frontiers in Environmental Science

ORIGINAL RESEARCH
published: 11 February 2020
doi: 10.3389/fenvs.2020.00013

Cover Cropping May Alter Legacy Phosphorus Dynamics Under Long-Term Fertilizer Addition

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Use of cover crops in an integrated agricultural system can reduce demand of inorganic phosphorus (P) fertilizers, where the subsequent crops can take up P accumulated in cover crops biomass after the decomposition. In this research we hypothesized that some cover crops can take up P from less labile fractions and recycle it back to the soil through plant residues resulting in better P use efficiency of the system; cover crops are capable of P uptake from subsurface layers which leads to the accumulation of this P on the surface after the decomposition of their residues; and cover crop species respond differently to distinct inorganic P sources. To examine these hypotheses, a field experiment was conducted over nine successive years in South Brazil. The experimental treatments were established as a split-plot randomized block design, in a 3 × 6 factorial scheme, considering three P treatments [no-P, single superphosphate (SSP), and rock phosphate (RP)] as main plot and six cover crop treatments (common vetch, white lupin, fodder radish, ryegrass, black oat, and a fallow) as subplots. Soil samples were collected from the depths of 0–5, 5–10, and 10–15 cm and analyzed by Hedley P fractionation. In P-unfertilized cropping system, the amounts of labile and mod-labile P fractions were not modified by cover crops related to fallow. When inorganic P fertilizers were applied, the amount of labile and mod-labile P pools under fallow were higher than under cover crops in 5–10 cm depth. Black oat and common vetch cycled more labile P under SSP and RP, respectively. Common vetch and ryegrass resulted in the highest accumulation of organic P on the surface under SSP and RP, respectively. Black oat was capable to change P extracted by 1.0 M HCl to more labile forms. Fodder radish showed the highest P uptake in comparison with other cover crops. The higher P balance efficiency of the system was achieved under SSP in comparison with RP application but it seems that cover crops are more effective at improving the efficiency under RP compared to SSP.

Keywords: common vetch (*Vicia sativa*), white lupin (*Lupinus albus*), fodder radish (*Raphanus sativus*), ryegrass (*Lolium multiflorum*), black oat (*Avena strigosa*), Hedley P fractionation

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Soil Processes, a section of the journal Frontiers in Environmental Science

Received: *30 October 2019*
Accepted: *22 January 2020*
Published: *11 February 2020*

Citation:
Soltangheisi A, Teles APB, Sartor LR and Pavinato PS (2020) Cover Cropping May Alter Legacy Phosphorus Dynamics Under Long-Term Fertilizer Addition. Front. Environ. Sci. 8:13. doi: 10.3389/fenvs.2020.00013

INTRODUCTION

Phosphorus is the least mobile plant nutrient and a key limiting factor for crop development in agricultural systems (Hinsinger, 2001) and terrestrial ecosystems (Frossard et al., 1995). World P resources are finite and non-renewable, mostly concentrated in regions with territorial conflicts (Cordell and White, 2014). A sudden 800% rise in P fertilizer prices in 2008 gave rise to concern about the depletion of P resources (Cordell and White, 2011).

Phosphorus is being applied in high quantities to agricultural fields while the major part of it is being fixed by soil mineral particles and converted to soil pools with low plant availability over time (Takeda et al., 2009; Richardson et al., 2011), resulting in the high accumulation of legacy P in agricultural soils (Holford, 1997; Pearse et al., 2007). Withers et al. (2018) estimated that 106 Tg of legacy P will be accumulated in Brazilian agricultural lands by 2050 if current rate of inorganic P fertilizer use continues. Some plants like white lupin (*Lupinus albus*), pigeon pea (*Cajanus cajan*), rye (*Secale cereale*), and wheat (*Triticum aestivum*) are capable of using these less labile P fractions through different acquisition strategies and accumulate it in their aboveground plant parts (Braum and Helmke, 1995; Kamh et al., 1999; Raghothama, 1999; Wasaki et al., 2008, 2009). These crops can be used as cover crops in an integrated agricultural system (Vanlauwe et al., 2000; Horst et al., 2001), where the subsequent crops can take up P accumulated in their biomass after the decomposition, resulting in reduced demand of inorganic P fertilizers. Phosphorus released from cover crops after decomposition can be: (1) plant available [resin P and P extracted by 0.5 M $NaHCO_3$ in Hedley P fractionation (Hedley et al., 1982)]; (2) immobilized in microbial biomass (moderately labile for plant uptake); (3) transformed into soil organic P (moderately to poorly available for plant uptake) (Daroub et al., 2000; Sugito et al., 2001); and (4) lost from soils via erosion, runoff or leaching (Maltais-Landry and Frossard, 2015). Iyamuremye et al. (1996) observed that most of P released to the soil after mineralization of crop residues was accumulated in NaOH extractable P derived from Hedley P fractionation. Cover crops which are capable of acquiring higher amounts of P and subsequently release it from their residues in plant-available forms are more efficient in stimulating soil P cycling (Damon et al., 2014). The contribution of cover crop residues to P uptake by the main crop depends on residue type and management, and soil conditions (Thibaud et al., 1988; Nachimuthu et al., 2009). Revealed by isotope labeling, this contribution can be less than 25% when the residues are not incorporated to the soil (Noack et al., 2014). Residues with high C:N ratio, high lignin and low cellulose contents like the species of the Poaceae family including black oat (*Avena strigosa*) and rye decompose slower and release smaller amounts of P (Ferreira et al., 2014), while residues with low C:N ratio, low lignin and high cellulose contents like the species of Brassicaceae family, including oilseed radish (*Raphanus sativus*) decompose faster and release higher amounts of P to the soil (Doneda et al., 2012).

