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Yield and Uptake of Phosphorus by Wheat and Canola Grown after Two Years of Forage Legume and Annual Crops

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Abstract

Annual legumes have been shown to enhance the growth and phosphorus (P) uptake by following rotational crops. However, there is lack of information on the effect of perennial forage legumes included in rotation for a short duration on yield and P uptake of crops like wheat (*Triticum aestivum* L.) and canola (*Brassica napus* L.) grown after the forage legume. A field study was conducted in four soil zones of Saskatchewan, Canada to assess: 1) the effect of two years of forage legume versus annual cereal, oilseed and grain legume on yield and P uptake of wheat and canola grown in the two subsequent years and 2) the effect of the complete four-year rotation on soil P dynamics and P balance. Four different crop sequences (alfalfa-alfalfa, red clover-red clover, barley-pea and barley-flax) employed over the first two years of crop rotation were compared as treatments followed by wheat and canola. Wheat grain yield was improved 32% - 60% by alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) rotations at three of the four sites ($P = 0.008$, $P = 0.001$, $P < 0.0001$) compared to annual grains, while grain P uptake was enhanced 38% - 43% by red clover and alfalfa rotation at two sites ($P = 0.013$, $P = 0.033$). In the following year, positive yield benefits (55% - 64%) of having two years of alfalfa and red clover were observed at three sites. Four years of continuous cropping with a limited addition of fertilizer P resulted in a negative soil P balance and significant depletion of soil P fertility at all locations.

Keywords

Yield, Phosphorus Uptake, Wheat, Canola, Forage Legume, Crop Rotations

1. Introduction

Phosphorus (P) is an essential macronutrient for plant growth and crop production due to its important role in energy metabolism and biosynthesis of nucleic acids and cell membranes [1] [2]. 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 [3] [4]. Much of the P in soil is not immediately available for plant uptake due to adsorption and precipitation of available P into less soluble and more stable P forms through various chemical reactions in the soil [3]. Thus, P deficiency is a major nutritional constraint for primary crop production in many N fertilized agricultural soils. Continuous annual application of P fertilizer is a common practice for correcting P deficiency and sustaining higher crop yield in many P-limited soils. However, complete reliance on chemical fertilizers, in the long-run, is not considered to be an economically feasible and environmentally sustainable practice. Phosphorus fertilization increases the investments in crop production and enhances the risk of environmental pollution such as eutrophication and ground water contamination in some environments [5] [6]. The identification and incorporation of suitable crop species that are efficient in P mobilization is considered to be one of the promising agronomic approaches to access insoluble native soil P reserves and to capitalize on the use of accumulated residual soil P reserves [7] [8].

Legumes play an important role in agriculture and natural ecosystems [2]. A legume in rotation not only enhances soil physical properties and N supply, but also has been shown to improve soil P availability [9] [10] [11]. 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 [4]; (ii) secretion of carboxylic acids such as malate and citrate [12]; and (iii) exudation of root or microbially derived P solubilizing enzymes such as acid phosphatases and phytases [13] [14]. In addition, legumes are also capable of improving spatial access to soil P through their unique morphological root traits and symbiotic associations with arbuscular mycorrhizal fungi [15] [16].

Previous research has shown that some legumes are not only capable of mobilizing available soil P for their own requirements, but are also able to enhance the growth and P uptake of following crop species [17] [18] [11]. In particular, legumes like white lupin (*Lupinus albus* L.), chickpea (*Cicer arietinum* L.), field pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) revealed superior ability in enhancing the growth and P uptake of following wheat and maize (*Zea mays* L.) [19] [18]. According to Kamh *et al.* [17], white lupin has a positive effect on the growth and P uptake of subsequent wheat. In a field trial, Horst *et al.* [20] also observed a positive rotational effect of P-efficient leguminous crops on the less P-efficient cereal crops.

The effect of grain legumes as preceding crops on the growth and P uptake of following crop species has been intensively investigated [17] [20] [11]. However, the effect of perennial forage legumes like alfalfa on the 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.

Forage legumes are an essential component of sustainable agro-ecosystems [21] [22]. There are numerous benefits of including forage legumes into cropping systems 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 [22]. In addition, rotational effects of forage legumes on P availability have also been observed [23]. Perennial 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 [23]. Inclusion of forage legumes in rotation for two years may increase the availability of indigenous soil P and residual fertilizer P added in previous years, thereby enhancing the crop P uptake. The objectives of this study were to 1) assess the effect of two previous years of forage legume versus annual crop rotations on the yield and P uptake by wheat and canola grown in the next two years and 2) evaluate the effect of the different crop rotations on soil P availability and P balance in four soil zones of Saskatchewan, Canada.

2. Material and Methods

2.1. Site Description

The study was conducted at four agricultural research sites: 1) the Semiarid Prairie Agricultural Research Centre (SPARC) at Swift Current (50˚16'N 107˚44'W); 2) the Agriculture and Agri-Food Canada Research Farm (AAFC) at Saskatoon (52˚04'N, 108˚08'W); 3) the Western Beef Development Centre's (WBDC) Termuende Research Ranch at Lanigan (51˚51'N, 105˚02'W); and 4) the Melfort Research Farm (MRF) at Melfort (52˚08'N, 104 ˚06'W) Saskatchewan, Canada from 2010 to 2013 (**Figure 1**). The climate, long-term mean annual temperature, precipitation and soil classification at each site are described in **Table 1**. The soils were sampled prior to the experiment and initial soil characteristics were determined (**Table 2**). The soil texture was determined with a laser scattering particle size distribution analyzer (HORIBA LTD., Kyoto, Japan). Soil organic carbon (OC) content was analyzed by dry combustion using a LECO C632 carbon combustion analyzer (LECO® Corporation, St. Joseph, MI, USA) [24]. Soil pH and electrical conductivity (EC) were measured with a glass electrode using 1:2 soil:water suspensions [25]. The Lanigan site had a previous history of manure application, while other sites had not.

