop rotations were positively affected by alfalfa and red-clover rotations at Saskatoon, Lanigan and Melfort. But at Swift current, wheat grain yield decreased following two years of alfalfa rotation due to the greater soil moisture depletion by two years of alfalfa rotation. Wheat P uptake was significantly improved after two years of red-clover rotation at Lanigan, Swift Current and Melfort, whereas canola P uptake in year four was not affected by different crop rotations at all locations. The amount of soil available P and P supply rate measured after wheat and canola harvest were not affected by different crop rotations at all sites despite the greater P removal by forage legumes during the first two years of the four year crop rotation period at all sites and the enhanced P removal by wheat and canola crops following forage legume rotations at Lanigan and Melfort. These results suggest that forage legumes are able to maintain soil P fertility in the face of greater P removal by crops in rotation, at least in the short-term. Two years of crop rotation period might be too short a time period to produce significant changes in the various chemically extracted P fractions. The finding of lack of a significant effect of rotation treatment on the amounts of soil available P does not rule out the possibility that there is an impact, but variability prevented its detection. Including forage legumes in rotation has a beneficial effect on the grain yield of subsequently grown wheat and canola crops at Saskatoon, Lanigan and Melfort.

The second objective of this research work was to evaluate the effect of the different crop rotations over the four years on soil P dynamics and P balance. Four years of continuous cropping with a minimal addition of fertilizer P resulted in a significant reduction of soil P fertility over time at all locations according to the calculated P balance. Cropping with a very low addition of P fertilizer also depleted the available P in the top soil (0-15 cm) from 2012 to 2013 at the Saskatoon, Lanigan and Swift Current sites. Four years of continuous cropping with minimum addition of fertilizer P depleted soil P fertility over time at all locations even though the available P level between different crop rotation treatments were not significant at each sampling period. Therefore, it is important to add P in the form of fertilizer P or manure to sustain soil P fertility and maintain high crop yield over time.

6.3 Future research directions

This MSc research work addressed some important questions regarding the effect of forage legumes versus annual crops in short-term rotation on soil P forms, amounts and availability. However, there are some research gaps recognized that deserve further attention.

6.3.1 Phosphorus solubilizing mechanisms in the rhizosphere of legumes

The P solubilizing mechanisms that legumes may employ are important in determining P efficiency in legume crops and the influence of legumes on P uptake of subsequent crops. Further research needs to be conducted to elucidate the specific nature of these mechanism, focusing on root morphology, rhizosphere biological and chemical characteristics, and root exudates from perennial forage legumes, perennial grass species and annual crops in order to better define the nature of the intrinsic ability of forage legumes to mobilize P and enhance P uptake in comparison to annual crops. Such information would also be valuable in crop development research to assist breeders in selecting for desirable traits.

6.3.2 Crop rotation period

Further studies may consider the effects of several cycles of this rotation over a number of years. This study evaluated the effect of a single cycle of crop rotation using bulk soil samples. It may be useful to also do the P fractionation using rhizosphere soil, if such soil could be separated out for measurement easily and consistently. Sequential P fractionation was used in this study and many other studies to evaluate the effect of cropping systems and fertilizer application on soil P fractions and to determine the static P content, which makes it difficult to elucidate the actual P transformation mechanisms in detail. Using both the sequential extraction method and a radioisotope ^{32}P tracer technique (Vu et al., 2010) may be a more rewarding approach to follow the P transformations between the different P pools in soil over the short-term.

6.3.3 Forage legumes and soil moisture content

Further studies are warranted to examine the nature of soil moisture depletion by various forage legumes and annual crops grown under different soil-climatic conditions in Saskatchewan, and its relationship to P cycling. Previous studies showed that forage legumes deplete soil moisture to a greater extent in comparison to annual crops (Astatke et al., 1995; Zenter et al., 2003). In our study, we found that alfalfa depleted soil moisture to a greater extent compared to other annual crops resulting in a depression of yield of the following wheat crop only at the Swift Current site. More site-years of data are needed under different soil and climatic conditions to evaluate feasibility of this practice in the southern semi-arid prairie region.