We hypothesized that (1) some cover crops can take up P from less labile fractions and recycle it back to the soil through plant residues, resulting in better P use efficiency of the system; (2) cover crops are capable of P uptake from subsurface layers which leads to the accumulation of this P on the surface after the decomposition of their residues; and (3) cover crop species respond differently to distinct inorganic P sources, altering the dynamics of P in soil profile.

MATERIALS AND METHODS

Experimental Design and Management Practices

A field experiment was conducted over nine successive years from 2009 to 2017 at the Federal Technological University of Paraná (UTFPR), Dois Vizinhos, located in Paraná State, South Brazil, in 25°44′05″S, 53°03′31″W and 509 m above the sea level. The soil is a clayey Rhodic Hapludox (Soil Survey Staff, 1999) with low pH (5.2–5.4) and high organic matter content (∼40 g kg^{-1}) in the 0–10 cm depth layer (Pavinato et al., 2017). This soil is representative of large areas of soybean [*Glycine max* (L.) Merr.] and maize (*Zea mays*) cultivation in Brazil. The area had been cropped with summer grain crops (soybean/maize) and winter wheat or cover crops (oat) since 2001 under a no-tillage cultivation system. The available P level (anion exchange resin) in the soil at experiment establishment was considered low in the 0–5 (8.1 mg kg^{-1}) and 5–10 (9.7 mg kg^{-1}) cm layers, and very low in the 10–20 (4.8 mg kg^{-1}) cm layer according to Raij et al. (1997). Iron oxide content, mineral assemblage by X-ray diffraction and P maximum adsorption capacity (2201 mg kg^{-1}) were also determined in a soil sample collected from the 0–20 cm layer before trial establishment and the results are accessible at Teles et al. (2017).

The experimental treatments were established as a split-plot randomized block design, in a 3 × 6 factorial scheme (considering three P treatments as main plot and six cover crop treatments as subplots) with three replicates. Plots with the dimension of 5 × 5 m were established in January 2009. Phosphate fertilizer sources were always applied at sowing of summer crops (maize or beans), as rock phosphate (RP from Algeria: 9% soluble P_2O_5 and 29% total P_2O_5) and soluble phosphate (SSP: 18% soluble/total P_2O_5). Both phosphate fertilizers were broadcasted over the whole area without incorporation before establishment of every summer crop. A treatment without P addition was also included (Nil-P). Species of winter cover crops were: common vetch (*Vicia sativa*), white lupin (*Lupinus albus*), fodder radish (*Raphanus sativus*), ryegrass (*Lolium multiflorum*), black oat (*Avena strigosa*), and a fallow (occasional weeds, not identified).

In January 2009, the phosphate fertilizers were first applied to a common bean (*Phaseolus vulgaris* L.) crop at a rate of 135 kg ha^{-1} of soluble P_2O_5 either as SSP or RP according to the respective treatments. This rate included an allowance to build up the background level of plant-available P in the soil (<10 mg kg^{-1}). Each subsequent summer, from 2010 to 2015, maize was cultivated with P fertilizer application at a rate of 105 kg ha^{-1} yr^{-1} of soluble P_2O_5 to meet the grain yield of

8.0 Mg ha^{-1}, except in 2012/13 when soybean was established with the same fertilizer rate, although it was not harvested because of excessive rain in that season. In this way, the total amounts of P added were 335 and 1078 kg ha^{-1} by SSP and RP, respectively (**Table 1**). Winter cover crops did not receive P fertilizer, to enable assessment of the residual P uptake left by the preceding summer crop. Phosphorus fertilization was halted after 2015 as anion exchange resin P reached high levels (>50 mg kg^{-1}). Potassium fertilizer was applied to summer crops as potassium chloride at sowing, according to standard recommendations (40 kg ha^{-1} of K_2O annually), but was not applied to the winter cover crops since the soil K levels were considered high (Sociedade Brasileira de Ciência do Solo [SBCS], 2016). Winter cover crop species received N topdressing (40 kg ha^{-1} of N as urea), except vetch and lupin which are leguminous species and can fix atmospheric N_2. Each maize crop received 120 kg ha^{-1} of N as urea, applied in three dressings of 40 kg ha^{-1} of N, at sowing, at V4–V5 stage and at V7–V8 stage.

Both winter and summer crops were established under a no-till cultivation system. Cover crops were established in the first half of May in all 9 years. Pure live seed rates were 70 kg ha^{-1} of common vetch, 100 kg ha^{-1} of white lupin, 20 kg ha^{-1} of fodder radish, 20 kg ha^{-1} of ryegrass, and 60 kg ha^{-1} of black oat. Row spacing was 20 cm between lines, except white lupin which was sown in 40-cm rows. Sowing lines were opened with a hand chisel and the seeds were distributed one by one along each row by hand. The cover crops biomass was desiccated with glyphosate [*N*-(phosphonomethyl) glycine] (2 L a.i. ha^{-1}) in the first half of September and left on the soil surface. Maize in the summer was established in the first fortnight of October in each year, with a final density of about 60,000 plants per hectare. The hybrid CD308 (Coodetec, Dow Agrosciences, Cascavel, PR, Brazil) was cultivated in the first two years, and the hybrid AGN30A30 (Agromen, Agromen Sementes Ltda, Orlândia, SP, Brazil) in the other years. Control of weeds, pests, and diseases were performed according to the recommended protocols.