2.2. Experimental Design and Treatments

The field experiment was conducted in four soil zones (Brown, Dark Brown, Thin Black, and Dark Gray) of Saskatchewan that differ in OC content due to regional variations in climate and vegetation. Each site was set up as a randomized complete block design (n = 4). Four crop rotations were included as agronomic treatments (**Table 3**). Monthly average precipitation, temperature and long-term averages (30 year average) of four research sites are provided in **Table 4** and **Table 5**.

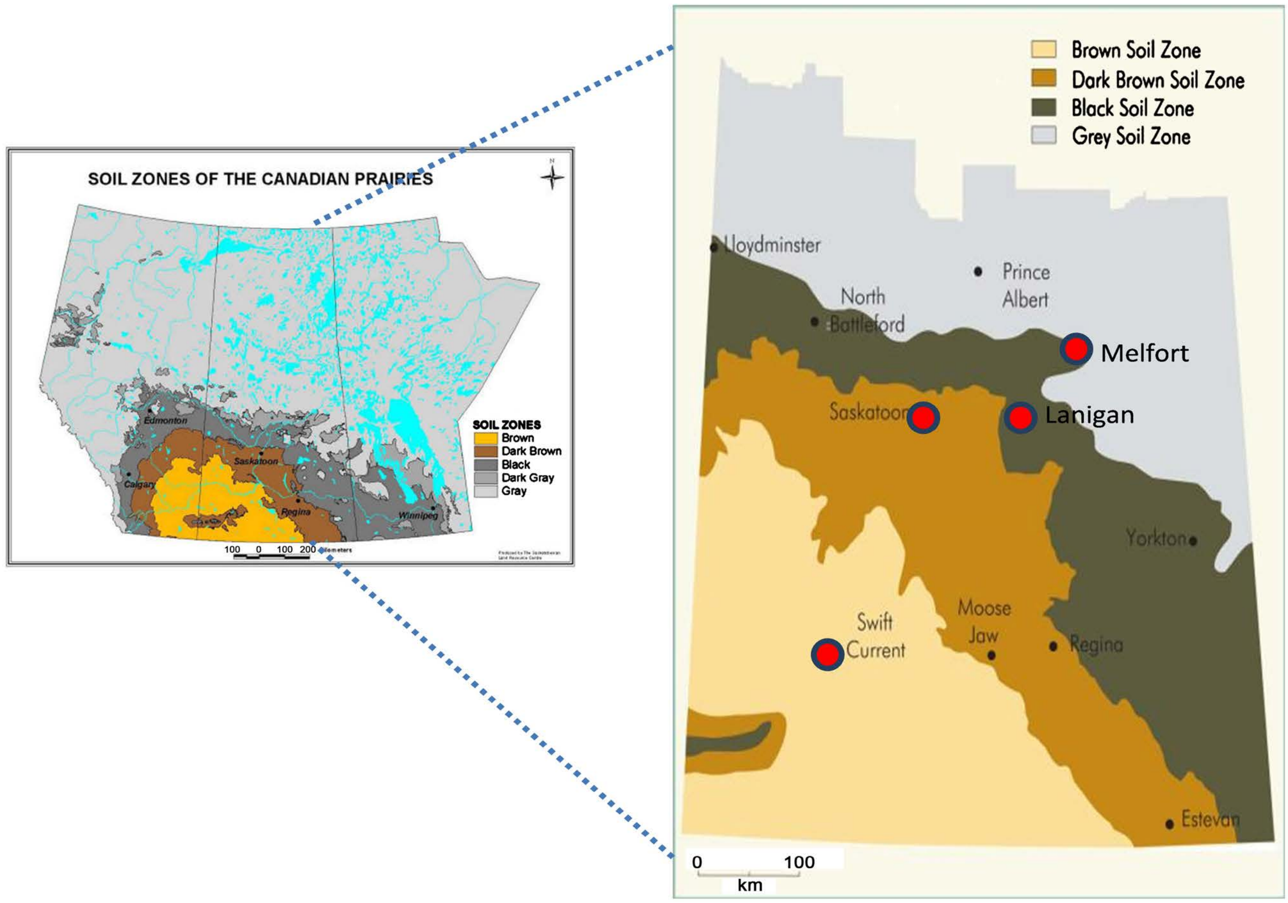

Figure 1. Map of Saskatchewan, Canada, showing major soil zones, and research locations (red dots) used in this study.

Table 1. Description of the four experimental sites.

Sites	Climate	Average annual Precipitation (mm)	Mean annual Temperature (˚C)	Soil classification†
Swift current	Semi-arid	357	4.3	Brown chernozem
Saskatoon	Semi-arid	391	4.0	Dark Brown chernozem
Lanigan	Semi-arid	449	2.7	Black chernozem
Melfort	Sub-humid	396	1.3	Black chernozem

†The soils at each site are classified according to the Canadian System of Soil Classification.

Table 2. Selected physiochemical properties of soils (0 - 30 cm) at the four experimental sites.

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

†EC is electrical conductivity; OC is organic matter content.

Table 3. Description of crop rotation treatments.

Rotation	2010	2011	2012	2013
1	Alfalfa	Alfalfa	Wheat	Canola
2	Red clover	Red clover	Wheat	Canola
3	Barley1	Pea	Wheat	Canola
4	Barley2	Flax	Wheat	Canola

Table 4. Monthly mean temperature (˚C) from April to October of 2010, 2011, 2012, 2013 and 30 years mean temperature (from 1971 to 2000) at the four study sites in Saskatchewan, Canada.