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8. APPENDIX

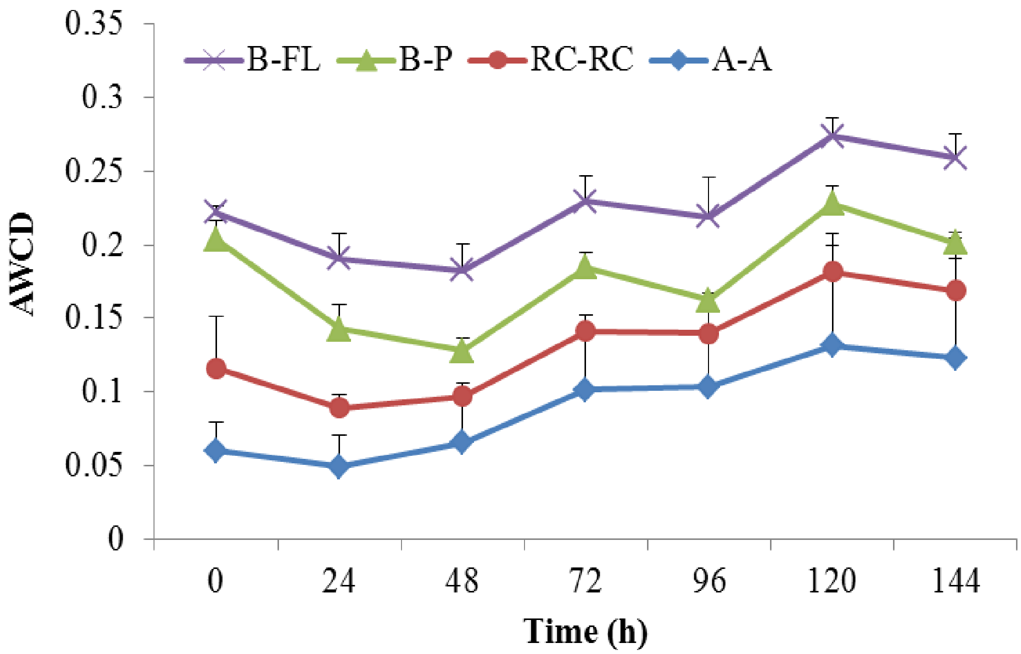

Fig. A1. Biolog EcoPlate™ average well color development (AWCD) over the 7 days incubation period displayed for four different crop rotations.

Table A1. Phosphorus fertilizer recommendations for barley and spring wheat on a medium to fine textured soil with a neutral pH, based on the Kelowna soil test method. Recommendations are given for each soil zone at medium (50%) seedbed soil moisture condition at seeding (Adapted from Phosphorus Fertilizer Application in Crop Production, 2013).

Soil test P (kg ha^{-1})	Brown	Dark Brown	Thin Black	Black	Gray Wooded
	---------------------------- P_2O_5 (kg ha^{-1}) ----------------------------				
0-11	39	45	51	51	51
11-22	34	39	45	45	45
22-33	28	33	39	39	39
33-45	22	28	34	34	34
45-56	17	23	28	28	28
56-67	17	17	23	23	23
67-78	17	17	23	17	17
78-90	17	17	17	17	17
90-101	0	0	0	0	0
>90	0	0	0	0	0

Table A2. Soil test rating for plant available P (Kelowna P) level (Adapted from Phosphorus Fertilizer Application in Crop Production, 2013).

Soil test level rating	Phosphorus (P) (kg ha^{-1})
Very low	0-23
Low	23-39
Medium	39-56
High	56-90
Very high	>90

Table A3. Standard error for forage yield of alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) and biomass of barley (*Hordeum vulgare* L.), peas (*Pisum sativum* L.) and flax (*Linum usitatissimum* L.) grown at four locations for two years in Saskatchewan, Canada. (Corresponding to table 3.5)

Year	Site	Alfalfa	Red clover	Barley1	Barley2
2010	Saskatoon	380	661	769	1188
	Lanigan	422	290	929	821
	Swift Current	357	120	280	203
	Melfort	222	420	928	362
		Alfalfa	Red clover	Peas	Flax
2011	Saskatoon	538	286	1518	433
	Lanigan	333	925	929	821
	Swift Current	273	953	161	144
	Melfort	414	211	172	233