TABLE 1 | Amounts of soluble and total P_2O_5 and total P applied with single superphosphate (SSP) and rock phosphate (RP) from 2009 to 2017.

Application time†	Soluble P_2O_5 (kg ha^{-1})		Total P_2O_5 (kg ha^{-1})		Total P (kg ha^{-1})	
	SSP	RP	SSP‡	RP$	SSP	RP
2009	135	135	135	435	59	190
2010	105	105	105	338	46	148
2011	105	105	105	338	46	148
2012	105	105	105	338	46	148
2013	105	105	105	338	46	148
2014	105	105	105	338	46	148
2015	105	105	105	338	46	148
2016	0	0	0	0	0	0
2017	0	0	0	0	0	0
Total	765	765	765	2463	335	1078

†*P sources were applied during the first fortnight of October each year.* ‡*SSP contains 18% soluble and total P_2O_5.* $*RP contains 9% soluble and 29% total P_2O_5.*

Soil and Plant Sampling and Analyses

Cover crop and maize tissue sampling was performed at flowering stage (for maize at VT stage), when the concentration of nutrients is normally at a high level, to determine dry matter (DM) accumulation and nutrient contents. For cover crops, aboveground biomass was collected from an area covering two row lines by 0.5 m long (0.40 m^2 for white lupin and 0.20 m^2 for other crops). Five subsamples were collected from each plot and thoroughly mixed to provide a composite plot sample. Maize was sampled by taking 10 representative plants from each plot. The crop samples were oven dried at 65°C for 72 h (or until constant weight), weighed (for DM determination), ground through a 2-mm sieve in a Wiley mill, and stored for later laboratory analysis. Concentration of P in plant tissues was determined according to the methodology described by Tedesco et al. (1995), and nutrient uptake (kg ha^{-1}) was calculated based on the total DM. The maize harvest was performed manually in early April of each year, by taking an area of four rows by 4 m in each plot. Grain yields were obtained after threshing the ears in a mechanical machine and reported at 13% grain moisture, a standard procedure for Brazilian crops (Elmore and Roeth, 1999).

Changes in soil P pools were evaluated in the 0–5, 5–10, and 10–15 cm soil layers, collected in September 2017, at management of the ninth cover crop cultivation, right before glyphosate application. Four soil subsamples were collected from each plot in the depths of 0–5, 5–10, and 10–15 cm and thoroughly mixed to provide a composite plot sample from each depth (0.5 kg). The soil samples were air-dried for 72 h (or until constant weight), ground through a 2-mm sieve and stored for later laboratory analysis. Chemical P fractionation was performed according to the procedure described by Hedley et al. (1982), with modifications by Condron et al. (1985) changing the original sonication step by 0.5 M NaOH extraction. This technique uses chemical extractants sequentially in the same sample to remove progressively from the most available to the most stable fractions of inorganic (Pi) and organic P (Po). Thus, the pools determined sequentially were as follows (Cross and Schlesinger, 1995): (i) labile pool, corresponding to the inorganic P extracted by anion exchange resin (P_{AER}) and the inorganic and organic P extracted by 0.5 M $NaHCO_3$ at pH 8.5 (Pi_{BIC} and Po_{BIC}); (ii) moderately labile (mod-labile) pool, corresponding to the inorganic and organic P extracted by 0.1 M NaOH ($Pi_{HID\text{-}0.1}$ and $Po_{HID\text{-}0.1}$), and inorganic P extracted by 1.0 M HCl (P_{HCl}); and (iii) non-labile pool, corresponding to the inorganic and organic P extracted by 0.5 M NaOH ($Pi_{HID\text{-}0.5}$ and $Po_{HID\text{-}0.5}$), besides residual digestion with $H_2SO_4 + H_2O_2$ in the presence of saturated $MgCl_2$ ($P_{residual}$). The P concentration in each extractant was measured according to Murphy and Riley (1962) procedure for acid extracts, and Dick and Tabatabai (1977) procedure for alkaline extracts. To measure organic P in alkali extracts, each fraction was digested with H_2SO_4 1:1 (acid:water) and ammonium persulfate 7.5%, and organic P was determined by the difference of concentration in the undigested (Pi) from the digested extract (total P).

Soil P pools to calculate P balance efficiency of the system were presented as kg ha^{-1} of P considering the soil bulk density of 1.2 g cm^{-3} to the depth of 15 cm. The amount of P exported from the field through the grains were calculated as a function of grain P concentration (%) and grain yield (kg ha^{-1}). Phosphorus balance efficiency was calculated by the following equation (Simpson et al., 2011; Boitt et al., 2018):

$$\text{P balance efficiency (\%)} = \frac{P_{\text{Output}}}{(P_{\text{Output}} + \Delta P_{\text{Soil}})} \times 100$$

To investigate the changes in the proportions of total P in different P fractions with time (**Figure 1**), data of 2009 and 2011 were obtained from Teles et al. (2017), data of 2014 were retrieved from Soltangheisi et al. (2018), and data of 2017 are the results of the current study. Each small square represents 1% of total P. Labile, mod-labile, and non-labile P fractions were shown with the shades of green, blue, and brown colors, respectively.

Data Analysis

Variance homogeneity and normality of data distribution were tested for each parameter before carrying out an analysis of variance (ANOVA). Data were transformed using Box-Cox techniques (Box and Cox, 1964) and outliers were removed when needed. Next, the data were submitted to ANOVA using PROC GLM to test the effect of phosphate fertilizer sources and cover crops on soil P fractions. When significant, means were compared using the *t*-test (LSD) ($p < 0.05$). When the interaction phosphate fertilizer source × cover crop was significant, means were tested using LSD ($p < 0.05$). All the statistical analyses were carried out using the SAS 9.3 program (Sas Institute, 2008).