Sites	Year		Apr.	May	June	Jule	Aug.	Sep.	Oct.†
	2010	Temp. (˚C)‡	6.1	7.8	15.4	17.0	16.8	10.7	7.9
	2011	Temp.	2.5	9.5	14.3	18.2	18.2	15.1	7.4
Swift current	2012	Temp.	5.5	9.9	16.8	20.0	19.3	14.2	2.9
	2013	Temp.	−0.9	12.6	15.5	16.8	19.2	15.2	4.0
	30-year	Ave. temp.‡	4.9	11.1	15.6	18.1	17.9	11.8	5.5
	2010	Temp. (˚C)	6.4	9.6	15.3	17.6	16.1	10.5	6.5
	2011	Temp.	3.1	10.9	15.5	18.4	17.2	14.7	6.5
Saskatoon	2012	Temp.	4.4	10.1	15.8	19.7	17.3	13.0	1.7
	2013	Temp.	−2.3	13.0	15.5	17.4	18.9	15.2	3.3
	30-year	Ave. temp.	4.7	11.8	16.0	18.3	17.6	11.5	4.5
	2010	Temp. (˚C)	5.7	9.0	15.7	17.8	16.3	10.3	6.4
	2011	Temp.	2.4	10.2	14.8	17.8	17.2	13.5	6.4
Lanigan	2012	Temp.	5.0	10.1	16.2	19.8	16.8	11.7	1.3
	2013	Temp.	−3.9	12.3	15.6	16.6	17.5	13.8	2.8
	30-year	Ave. temp.	4.0	11.3	15.9	18.1	17.3	11.3	4.3
	2010	Temp. (˚C)	5.8	9.1	15.3	17.4	16.0	9.5	6.0
	2011	Temp.	1.5	10.1	15.4	17.6	17.0	13.8	5.7
Melfort	2012	Temp.	2.6	9.6	15.2	18.9	17.1	12.4	1.1
	2013	Temp.	−3.9	12.0	15.4	16.4	17.7	14.4	2.8
	30-year	Ave. temp.	2.5	10.8	15.7	17.4	16.4	10.5	3.6

†The 30 years average temperature is not available. ‡Temp. (˚C) is temperature; Ave. temp. (˚C) is average temperature.

Alfalfa (*Medicago sativa* L. 2065 M), red clover (*Trifolium pratense* L. Belle) and barley (*Hordeum vulgare* L. CDC Copeland) were seeded in June, 2010. The seeding rate of forage legumes was 9 kg/ha at Saskatoon, Lanigan and Melfort, but at Swift Current it was 4.5 kg/ha. Lower seeding rate was recommended based on moisture limitation in the Brown soil zone in the Swift Current region (Forage crop production guide, 2012). The barley was seeded at the recommended rate at all sites. The plot sizes varied on each experimental site: Saskatoon 37.5 m^2

Table 5. Monthly mean precipitation (mm) from April to October of 2010, 2011, 2012, 2013 and 30 years mean precipitation (from 1971 to 2000) at the four study sites in Saskatchewan, Canada.

Sites	Year		Apr.	May	June	July	Aug.	Sep.	Oct.†
Swift current	2010	Precip. (mm)‡	33.7	93.6	121.5	71.5	85.0	99.7	8.4
	2011	Precip.	25.4	56.9	117.3	68.0	30.4	10.6	.
	2012	Precip.	42.0	101.9	113.4	22.0	10.9	6.8	.
	2013	Precip.	11.8	11.2	103.0	50.4	13.5	42.8	.
	30-year	Ave. Precip.‡	22.3	49.5	66.0	52.0	39.9	30.2	16.2
Saskatoon	2010	Precip. (mm)	72.6	128.5	169.0	46.0	43.7	87.9	12.2
	2011	Precip.	0.6	17.5	94.4	68.6	16.5	6.0	.
	2012	Precip.	27.3	108.3	121.1	80.9	48.5	8.0	.
	2013	Precip.	6.2	15.2	115.9	35.2	14.7	14.9	.
	30-year	Ave. Precip.	24.2	43.6	60.5	57.3	35.4	30.6	16.9
Lanigan	2010	Precip. (mm)	64.1	139.0	71.4	80.8	51.6	10.2	25.0
	2011	Precip.	12.3	20.8	54.2	114.6	54.2	11.5	.
	2012	Precip.	24.3	86.1	89.4	72.0	7.4	0.0	.
	2013	Precip.	3.4	36.8	107.8	47.2	12.9	50.7	.
	30-year	Ave. Precip.	30.3	53.5	83.9	66.1	53.0	42.6	28.0
Melfort	2010	Precip. (mm)	140.6	66.6	113.2	63.6	56.8	92.0	18.4
	2011	Precip.	8.1	10.5	103.5	73.3	10.7	1.1	.
	2012	Precip.	24.7	55.2	112.3	97.8	68.1	12.6	.
	2013	Precip.	3.0	18.0	96.9	100.0	10.6	17.0	.
	30-year	Ave. Precip.	24.5	45.6	65.8	75.7	56.8	39.9	24.7

†Precipitations for October 2011, 2012 and 2013 are not available from the recording station. ‡Precip. is precipitation; Ave. precip. is average precipitation.

(6.25 m × 6 m), Lanigan 36 m^2 (6 m × 6 m), Swift Current 48 m^2 (6 m × 8 m) and Melfort 51.1 m^2 (7 m × 7.3 m). In 2011, pre-established perennial forage legumes were grown continually, while the annual legume peas (*Pisum sativum* L. Golden) and oil-seed flax (*Linum usitatissimum* L. CDC Bethune) were seeded at the recommended rate on plots that were previously sown to barley. Wheat (*Triticum aestivum* L. Unity VB) was seeded at a recommended rate in the first week of June, 2012 and Canola (*Brassica napus* cv. LL130) was seeded in late May of the following year.