Table A4. Standard error for the P uptake by alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) and total P uptake (grain+straw) by barley (*Hordeum vulgare* L.), peas (*Pisum sativum* L.) and flax (*Linum usitatissimum* L.) grown at four locations for two years in Saskatchewan, Canada. (Corresponding to table 3.6)

Year	Sites	Alfalfa	Red clover	Barley1	Barley2
2010	Saskatoon	0.78	1.02	1.20	0.91
	Lanigan	0.92	0.68	1.51	1.94
	Swift Current	0.47	0.31	1.29	1.40
	Melfort	0.49	0.69	0.87	2.11
		Alfalfa	Red clover	Peas	Flax
2011	Saskatoon	1.77	0.88	1.59	0.89
	Lanigan	0.83	1.02	1.19	1.25
	Swift Current	1.16	1.75	0.18	0.15
	Melfort	0.98	0.57	1.06	1.09

Table A5. Standard error for the P removals by alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), barley (*Hordeum vulgare* L.), peas (*Pisum sativum* L.) and flax (*Linum usitatissimum* L.) grown at four locations for two years in Saskatchewan, Canada. (Corresponding to table 3.7)

Year	Sites	Alfalfa	Red clover	Barley1	Barley2
2010	Saskatoon	0.78	1.02	0.09	0.17
	Lanigan	0.92	0.68	1.51	1.94
	Swift Current	0.47	0.31	0.70	0.68
	Melfort	0.49	0.69	0.58	0.55
		Alfalfa	Red clover	Peas	Flax
2011	Saskatoon	1.77	0.88	1.27	0.93
	Lanigan	0.83	1.02	0.15	0.15
	Swift Current	1.16	1.75	0.03	0.03
	Melfort	0.98	0.57	1.02	0.99
		A-A	RC-RC	B-P	B-FL
2010+2011	Saskatoon	2.31	1.74	1.25	0.91
	Lanigan	1.25	1.04	1.60	1.96
	Swift Current	0.76	2.05	0.69	0.67
	Melfort	1.30	1.35	1.36	1.40

† A-A is alfalfa-alfalfa; RC-RC is red clover-red clover; B-P is barley-pea and B-FL is barley-flax.

Table A6. Standard error for gravimetric soil moisture contents (% water by weight) in the soil profile after two years of forage legume and annual crop rotations at four sites in Saskatchewan, Canada. (Corresponding to table 4.2)

Sampling depth	Sites	A-A†	RC-RC	B-P	B-FL
0-15 cm	Saskatoon	1.19	2.42	3.72	1.41
	Lanigan	2.18	0.98	1.36	2.23
	Swift Current	1.43	2.00	1.62	0.77
	Melfort	0.90	0.30	0.37	0.78
15-30 cm	Saskatoon	2.36	2.57	2.52	1.49
	Lanigan	1.16	1.80	1.10	2.30
	Swift Current	2.04	1.61	2.98	2.77
	Melfort	2.42	0.44	1.35	4.26
30-60 cm	Saskatoon	0.83	1.31	1.50	1.88
	Lanigan	1.63	2.63	1.87	1.32
	Swift Current	3.01	3.77	2.90	2.00
	Melfort	2.76	0.84	5.19	0.91

† A-A is alfalfa-alfalfa; RC-RC is red clover-red clover; B-P is barley-pea and B-FL is barley-flax.

Table A7. Standard error for distribution of inorganic phosphorus (Pi) and organic phosphorus (Po) fractions (mg P kg^{-1}) in soils after two years of forage legume and annual crop rotations at four sites in Saskatchewan, Canada. (Corresponding to table 4.3)