RESULTS

Soil P Lability

Labile, mod-labile and non-labile P pools were influenced by the interaction of cover crops × P sources in 0–5 and 5–10 cm depth layers (**Table 2**). In 10–15 cm soil layer, cover crops did not affect P lability, while mod-labile and non-labile P were increased by P application (**Table 2**).

Labile P pool content increased with P application under all cover crops including fallow in both 0–5 and 5–10 cm layers, regardless of P sources (SSP and RP), except under white lupin in 5–10 cm which was similar to nil-P (**Table 2**). Under ryegrass cultivation, the highest value of labile P was recorded when RP applied, followed by SSP and nil-P in 0–5 cm depth, while in 5–10 cm the highest was observed under SSP, followed by RP and nil-P. Otherwise, for black oat it was the opposite, with higher values under SSP compared to RP in both layers. Cover crops did not modify the amount of labile P under nil-P in all depths evaluated. Under SSP, the highest labile P concentration was recorded under black oat cultivation in soil surface layer and under fallow in subsurface layer of 5–10 cm.

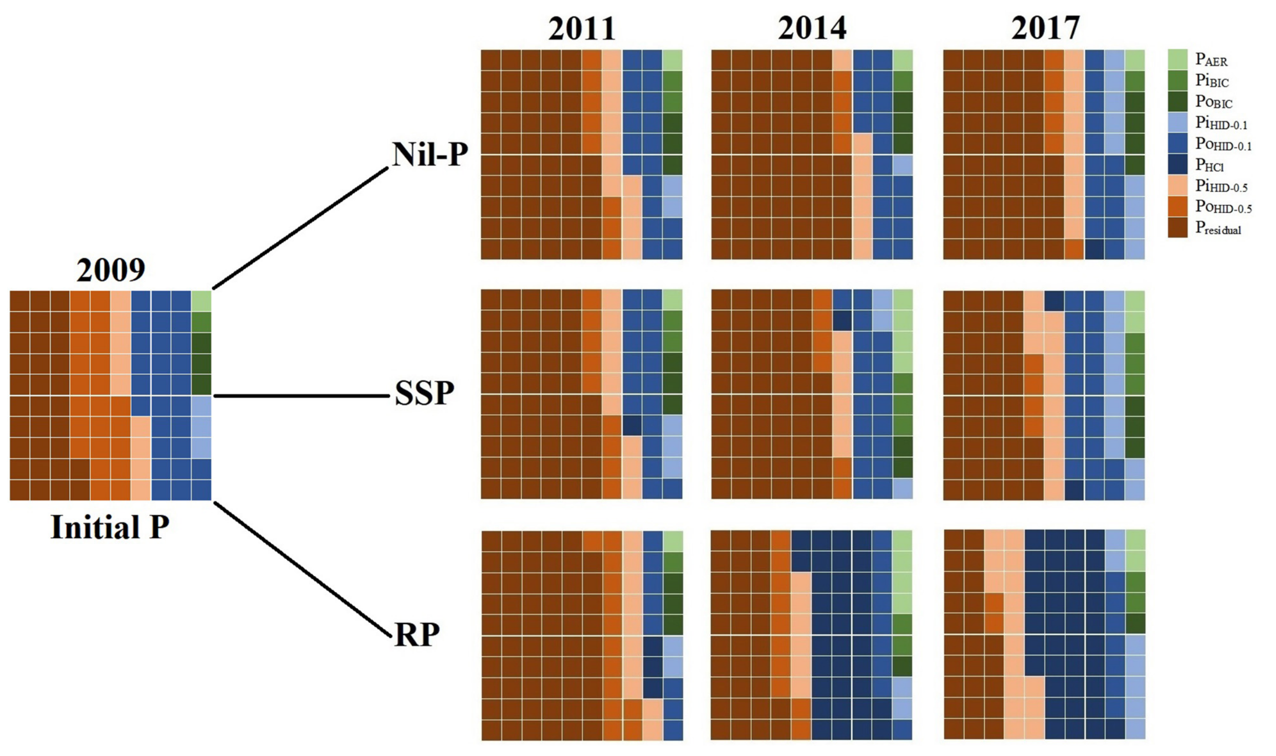

FIGURE 1 | Proportions of each P fraction under phosphate sources in 0–5 cm soil layer from 2009 to 2017. Each small square represents 1% of total P. Labile, mod-labile, and non-labile P fractions were shown with the shades of green, blue, and brown colors, respectively. 2009 and 2011 data were obtained from Teles et al. (2017). 2014 data were obtained from Soltangheisi et al. (2018). Data of 2017 are the results of the current study.

TABLE 2 | Labile, mod-labile and non-labile P in three soil layers under phosphate sources and cover crops after the 9th cultivation cycle of successive cover crops and cash crops.