In the first year of the rotation (2010), a recommended rate of urea (46-0-0) (50 kg N ha^{-1}) was applied with barley (on non-legume plots) at the Swift Current, Saskatoon, and Lanigan sites, whereas at the Melfort site, 70 kg N/ha ammonium nitrate (34-0-0) was applied prior to seeding. A recommended rate (15 kg P_2O_5/ha) of monoammonium phosphate (11-52-0) was side banded on all plots. In the second year of the crop rotation (2011), flax was seeded with 70 kg/ha urea (46-0-0), but other plots did not receive N or P fertilizer. Field pea seed was inoculated with a recommended rate of commercial Rhizobium inoculant (liquid formulation) immediately prior to seeding but was not fertilized. In 2012 and 2013, N or P fertilizer was not applied to any of the plots. Potassium sulfate fertilizer (100 kg/ha) (0-0-50) (equivalent to 20 kg S/ha) was applied uniformly on all plots to ensure that sulfur (S) deficiency did not limit canola growth. Every year, weeds were controlled by application of an appropriate post-emergent herbicide. Alfalfa and red clover hay was harvested once in the year of establishment (2010), and was harvested two to three times in the second year (2012) according to the growing conditions at each site. The above ground biomass of annual crops (barley, peas, flax, wheat and canola) was hand harvested from two 1 m^2 areas per plot, air dried and the biomass (grain + straw) was measured. The grain yields of these crops were determined by harvesting a 10 m^2 area from each plot using a Wintersteiger™ plot combine. In 2010, barley biomass (straw + grain) at Lanigan was harvested as green-feed hay because the grain did not mature sufficiently to harvest due to cold weather conditions.

2.3. Soil Sampling and Analyses

Soil samples were taken from all plots at: April and October of 2012 and 2013. At each site, three core samples were randomly taken from each plot using a core soil sampler (diameter 4.5 cm and length 100 cm) and composited for each depth increment (0 - 15, 15 - 30 and 30 - 60 cm). Samples were then air-dried, ground, passed through a 2 mm sieve and stored at room temperature prior to further analyses.

2.4. Extractable Soil P and P Supply Rate

Available soil P was extracted with modified Kelowna solution (composed of 0.025 M acetic acid, 0.25 M ammonium acetate and 0.015 M ammonium fluoride with a measured pH of 4.9) [26]. Three grams of soil were placed in a 250 mL extraction bottle with 30 mL of modified Kelowna solution and the mixture was shaken on a rotary shaker (160 rpm) for 5 min. The suspension was then filtered (VWR #454) and stored in vials in the cooler. The P concentration in the extracts was then determined colorimetrically using a Technicon Autoanalyzer II (Technicon Industrial Systems, 1973) [26]. Soil P supply rate was determined using a "sandwich" test [27]. 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 h, 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 solution. The P ion concentration in the eluents was measured colorimetrically using a Technicon Autoanalyzer II (Technicon Industrial Systems, 1973) [26].

2.5. Plant Analysis

Plant samples collected after each crop growing season were air-dried and ground to determine P content. A 0.25 g of ground sub-sample from each replicate was digested using a H_2SO_4-H_2O_2 digestion method [28]. The P concentration in the digested solution was determined colorimetrically using a Technicon Autoanalyzer II (Technicon Industrial Systems, 1973) [26]. Plant P uptake (grain and straw) (kg/ha) was calculated by multiplying the grain and straw P concentration by grain and straw yield respectively. Plant P removal was calculated after each crop growing season based on the plant P concentration and harvested biomass.

2.6. Statistical Analysis

Treatment means at each site were compared 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 rotation) 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 $P < 0.10$. Experimental sites were not replicated among the locations making statistical inter-site comparison invalid.

3. Results

3.1. Wheat Grain Yield

Wheat grain yield was significantly affected by different crop rotations at all locations ($P = 0.025$, $P = 0.008$, $P = 0.001$, $P < 0.001$) (**Table 6**). At Saskatoon and Melfort, wheat grain yield was greater after two years of red clover and alfalfa rotations in comparison to barley-flax rotation ($P = 0.008$, $P < 0.001$); At Lanigan, wheat produced higher grain yield following alfalfa, red clover and barley-pea rotations relative to barley-flax rotation ($P = 0.001$). At Swift Current, however, yield depression occurred in wheat following two years of alfalfa rotation compared to the other crop rotations ($P = 0.025$), likely due to less precipitation at the Swift Current site over the course of the study compared to the other sites. The Swift Current site is located in the Brown soil-climatic zone, which is the driest of the four soil zones in the agricultural region of Saskatchewan (**Figure 1**).

Legumes grown in 2010 and 2011 had a significant positive effect on 2013 canola grain yields at Saskatoon, Lanigan and Melfort sites ($P < 0.10$) (**Table 6**). Canola grain yield was higher in the red clover rotation relative to barley-flax rotation in Saskatoon ($P = 0.065$) and Lanigan; ($P = 0.091$). At Melfort, the grain yield was relatively higher in the rotation with two years of alfalfa when compared to barley-pea and barley-flax rotations ($P = 0.003$). At Swift Current, crop rotations did not have a significant impact on canola grain production ($P = 0.165$).

3.2. Wheat and Canola Grain P Uptake

Wheat grain P uptake was significantly different among crop rotations at Lanigan and Melfort ($P < 0.10$), but at the other two locations crop rotation did not significantly affect wheat grain P uptake ($P > 0.10$) (Table 7). At Lanigan, P uptake of wheat grain was highest after two years of red clover and barley-pea

Table 6. Effect of two years of forage legume (alfalfa-alfalfa; red clover-red clover) and annual crop (barley-pea; barley-flax) rotation on grain yields of following wheat (2012) and canola (2013) crops at four sites in Saskatchewan, Canada.