Sites	P fractions	A-A†	RC-RC	B-P	B-FL
Saskatoon	Resin-Pi	4.65	5.87	1.75	5.12
	$NaHCO_3$-Pi	3.46	3.91	3.79	9.40
	$NaHCO_3$-Po	2.31	1.66	3.85	2.23
	NaOH-Pi	6.05	10.67	9.89	9.54
	NaOH-Po	7.95	20.66	10.50	27.16
	HCl-Pt	42.61	18.59	9.65	34.01
	Residual-Pt	10.13	20.46	10.17	24.26
Lanigan	Resin-Pi	10.10	10.33	8.21	7.25
	$NaHCO_3$-Pi	8.35	28.39	29.91	18.45
	$NaHCO_3$-Po	4.63	9.95	4.35	6.09
	NaOH-Pi	12.86	8.06	50.94	31.09
	NaOH-Po	85.94	58.75	57.00	64.25
	HCl-Pt	68.89	31.66	34.95	21.79
	Residual-Pt	13.56	6.22	17.70	18.49
Swift Current	Resin-Pi	4.75	11.79	9.17	4.25
	$NaHCO_3$-Pi	5.45	8.45	7.08	6.65
	$NaHCO_3$-Po	4.98	7.83	2.11	10.19
	NaOH-Pi	13.25	20.66	19.56	13.52
	NaOH-Po	18.79	38.98	7.40	26.37
	HCl-Pt	53.17	35.27	38.78	23.64
	Residual-Pt	25.24	42.02	13.92	36.37
Melfort	Resin-Pi	6.24	9.55	6.69	2.71
	$NaHCO_3$-Pi	4.64	7.62	6.98	7.61
	$NaHCO_3$-Po	4.26	2.70	5.18	1.80
	NaOH-Pi	15.87	22.27	23.62	10.03
	NaOH-Po	83.95	64.81	27.52	41.91
	HCl-Pt	7.44	68.90	18.48	13.45
	Residual-Pt	13.79	13.92	35.98	55.60

† A-A is alfalfa-alfalfa; RC-RC is red clover-red clover; B-P is barley-pea and B-FL is barley-flax.

Table A8. Standard error for wheat grain and biomass yield at four sites in Saskatchewan, Canada. (Corresponding to table 5.5)

	Sites	A-A-W†	RC-RC-W	B-P-W	B-FL-W
Wheat grain	Saskatoon	178	209	211	164
	Lanigan	82	187	29	87
	Swift Current	197	138	83	114
	Melfort	16	75	165	53
Total biomass	Saskatoon	195	203	161	116
	Lanigan	676	89	1070	826
	Swift Current	187	177	115	39
	Melfort	457	263	184	324

† A-A-W is alfalfa-alfalfa-wheat; RC-RC-W is red clover-red clover-wheat; B-P-W is barley-pea-wheat; B-FL-W is barley-flax-wheat.

Table A9. Standard error for P concentration in wheat straw and grain at four sites in Saskatchewan, Canada. (Corresponding to table 5.6)

	Sites	A-A-W†	RC-RC-W	B-P-W	B-FL-W
Wheat straw	Saskatoon	0.01	0.13	0.26	0.16
	Lanigan	0.08	0.04	0.06	0.07
	Swift Current	0.10	0.13	0.03	0.03
	Melfort	0.10	0.03	0.06	0.06
Wheat grain	Saskatoon	0.03	0.70	0.14	0.10
	Lanigan	0.18	0.09	0.16	0.24
	Swift Current	0.26	0.16	0.19	0.14
	Melfort	0.21	0.48	0.18	0.33

† A-A-W is alfalfa-alfalfa-wheat; RC-RC-W is red clover-red clover-wheat; B-P-W is barley-pea-wheat; B-FL-W is barley-flax-wheat.

Table A10. Standard error for wheat straw, grain and total P uptake at four sites in Saskatchewan, Canada. (Corresponding to table 5.7)

	Sites	A-A-W†	RC-RC-W	B-P-W	B-FL-W
Wheat straw	Saskatoon	0.36	0.97	2.28	1.40
	Lanigan	1.01	0.37	0.85	0.69
	Swift Current	0.04	0.03	0.01	0.01
	Melfort	0.39	0.30	0.38	0.62
Wheat grain	Saskatoon	0.64	2.03	0.59	0.56
	Lanigan	0.64	0.70	0.34	0.65
	Swift Current	0.84	0.71	0.33	0.42
	Melfort	0.75	1.77	1.10	1.02
Wheat total	Saskatoon	0.31	2.64	1.69	1.19
	Lanigan	1.59	0.44	0.81	0.61
	Swift Current	0.52	0.74	0.34	0.57
	Melfort	0.44	1.56	0.76	0.77

† A-A-W is alfalfa-alfalfa-wheat; RC-RC-W is red clover-red clover-wheat; B-P-W is barley-pea-wheat; B-FL-W is barley-flax-wheat.