	P fractions/phosphate source/depth											
	Labile P				Mod-labile P				Non-labile P			
	Nil-P	SSP	RP	Mean	Nil-P	SSP	RP	Mean	Nil-P	SSP	RP	Mean
Cover crops	mg kg^{-1}											
0–5 cm												
Fallow	73^{Ba}	150^{Ab}	131^{Aab}	118	287^{Ba}	505^{Ba}	1803^{Aab}	865	826^{Cbc}	910^{Ba}	1297^{Ab}	1011
Common vetch	67^{Ba}	134^{Abc}	158^{Aa}	120	292^{Ba}	544^{Ba}	2005^{Aa}	947	802^{Cc}	959^{Ba}	1362^{Aa}	1041
White lupin	69^{Ba}	147^{Ab}	136^{Aab}	117	271^{Ca}	514^{Ba}	1534^{Ac}	773	914^{Ba}	929^{Ba}	1352^{Aa}	1065
Fodder radish	74^{Ba}	116^{Ac}	115^{Ab}	102	298^{Ba}	48^{Ba}	1841^{Aab}	873	858^{Cb}	949^{Ba}	1394^{Aa}	1067
Ryegrass	78^{Ba}	110^{Bc}	140^{Aab}	109	328^{Ba}	477^{Ba}	1656^{Abc}	820	876^{Bab}	896^{Ba}	1352^{Aa}	1041
Black oat	75^{Ca}	180^{Aa}	132^{Bab}	129	284^{Ca}	567^{Ba}	1093^{Ad}	648	827^{Cbc}	914^{Ba}	1243^{Ac}	995
Mean	73	140	135		293	515	1655		851	926	1333	
5–10 cm												
Fallow	56^{Ba}	108^{Aa}	94^{Aa}	86	232^{Ca}	401^{Ba}	1047^{Aa}	560	780^{Ca}	879^{Bb}	1191^{Aa}	950
Common vetch	50^{Ba}	76^{Abc}	76^{Ab}	67	260^{Ca}	335^{Bab}	417^{Ab}	337	822^{Ba}	875^{Bb}	1032^{Abc}	910
White lupin	49^{Aa}	60^{Ad}	58^{Ad}	56	232^{Ba}	257^{Abc}	306^{Ac}	265	782^{Ca}	851^{Bb}	937^{Ad}	857
Fodder radish	53^{Ba}	68^{Acd}	5 9^{Abed}	60	239^{Aa}	295^{Abc}	30g^{Ac}	281	789^{Ba}	898^{Aab}	959^{Ad}	882
Ryegrass	52^{Ca}	89^{Ab}	70^{Bbc}	70	263^{Ca}	390^{Ba}	484^{Ab}	379	834^{Ca}	949^{Ba}	1041^{Ab}	941
Black oat	52^{Ba}	91^{Ab}	62^{Bcd}	68	219^{Ba}	347^{Aab}	315^{Ac}	294	807^{Ba}	902^{Aab}	962^{Acd}	890
Mean	52	82	70		241	338	480		802	892	1020	
10–15 cm												
Fallow	70	60	48	59ns	216	255	224	232ns	801	812	808	807^{ns}
Common vetch	54	52	73	60	170	287	223	227	769	847	946	854
White lupin	49	58	56	54	219	276	250	248	708	777	913	799
Fodder radish	45	53	60	53	209	264	233	235	763	851	829	814
Ryegrass	44	55	54	51	191	239	245	225	749	783	832	788
Black oat	39	63	71	58	175	268	252	232	739	799	823	787
Mean	50^{ns}	57	60		197^{B}	265^{A}	238^{A}		755^{C}	812^{B}	859^{A}	

Within each depth and P fraction, means followed by the same capital letter in line and tiny in column were not significantly different at $p < 0.05$ by LSD test. Nil-P, without phosphate application; SSP, single superphosphate; RP, rock phosphate; ns, non-significant.

Under RP application the highest labile P was observed under common vetch in 0–5 cm soil layer, and under fallow in 5–10 cm depth (**Table 2**).

Mod-labile P pool was the highest under RP application for all cover crop treatments in the top surface layer (**Table 2**). This P pool was changed by phosphate sources, with substantial increase under RP, followed by SSP application. Under nil-P, cover crops did not modify mod-labile P in all soil layers. The same trend was observed under SSP application in 0–5 and 10–15 cm depths, while in 5–10 cm layer the highest and the lowest mod-labile P pool was detected under fallow and white lupin, respectively. Under RP application, mod-labile P reached a maximum under common vetch cultivation in 0–5 cm, being 1.8 times higher than under black oat which was the lowest in this layer. In 5–10 cm depth, the highest mod-labile P was recorded under fallow, 2.6 times higher than the average of the cover crops. In 10–15 cm the mod-labile P was affected only by P sources being higher under P application compared to nil-P, irrespective of the source (**Table 2**).

In general, non-labile P pool was the highest under RP application for all soil depths investigated, except for fodder radish and black oat in 5–10 cm layer in which SSP and RP were similar, but both higher than nil-P (**Table 2**). Under nil-P, the highest amount of non-labile P pool in 0–5 cm was recorded for white lupin and the lowest for common vetch, while in 5–10 cm it was not affected by cover crops. Under SSP application non-labile P pool was not affected by cover crops in 0–5 and 10–15 cm layers, while it reached a maximum under ryegrass in 5–10 cm depth. Under RP application the lowest non-labile P was observed under black oat in 0–5 cm, while the highest was measured under fallow in 5–10 cm. Although cover crops did not influence non-labile P in 10–15 cm depth, it was affected by P sources, being the highest under RP followed by SSP and nil-P (**Table 2**).

Organic, Inorganic, and Total P

Inorganic P was affected by the interaction between P sources and cover crops in all depths (**Table 3**). The highest amount of inorganic P was measured under RP application for all cover crops and in all soil depths investigated, while in general significant differences were not observed between SSP and nil-P (**Table 3**). Cover crops did not influence inorganic P under nil-P in 0–5 and 5–10 cm soil layers, while in the deepest soil layer

TABLE 3 | Inorganic, organic and total P in three soil layers under phosphate sources and cover crops after the 9th cultivation cycle of successive cover crops and cash crops.