	Treatment	Sites			
		Swift Current	Saskatoon	Lanigan	Melfort
Wheat grain yield (kg ha^{-1})	A-A-W†	1304 ± 197[b]§	2529 ± 178[ab]	2283 ± 82[a]	3623 ± 16[a]
	RC-RC-W	1856 ± 138[a]	2792 ± 209[a]	2535 ± 187[a]	3377 ± 75[ab]
	B-P-W	1739 ± 83[ab]	1982 ± 211[bc]	2515 ± 29[a]	3204 ± 165[b]
	B-FL-W	2073 ± 114[a]	1740 ± 164[c]	1736 ± 87[b]	2501 ± 53[c]
Canola grain yield (kg ha^{-1})	A-A-W-C‡	1301 ± 216[a]	1995 ± 122[ab]	1934 ± 111[ab]	3455 ± 37[a]
	RC-RC-W-C	946 ± 176[a]	2356 ± 196[a]	2352 ± 312[a]	2791 ± 186[ab]
	B-P-W-C	764 ± 99[a]	1608 ± 186[ab]	1815 ± 173[ab]	2319 ± 378[b]
	B-FL-W-C	1056 ± 112[a]	1508 ± 321[b]	1509 ± 200[b]	2109 ± 206[b]

†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. ‡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. §Values presented are means ± standard error. Means with a different superscript letter in the same column for a crop and site are significantly different ($P < 0.10$).

Table 7. Effect of two years of forage legume (alfalfa-alfalfa; red clover-red clover) and annual crop (barley-pea; barley-flax) rotation on grain P uptake of following wheat (2012) and canola (2013) crops at four sites in Saskatchewan, Canada.

	Treatment	Sites			
		Swift current	Saskatoon	Lanigan	Melfort
Wheat grain P uptake (kg ha^{-1})	A-A-W†	4.5 ± 0.84[a]	9.5 ± 0.64[a]	9.5 ± 0.64[ab]§	16.1 ± 0.75[a]
	RC-RC-W	6.9 ± 0.71[a]	8.0 ± 2.03[a]	11.0 ± 0.70[a]	15.4 ± 1.77[a]
	B-P-W	5.8 ± 0.33[a]	7.4 ± 0.59[a]	10.4 ± 0.34[a]	14.7 ± 1.10[ab]
	B-FL-W	5.5 ± 0.42[a]	7.2 ± 0.56[a]	7.7 ± 0.65[b]	11.2 ± 1.02[b]
Canola grain P uptake (kg ha^{-1})	A-A-W-C‡	8.0 ± 1.17[a]	9.7 ± 0.85[a]	10.4 ± 0.87[a]	17.2 ± 1.02[a]
	RC-RC-W-C	7.2 ± 1.58[a]	10.2 ± 1.08[a]	12.3 ± 1.84[a]	14.3 ± 1.81[ab]
	B-P-W-C	5.7 ± 0.81[a]	8.0 ± 0.64[a]	10.3 ± 1.11[a]	12.0 ± 2.52[b]
	B-FL-W-C	7.6 ± 0.68[a]	7.8 ± 1.59[a]	8.2 ± 1.33[a]	12.71.64[b]

†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. ‡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. §Values presented are means ± standard error. Means with a different superscript letter in the same column for a crop and site are significantly different ($P < 0.10$).

rotations; but it was lowest after a barley-flax rotation (P = 0.013). At Melfort, wheat grain P uptake was greatest following alfalfa and red clover rotations but lowest following a barley-flax rotation (P = 0.033). Crop rotations did not significantly affect canola grain P uptake at Swift Current, Saskatoon and Lanigan (P = 0.502, P = 0.369, P = 0.262) (Table 7). At Melfort, canola grain P uptake was significantly higher following two years of alfalfa rotation compared to barley-pea and barley-flax rotations (P = 0.045) (Table 7).

Wheat grain P concentration was not significantly affected by crop rotations at Saskatoon, Lanigan and Melfort (P = 0.16, P = 0.484, P = 0.987) while canola grain P concentration was significantly affected by Crop rotation at three sites: Swift Current, Saskatoon and Melfort (P = 0.039, P = 0.002, P = 0.086) (Table 8).

3.3. Extractable Soil P and P Supply Rate after Wheat and Canola Growth

After two years of different crop rotations followed by a wheat crop, there were no significant differences in modified Kelowna extractable P or P supply rate measured at two depths (0 - 15 cm, 15 - 30 cm; data from 15-30 cm were not included) among any of the site rotations (P > 0.10) (Figure 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.

The soil P availability measured at two depths: 0 - 15 and 15 - 30 cm in the fall of 2013 after the canola harvest again revealed no significant differences in

Table 8. Effect of rotation (alfalfa-alfalfa; red clover-red clover, barley-pea; barley-flax) on grain P concentrations of following wheat (2012) and canola (2013) crops at four sites in Saskatchewan, Canada.

	Treatment	Sites			
		Swift current	Saskatoon	Lanigan	Melfort
Wheat grain P concentration (mg P g^{-1})	A-A-W†	3.45 ± 0.26^{a}§	3.76 ± 0.03^{a}	4.13 ± 0.18^{a}	4.44 ± 0.21^{a}
	RC-RC-W	3.72 ± 0.16^{a}	2.93 ± 0.70^{a}	4.34 ± 0.09^{a}	4.54 ± 0.48^{a}
	B-P-W	3.37 ± 0.19^{a}	3.77 ± 0.14^{a}	4.15 ± 0.16^{a}	4.57 ± 0.18^{a}
	B-FL-W	2.62 ± 0.14^{b}	4.16 ± 0.10^{a}	4.43 ± 0.24^{a}	4.47 ± 0.33^{a}
Canola grain P concentration (mg P g^{-1})	A-A-W-C‡	6.34 ± 0.56^{b}	4.84 ± 0.20^{a}	5.36 ± 0.13^{a}	4.96 ± 0.27^{c}
	RC-RC-W-C	7.44 ± 0.32^{a}	4.30 ± 0.11^{b}	5.18 ± 0.11^{a}	5.09 ± 0.28^{bc}
	B-P-W-C	7.41 ± 0.14^{a}	5.06 ± 0.24^{a}	5.64 ± 0.11^{a}	5.61 ± 0.25^{a}
	B-FL-W-C	7.26 ± 0.12^{ab}	5.26 ± 0.25^{a}	5.44 ± 0.33^{a}	5.43 ± 0.25^{ab}

†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. ‡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. §Values presented are means ± standard error. Means with a different superscript letter in the same column for a crop and site are significantly different (P < 0.10).