Table A11. Standard error for gravimetric soil moisture contents (% water by weight) measured in fall 2012 in the soil profile after different crop rotations at three sites in Saskatchewan, Canada. (Corresponding to table 5.8)

Sampling depth	Sites	A-A-W†	RC-RC-W	B-P-W	B-FL-W
0-15 cm	Saskatoon	1.24	0.39	1.14	1.18
	Lanigan	4.14	4.90	0.34	0.75
	Melfort	0.88	1.31	1.55	0.35
15-30 cm	Saskatoon	3.10	4.02	1.50	0.62
	Lanigan	0.73	1.29	1.20	0.14
	Melfort	0.65	1.52	1.59	0.61
30-60 cm	Saskatoon	4.00	4.08	5.52	5.11
	Lanigan	1.06	3.49	1.52	4.28
	Melfort	0.36	0.94	1.04	1.43

† A-A-W is alfalfa-alfalfa-wheat; RC-RC-W is red clover-red clover-wheat; B-P-W is barley-pea-wheat; B-FL-W is barley-flax-wheat.

Table A12. Standard error for canola grain and biomass yields at four sites in Saskatchewan, Canada. (Corresponding to table 5.9)

	Sites	A-A-W-C†	RC-RC-W-C	B-P-W-C	B-FL-W-C
Canola grain	Saskatoon	122	196	186	321
	Lanigan	111	312	173	200
	Swift Current	216	176	99	112
	Melfort	37	186	378	206
Canola straw	Saskatoon	279	631	399	397
	Lanigan	629	1108	1610	1523
	Swift Current	634	780	1041	734
	Melfort	473	222	299	232

† A-A-W-C is alfalfa-alfalfa-wheat-canola; RC-RC-W-C is red clover-red clover-wheat-canola; B-P-W-C is barley-pea-wheat-canola; B-FL-W-C is barley-flax-wheat-canola.

Table A13. Standard error for P concentration in canola straw and grain at four sites in Saskatchewan, Canada. (Corresponding to table 5.10)

	Sites	A-A-W-C†	RC-RC-W-C	B-P-W-C	B-FL-W-C
Canoal straw	Saskatoon	0.05	0.06	0.07	0.02
	Lanigan	1.81	0.09	1.27	0.13
	Swift Current	0.14	0.11	0.08	0.15
	Melfort	0.04	0.04	0.06	0.11
Canola grain	Saskatoon	0.20	0.11	0.24	0.25
	Lanigan	0.13	0.11	0.11	0.33
	Swift Current	0.56	0.32	0.14	0.12
	Melfort	0.27	0.28	0.25	0.25

† A-A-W-C is alfalfa-alfalfa-wheat-canola; RC-RC-W-C is red clover-red clover-wheat-canola; B-P-W-C is barley-pea-wheat-canola; B-FL-W-C is barley-flax-wheat-canola.

Table A14. Standard error for canola straw, grain and total P uptake at four sites in Saskatchewan, Canada. (Corresponding to table 5.11)

	Sites	A-A-W†	RC-RC-W	B-P-W	B-FL-W
Canola straw	Saskatoon	0.33	0.72	0.37	0.30
	Lanigan	4.62	1.02	2.29	0.96
	Swift Current	1.11	1.21	0.64	1.02
	Melfort	0.02	0.06	0.08	0.03
Canola grain	Saskatoon	0.85	1.08	0.64	1.59
	Lanigan	0.87	1.84	1.11	1.33
	Swift Current	1.17	1.58	0.81	0.68
	Melfort	1.02	1.81	2.52	1.64
Canola total	Saskatoon	1.09	1.66	0.79	1.48
	Lanigan	4.50	1.16	2.41	1.32
	Swift Current	1.90	2.68	1.28	1.66
	Melfort	0.94	1.80	2.47	1.63

† A-A-W-C is alfalfa-alfalfa-wheat-canola; RC-RC-W-C is red clover-red clover-wheat-canola; B-P-W-C is barley-pea-wheat-canola; B-FL-W-C is barley-flax-wheat-canola.