	P fractions/phosphate source/depth											
	Inorganic P				**Organic P**				**Total P**			
	Nil-P	**SSP**	**RP**	**Mean**	**Nil-P**	**SSP**	**RP**	**Mean**	**Nil-P**	**SSP**	**RP**	**Mean**
Cover crops	**mg kg^{-1}**											
0–5 cm												
Fallow	865^{Ba}	1141^{Ba}	3041^{Aab}	1682	307^{Ba}	424^{Ab}	190^{Cc}	307	1172^{Ca}	1565^{Bb}	3231^{Aab}	1989
Common vetch	881^{Ba}	1120^{Ba}	3343^{Aa}	1781	288^{Ba}	519^{Aa}	163^{Cc}	323	1169^{Ca}	1693^{Ba}	3506^{Aa}	2105
White lupin	985^{Ba}	1111^{Ba}	2639^{Ac}	1578	268^{Ca}	471^{Aab}	384^{Ba}	374	1253^{Ba}	1582^{Bb}	3023^{Ab}	1953
Fodder radish	921^{Ba}	1139^{Ba}	3009^{Aab}	1690	309^{Ba}	408^{Ab}	340^{ABab}	352	1230^{Ca}	1547^{Bb}	3349^{Aa}	2042
Ryegrass	941^{Ba}	1058^{Ba}	2783^{Abc}	1594	332^{Ba}	407^{Ab}	364^{ABab}	368	1273^{Ba}	1465^{Bb}	3147^{Aab}	1962
Black oat	890^{Ca}	1164^{Ba}	2165^{Ad}	1406	295^{Ba}	497^{Aa}	303^{Bb}	365	1185^{Ca}	1661^{Ba}	2468^{Ac}	1771
Mean	914	1122	2830		300	454	291		1214	1577	3121	
5–10 cm												
Fallow	803^{Ca}	1048^{Bab}	2040^{Aa}	1297	265^{Aa}	290^{Ab}	292^{Aab}	282	1068^{Ca}	1338^{Bab}	2332^{Aa}	1579
Common vetch	878^{Ba}	1015^{Bab}	1197^{Abc}	1030	254^{Ba}	275^{ABbc}	328^{Aa}	286	1132^{Ca}	1290^{Babc}	1525^{Ab}	1316
White lupin	835^{Ba}	950^{Bb}	1085^{Ac}	957	228^{Aa}	219^{Ad}	216^{Ac}	221	1063^{Ba}	1169^{Bc}	1301^{Ac}	1178
Fodder radish	831^{Ba}	1025^{Aab}	1118^{Ac}	991	250^{Aa}	237^{ABcd}	207^{Bc}	231	1081^{Ba}	1262^{Abc}	1325^{Ac}	1223
Ryegrass	894^{Ca}	1141^{Ba}	1337^{Ab}	1124	255^{Aa}	278^{Abc}	254^{Abc}	262	1149ca	1419^{Ba}	1591^{Ab}	1386
Black oat	843^{Ba}	975^{ABb}	1086^{Ac}	968	235^{Ba}	354^{Aa}	252^{Bbc}	280	1078^{Ba}	1329^{Aab}	1338^{Ac}	1248
Mean	847	1026	1311		248	276	258		1095	1301	1569	
10–15 cm												
Fallow	883^{Aa}	893^{Aab}	879^{Ab}	885	203	234	202	213ns	1086^{Aa}	1127^{Aab}	1081^{Ab}	1098
Common vetch	820^{Bab}	948^{Aa}	830^{Bb}	866	173	238	257	223	993^{Bb}	1186^{Aa}	1087^{Bb}	1089
White lupin	784^{Cb}	881^{Bab}	985^{Aa}	883	192	230	230	217	976^{Bb}	1111^{Aab}	1215^{Aa}	1101
Fodder radish	832^{Bab}	936^{Aa}	903^{Ab}	890	185	232	219	212	1017^{Bab}	1168^{Aa}	1122^{Aab}	1102
Ryegrass	809^{Bb}	865^{ABb}	894^{Ab}	856	175	211	232	206	984^{Bb}	1076^{Ab}	1126^{Aab}	1062
Black oat	776^{Bb}	923^{Aab}	910^{Aab}	870	177	216	235	209	953^{Bb}	1139^{Aab}	1145^{Aab}	1079
Mean	817	908	900		184^{B}	227^{A}	229^{A}		1002	1135	1129	

Within each depth and P fraction, means followed by the same capital letter in line and tiny in column were not significantly different at $p < 0.05$ by LSD test. Nil-P, without phosphate application; SSP. single superphosphate; RP, rock phosphate; ns, non-significant.

the highest amount was recorded under fallow (**Table 3**). While cover crops did not modify inorganic P under SSP application in soil surface layer, some small changes were observed in 5–10 and 10–15 cm depths. When RP was applied, the highest amount of inorganic P was detected under common vetch, 54% higher than under black oat which was the lowest in 0–5 cm soil layer. In 5–10 cm the amount of inorganic P under fallow was 1.8 times higher than the average of the cover crops (**Table 3**).