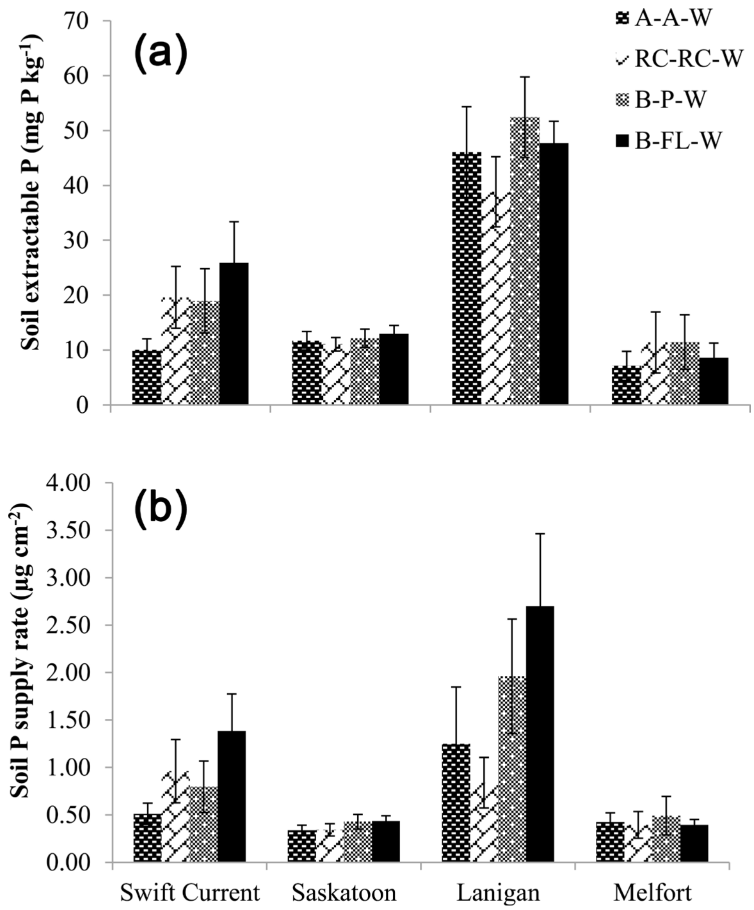

Figure 2. The concentrations of soil extractable P (a) and P supply rate (b) (0 - 15 cm) measured in the fall of 2012 in the four different crop rotations at the four sites in Saskatchewan, Canada. (A-A-W = alfalfa-alfalfa-wheat; RC-RC-W = red clover-red clover-wheat; B-P-W = barley-pea-wheat; B-FL-W = barley-flax-wheat). Error bars represent standard errors (n = 4).

modified Kelowna extractable P or P supply rate among different crop rotations at all four sites (only the surface soil P data are shown) ($P > 0.10$) (**Figure 3**). Greater canola 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 to anion exchange membrane at all sites ($P > 0.10$) (**Figure 3**).

3.4. Crop P Removal and P Balance over a Four-Year Rotational Cycle

After a four-year crop rotation, P balance (surplus or deficit) was calculated as the difference between the total P added from external sources (fertilizer) during the rotational cycle and harvested P removed from the system in crop biomass (**Table 9**). Four years of cropping with the addition of a small amount of P fertilizer added to each treatment at the start of the rotational cycle in 2010 (15 kg P_2O_5 ha^{-1}) resulted in a continuous drain on the soil P pool at all locations

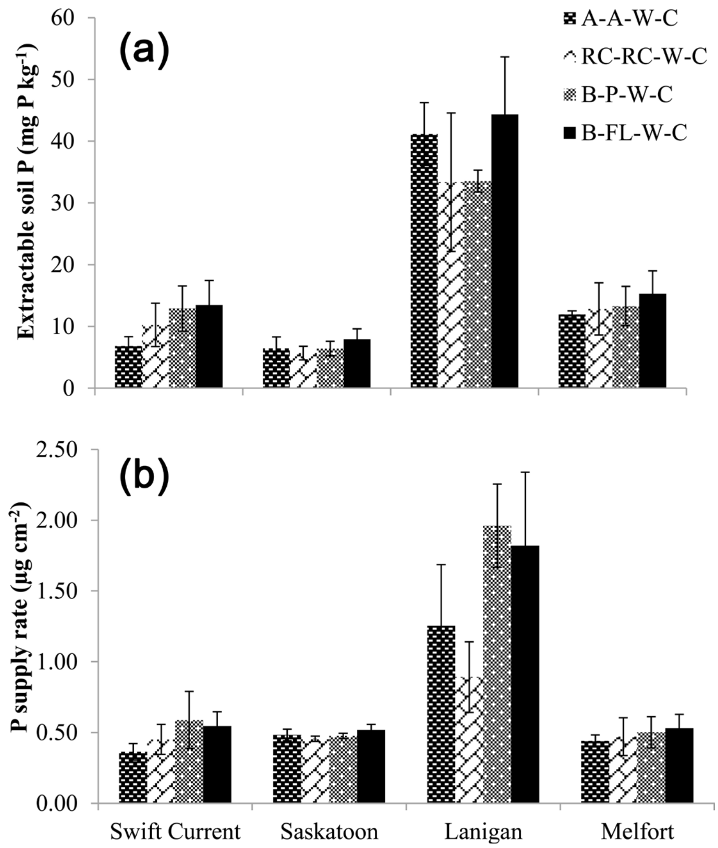

Figure 3. The concentrations of soil extractable P (a) and P supply rate (b) (0 - 15 cm) measured in fall 2013 in four different crop rotations at the four sites in Saskatchewan, Canada. (A-A-W-C = alfalfa-alfalfa-wheat-canola; RC-RC-W-C = red clover-red clover-wheat-canola; B-P-W-C = barley-pea-wheat-canola; B-FL-W-C = barley-flax-wheat-canola). Error bars represent standard errors (n = 4).