Table A15. Standard error for gravimetric soil moisture contents (% water by weight) measured in fall 2013 in the soil profile after different crop rotations at four sites in Saskatchewan, Canada. (Corresponding to table 5.12)

Sampling depth	Sites	A-A-W-C†	RC-RC-W-C	B-P-W-C	B-FL-W-C
0-15 cm	Saskatoon	1.04	0.65	0.47	0.97
	Lanigan	0.42	0.60	1.20	1.72
	Swift Current	0.28	5.28	1.65	1.31
	Melfort	0.67	0.77	0.94	2.83
15-30 cm	Saskatoon	1.21	1.03	0.45	0.71
	Lanigan	0.59	3.89	0.76	3.98
	Swift Current	5.54	1.01	1.35	0.88
	Melfort	1.07	0.60	1.48	0.81
30-60 cm	Saskatoon	1.14	5.91	1.31	5.12
	Lanigan	1.17	2.77	0.66	2.07
	Swift Current	2.04	1.51	1.50	1.35
	Melfort	0.94	0.89	0.74	1.07

† A-A-W-C is alfalfa-alfalfa-wheat-canola; RC-RC-W-C is red clover-red clover-wheat-canola; B-P-W-C is barley-pea-wheat-canola; B-FL-W-C is barley-flax-wheat-canola.

Table A16. Standard error for phosphorus balance from 2010 to 2013 in the four different crop rotations at the four sites in Saskatchewan, Canada. (Corresponding to table 5.13)

Sites	Treatment	P balance
Saskatoon	A-A-W-C†	3.51
	RC-RC-W-C	3.72
	B-P-W-C	1.10
	B-FL-W-C	2.53
Lanigan	A-A-W-C	1.10
	RC-RC-W-C	1.60
	B-P-W-C	1.43
	B-FL-W-C	0.98
Swift Current	A-A-W-C	2.37
	RC-RC-W-C	4.31
	B-P-W-C	1.64
	B-FL-W-C	1.64
Melfort	A-A-W-C	1.69
	RC-RC-W-C	3.48
	B-P-W-C	5.66
	B-FL-W-C	1.19

† A-A-W-C is alfalfa-alfalfa-wheat-canola; RC-RC-W-C is red clover-red clover-wheat-canola; B-P-W-C is barley-pea-wheat-canola; B-FL-W-C is barley-flax-wheat-canola.

Table A17. Standard error for the changes in extractable (modified Kelowna) soil P in the top soil (0-15 cm) over 2012 and 2013 in Saskatoon, Lanigan, Swift Current and Melfort (corresponding to Fig5.7).

Site	Treatments	Spring 2012	Fall 2012	Spring 2013	Fall 2013
Saskatoon	A-A-W-C†	1.81	1.67	2.13	1.88
	RC-RC-W-C	2.22	1.24	1.59	1.10
	B-P-W-C	0.90	1.67	1.17	1.19
	B-FL-W-C	1.38	1.55	0.83	1.72
Lanigan	A-A-W-C	4.98	8.27	5.25	5.08
	RC-RC-W-C	4.81	6.37	4.04	11.22
	B-P-W-C	7.52	7.35	2.89	1.79
	B-FL-W-C	4.83	4.00	3.79	9.28
Swift Current	A-A-W-C	3.45	2.01	1.94	1.51
	RC-RC-W-C	4.83	5.63	4.28	3.51
	B-P-W-C	4.71	5.86	4.56	3.68
	B-FL-W-C	1.66	7.49	4.06	3.97
Melfort	A-A-W-C	1.21	2.63	1.68	0.59
	RC-RC-W-C	2.50	5.55	3.55	4.23
	B-P-W-C	3.54	4.95	2.64	3.19
	B-FL-W-C	0.41	2.68	2.19	3.69

† A-A-W-C is alfalfa-alfalfa-wheat-canola; RC-RC-W-C is red clover-red clover-wheat-canola; B-P-W-C is barley-pea-wheat-canola; B-FL-W-C is barley-flax-wheat-canola.