Organic P was influenced by P sources × cover crops interactions in 0–5 and 5–10 cm soil layers, while it was only affected by P sources in 10–15 cm (**Table 3**). In the 0–5 cm layer organic P was the highest under SSP for all the cover crop treatments in comparison with nil-P and RP. Under nil-P cover crops did not affect the amount of organic P in all depths. Under SSP the highest amount of organic P was recorded under common vetch and black oat in 0–5 cm, and under black oat in 5–10 cm, being 62% higher than under white lupin which showed the lowest content of organic P in this depth. Under RP organic P reached a maximum when ryegrass was cultivated, while the lowest was observed for common vetch in 0–5 cm. In 5–10 cm the highest and the lowest organic P were measured under common vetch and fodder radish, respectively (**Table 3**). While the content of organic P increased by phosphate application compared to nil-P in 10–15 cm soil layer, it was not different among P sources (SSP and RP) (**Table 3**).

Total P in soil surface layer followed the trend: RP > SSP > nil-P except under white lupin and ryegrass in which there were no differences between SSP and nil-P (**Table 3**). The same trend was observed in 5–10 cm depth except under white lupin in which there was not significant difference between SSP and nil-P, and under fodder radish and black oat in which significant differences were observed between SSP and RP (**Table 3**). In 10–15 cm the total P was similar among SSP and RP, while both P sources were higher than nil-P except for fallow in which total P was the same under P sources and nil-P. Under nil-P, cover crops did not influence total P in 0–5 and 5–10 cm depths, while in 10–15 cm the highest amount was under fallow compared to cover crops (**Table 3**). When SSP was applied, the highest total P content in soil surface layer was recorded under common vetch and black oat as cover crops. In 5–10 cm, the highest total P was measured under ryegrass, being 21% higher than white lupin which was the lowest one

(**Table 3**). Under RP application, the highest and the lowest total P contents were observed for common vetch and black oat, respectively, with a difference of 1038 mg kg^{-1} in 0–5 cm. In 5–0 cm, total P under fallow was 1.6 times higher than the average of all cover crops. Among cover crops in this depth, total P under common vetch and ryegrass was higher than the others (**Table 3**).

Proportions of P Fractions

The proportion of total P in P_{AER} was not changed under nil-P and constituted 1% of total P in all years averaged among cover crops (**Figure 1**). When phosphates were applied (SSP and RP), P_{AER} proportion increased to 4% in 2014 and then decreased to 2% in 2017 for both P sources as a consequence of no P fertilization in the last 2 years. Proportion of Pi_{BIC} followed the same trend but it did not exceed 3% of total P (**Figure 1**). When RP was applied, Po_{BIC} was reduced from 3% in 2009 to 1% of total P in 2014 and 2017. Labile P pool (sum of green colors) constituted 6, 8, and 5% of total P under nil-P, SSP, and RP in 2017, after seven consecutive years of P application and 2 years under the use of legacy P (**Figure 1**).

The proportion of $Pi_{HID\text{-}0.1}$ was enhanced from 3% in 2009 to 9, 10, and 7% of total P under nil-P, SSP, and RP, respectively, in 2017 (**Figure 1**). $Po_{HID\text{-}0.1}$ proportion decreased from 2009 to 2017 in all years and under all P sources (nil-P, SSP, and RP) (**Figure 1**). P_{HCl} was negligible in 2009 and its proportion was not affected by SSP application over the time, while RP increased its proportion to 3, 34, and 38% in 2011, 2014, and 2017, respectively (**Figure 1**). Proportion of mod-labile P pool (sum of the shades of blue colors) was reduced with time under nil-P, from 31% in 2009 to 24% in 2017 (**Figure 1**). When P sources were applied, this proportion was lessened in the first 2 years of P application (in 2011), and then enhanced reaching 33 and 52% of total P under SSP and RP, respectively, in 2017 (**Figure 1**).

The proportion of $Pi_{HID\text{-}0.5}$ was not changed from 2009 to 2017 under nil-P (9% of total P), while it was enhanced with P application (**Figure 1**).%$Po_{HID\text{-}0.5}$ reduced with time from 23% in 2009 to 6, 4, and 2% under nil-P, SSP, and RP, respectively, in 2017 (**Figure 1**). $P_{residual}$ constituted 32% of total P in 2009 (**Figure 1**). Under nil-P, its proportion increased to 55% after 9 years. When SSP was applied, its proportion increased to 57% after 2 years (in 2011), and then reduced with time reaching 42% of total P in 2017. Under RP application, %$P_{residual}$ enhanced to 59% in 2011, and then reduced with time, reaching the same levels of the initial amount in 2014 (32%) and reducing to 25% in 2017, being 7% lower than the initial condition. Proportion of non-labile P pool (sum of the shades of brown colors) increased with time under nil-P from 64% of total P in 2009 to 70% in 2017 (**Figure 1**). When P sources were applied, it was increased in the first 2 years (2011), and then gradually reduced reaching 59 and 43% of total P under SSP and RP application, respectively, in 2017 (**Figure 1**). The latter was dropped more due to the proportional increase in mod-labile P pool under RP.

P Balance Efficiency and P Uptake by Cover Crops

In general, P balance efficiency was 2.2 times higher under SSP application (48% averaged among cover crops) compared to RP application (22% averaged among cover crops) (**Table 4**). Under SSP application, white lupin (55%) and ryegrass (54%) showed the best P balance efficiency, higher than other species and fallow, while under RP application all cover crops (>19%) had P balance efficiency higher than fallow (15%). Among cover crops, black oat resulted in the highest P balance efficiency (27%) under RP application compared to other species (**Table 4**).

Cumulative maize grain yield from 2009 to 2017 was enhanced with P application related to nil-P, while different P sources and cover crops did not influence it (**Supplementary Table S1**).

TABLE 4 | P inputs, pools, outputs, and balance efficiency after 9 years in the cropping system receiving single superphosphate (SSP) and rock phosphate (RP).