(**Table 9**). Crop rotations significantly affected crop total P removal and P balance at each site ($P < 0.10$). Among the 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, 2013) revealed that cropping with a very low addition of P fertilizer resulted in the depletion of available P in the top soil (0-15 cm) from spring 2012 to fall 2013 at the Swift Current, Saskatoon and Lanigan sites (**Figure 4**). At the Melfort site, soil available P diminished quickly from the spring 2012 to fall 2012, then unlike other sites, it increased from the fall of 2012 to fall of 2013 (**Figure 4**).

4. Discussion

4.1. Wheat and Canola Yield

In this experiment, wheat and canola grain yields were generally greater following

Table 9. Phosphorus balance from 2010 to 2013 in the four different crop rotations at the four sites in Saskatchewan, Canada.

Sites	Treatment	Fertilizer P applied‡	P removed in biomass	P balance§
			(kg ha^{-1})	
Swift current	A-A-W-C†	6.6	27.5 ± 2.37^{a}¶	−21.0^{b}
	RC-RC-W-C	6.6	24.4 ± 4.31^{ab}	−17.9^{ab}
	B-P-W-C	6.6	17.4 ± 1.64^{b}	−10.9^{a}
	B-FL-W-C	6.6	18.0 ± 1.64^{b}	−11.5^{a}
Saskatoon	A-A-W-C	6.6	49.4 ± 3.51^{a}	−42.8^{b}
	RC-RC-W-C	6.6	43.2 ± 3.72^{a}	−36.6^{b}
	B-P-W-C	6.6	22.1 ± 1.10^{b}	−15.5^{a}
	B-FL-W-C	6.6	24.7 ± 2.53^{b}	−18.1^{a}
Lanigan	A-A-W-C	6.6	54.5 ± 1.10^{a}	−47.9^{d}
	RC-RC-W-C	6.6	48.3 ± 1.60^{b}	−41.7^{c}
	B-P-W-C	6.6	22.9 ± 1.43^{c}	−16.4^{b}
	B-FL-W-C	6.6	17.0 ± 0.98^{d}	−10.4^{a}
Melfort	A-A-W-C	6.6	52.3 ± 1.69^{a}	−45.7^{b}
	RC-RC-W-C	6.6	48.7 ± 3.48^{ab}	−42.2^{ab}
	B-P-W-C	6.6	43.5 ± 5.66^{b}	−37.0^{a}
	B-FL-W-C	6.6	42.4 ± 1.19^{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_2O_5 ha^{-1}) was applied to all plots during the first year of the crop rotations (spring 2010). Thereafter no fertilizer P was applied to any plots. §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 for a site are significantly different ($P < 0.10$).

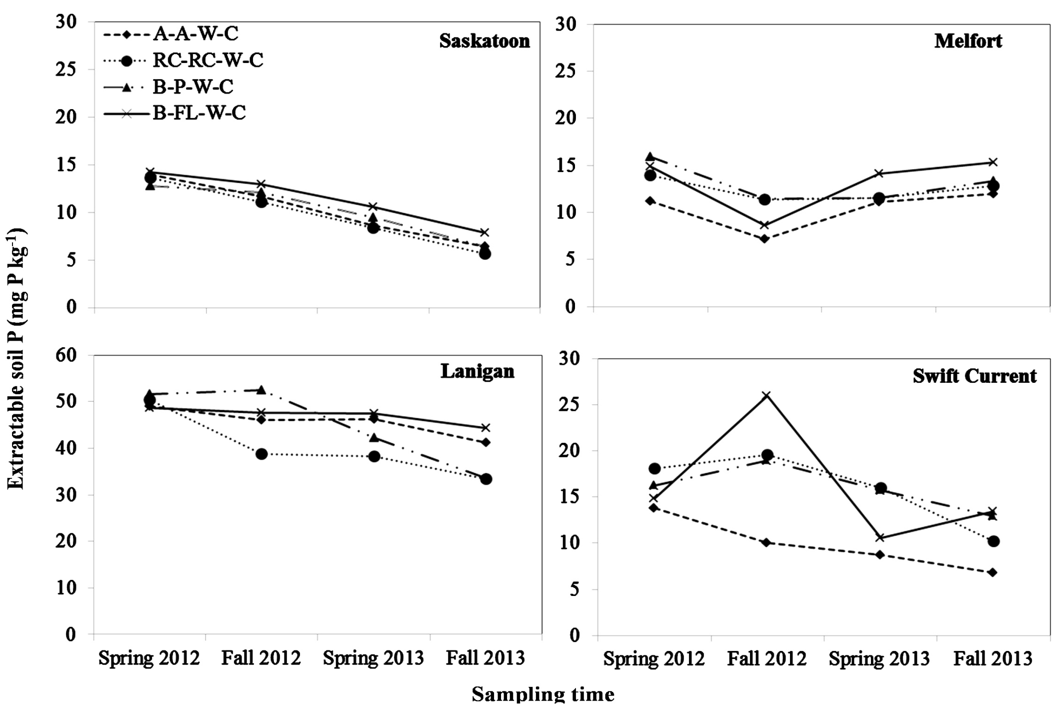

Figure 4. Changes in extractable (modified Kelowna) soil P in the top soil (0 - 15 cm) over 2012 and 2013 at four sites (Saskatoon, Melfort, Lanigan and Swift Current), Saskatchewan Canada. A-A-W-C is alfalfa-alfalfa-wheat-canola; RC-RC-W-C is red clover-red clover-wheat-canola; B-P-W-C is barley-pea-wheat-canola; B-FL-W-C is barley-flax-wheat-canola. Note the different scale at Lanigan.

forage legume rotations relative to an annual non-legume rotation (barley-flax) (**Table 6**). Increased growth and biomass production of wheat after legumes was also observed in pot experiments conducted in western and southern Australia [18] [11] and in a field experiment conducted in northern Nigeria [20]. The increased grain yields of wheat and canola grown after forage legume rotations can be attributed to enhanced N supply through biological N_2 fixation [10], improved soil physical and biological characteristics and lower disease incidence [9] [22]. At Swift Current, located in the dry Brown soil zone with semi-arid conditions, 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 [29]. In addition, improved soil P nutrition arising as a result of the legume crops (alfalfa and red clover) might be another contributing factor for positive rotational effects of legumes on the following wheat and canola grain yields. This is demonstrated by the enhanced P uptake of wheat following alfalfa and red clover rotation at Lanigan and Melfort and also improved P uptake of canola grain following alfalfa at Melfort. 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 crops in rotation [17] [20] [18] [11].

4.2. Wheat and Canola Grain P Uptake

The significantly higher P uptake by wheat grain following forage legume rotations at Lanigan and Melfort (**Table 7**) 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 [30] that would also contribute to increased demand for, and uptake of P. Improved P acquisition by wheat and canola could also originate from more robust root systems of these crops and modification of soil biological properties as a result of previous forage legume crops [20]. Wheat and canola grown on forage legume plots may have better root systems which enable them to explore a larger soil volume and take up greater amounts of soil P relative to wheat and canola crops grown on non-legume plots. Mycorrhizal infection rate can be of importance. Mycorrhizal infection rate of plants or populations of arbuscular mycorrhizal fungi (AM fungi) were not determined in our experiment, but Horst *et al.* [20] showed in a field study that mycorhizal infection was significantly enhanced after most legume crops compared to maize after maize. The mycorrhizal hyphae extend the root system and impart a better ability to take up immobile soil nutrients like P.

Even though forage legume rotations positively affected the grain yields of canola through enhanced soil conditions and nutrient availability at Saskatoon, Lanigan and Melfort, they only resulted in increased canola grain P uptake at Melfort (**Table 7**). The higher grain P uptake of canola following forage legume rotations at Melfort could be the reflection of increased canola grain yield. Plant P uptake was determined by two factors: P concentrations in plant tissues (grain

+ straw) and the biomass (grain + straw) yield. When canola grain P uptake was determined, the greater canola grain yield following forage legume rotations resulted in higher grain P uptake even though there was a dilution in canola grain P concentration due to greater biomass production (**Table 8**).

4.3. Extractable Soil P and P Supply Rate

The available P content and P supply rates measured after wheat and canola harvest were similar among rotations at all sites (**Figure 2**) regardless of 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 could be explained by the recycling of P via the extensive root system of the perennial forage legumes [31]. According to previous studies, a considerable amount of labile P is added to the soil through alfalfa root biomass turnover [31]. In a field experiment conducted in Michigan, Daroub *et al.* [31] estimated that alfalfa root could contribute 11.4 kg P ha^{-1} per year when the alfalfa root residue contains 0.2% P. Alamgir *et al.* [27] reported that soil P can be mobilized during legume residue (root and shoot residue) decomposition as legume residues contain more P (lower C:P ratios) than cereal crops and favours net P mineralization. The main advantage of legume root residues is the addition of organic matter to the soil [32] which can influence P availability mainly through the accumulation of organic P fractions [20]. The organic P fractions that build up have special importance for the maintenance of soil P availability because the slower decomposition and release of P from organic matter prevents rapid fixation of Pi, and better matches the P requirements of the subsequently grown crops [33] [34] [35]. Another possible reason might 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 [18] [36] [37], and that it can take a few years of a change in management practice to produce significant changes in the labile pool amounts.

4.4. Soil P and P Balance over the Four-Year Rotational Cycle

Soil extractable available P (modified Kelowna extractable P) declined gradually over the four year rotation period at the Saskatoon, Lanigan and Swift Current sites (**Figure 4**). It is anticipated that the net effect of greater crop P removal in forage legumes over several cycles of the rotation would eventually result in significantly lowered soil available P status. The observed depletion of soil available P over time in soils with low or no external P inputs such as these was also revealed in previous studies conducted in India and Missouri [38] [39]. At Melfort, the greater P depletion at the beginning of the crop rotation was mainly due to greater crop P removal (**Figure 4**). The increased available P level from the spring of 2013 to the fall of 2013 could be due to the replenishment of available P from other less available soil P fractions, especially from the organic P pool which is large in this soil and also addition of labile inorganic P through crop residue turnover due to the higher available soil moisture content at the site.

A four-year continuous cropping cycle with a very low P addition resulted in a negative P balance (deficit) for all crop rotation treatments at all four locations (**Table 9**). The greater soil P deficit in forage legume crop rotation treatments (alfalfa-alfalfa-wheat-canola and red clover-red clover-wheat-canola) compared to annual legume (barley-pea-wheat canola) and non-legume crop rotations (barley-flax-wheat-canola) 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 (data not shown). 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, especially in the forage legume rotations where P removal is higher, it is anticipated that P limitations will eventually arise. Therefore, it is critical to apply sufficient P to match the crop P removal over time in order to preserve the soil P fertility over the long-term.

5. Conclusion

The results of this four-year field experiment indicated that forage legumes for two years in rotation generally improve grain yield and P uptake of subsequently grown wheat and canola crops in the Black and Dark-Gray soil zones of Saskatchewan, Canada for the first cycle of the rotation. The 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 in the short-term. 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 could be an indication of a positive influence of forage legumes in short-term rotation on soil P availability. 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. The negative soil P balance arising from harvesting and removing P in crop biomass every year will likely need to be addressed through external P inputs in order to maintain soil P fertility over the long-term. Further research is needed to evaluate the effect of several cycles of this rotation over a number of years on soil P availability, and the impacts of the rotation on microbial communities and P dynamics in the rhizosphere.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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