	SSP						RP					
	Fallow	Common vetch	White lupin	Fodder radish	Ryegrass	Black oat	Fallow	Common vetch	White lupin	Fodder radish	Ryegrass	Black oat
	kg P ha^{-1}											
Inputs												
P added via inorganic fertilizer†	335	335	335	335	335	335	1078	1078	1078	1078	1078	1078
Pools												
Soil P pool‡	1325	1306	1268	1325	1299	1368	2215	1640	1820	1933	1879	1608
P in nil-P plots	1092	1082	1097	1109	1125	1072	1092	1082	1097	1109	1125	1072
ΔP_{soil}	233	224	171	216	174	296	1123	558	723	824	754	536
Outputs												
Grain P export§	213	196	211	195	202	186	202	201	223	199	204	197
P balance efficiency (%)	48	47	55	47	54	39	15	26	24	19	21	27

†Total P added via inorganic P fertilizers (SSP and RP) after 7 years (2009–2015). P sources were applied during the first fortnight of October each year. ‡Total soil P in 0–15 cm soil layer. §Total amount of P exported in grain in 9 years (2009–2017) as a function of P content in grain (%) and grain yield (kg ha^{-1}). Average grain yield from 2009 to 2017 were 4297, 6177, and 6416 kg ha^{-1} under nil-P, SSP, and RP, respectively. Average grain P content was 3.6 kg Mg^{-1}. ¶P Balance efficiency(%) = $\frac{P_{Output}}{(P_{Output} + \Delta P_{Soil})} \times 100$.

Considering the efficiency of cover crops to take up and mineralize (recycle) P over the time, fodder radish was the most profitable one with taking up 232 kg ha^{-1} of P in plant tissue from 2009 to 2017, being 2.1 times higher than the average of the other cover crops (112 kg ha^{-1} of P), while P uptake was not different among other cover crops (**Figure 2**). Averaged among cover crops, P uptake by them under P application (150–160 kg ha^{-1}) was higher than nil-P (100 kg ha^{-1}), with no changes between SSP and RP (**Figure 2**).

DISCUSSION

In P-unfertilized cropping system, the amounts of labile and mod-labile P fractions were not modified by cover crops related to fallow, showing that cover crops did not take up P from these fractions or they took it up and recycled it back to the soil through plant residues. As cover crops did not show any difference with fallow for non-labile P, the second scenario is more plausible meaning that they took up P from labile and mod-labile fractions and refilled those pools through their biomass. It has been observed that some plants like wheat (Wang et al., 2008; Vu et al., 2010) and common bean (Li et al., 2008) can take up greater amount of available P compared to other crops due to their large root system which explores greater volumes of soil and reducing rhizosphere pH by releasing protons, which potentially can alter soil P lability over the time with crop residue deposition. Acquiring P from non-labile fractions by lupin (Braum and Helmke, 1995; Bais et al., 2006; Le Bayon et al., 2006; Shane et al., 2008; Wang et al., 2008) and ruzigrass (*Urochloa ruziziensis*) (Almeida and Rosolem, 2016) has been previously reported by many researchers, presumably due to the excretion of high amounts of organic acids (Le Bayon et al., 2006), which was not observed in this study as the amount of non-labile P under white lupin cultivation was higher than fallow in soil surface layer, under both P sources and even under nil-P.

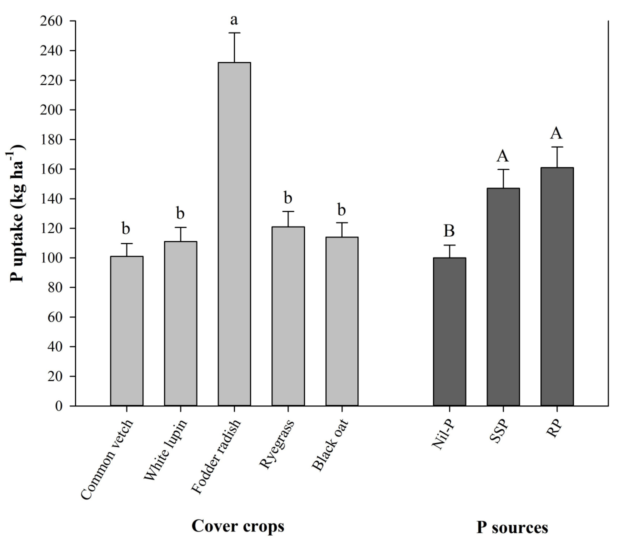

FIGURE 2 | Cumulative shoot P uptake by winter cover crops from 2009 to 2017 affected by cover crops and P sources. Mean values followed by the same tiny letter in cover crops and capital letter in P sources do not differ by *t*-test (LSD, $P < 0.05$).

When inorganic P fertilizers were applied, the amount of labile and mod-labile P pools under fallow were higher than under cover crops in the second soil layer evaluated (5–10 cm), showing that cover crops were able to take up P from less labile P fractions in subsurface layers and accumulate it on the surface after the decomposition of their residues (**Table 2**). Our research shows that their effectiveness is limited to 10 cm soil depth as we did not see changes in 10–15 cm depth. Black oat and common vetch cycled more labile P under SSP and RP, respectively. Contrary to our results, many studies did not observe the effect of cover crops on labile P fractions when available P was not limited (Kuo et al., 2005; Takeda et al., 2009; Rick et al., 2011).

The amount of organic P was also altered by the cultivation of distinct cover crops (**Table 3**). When SSP was applied, common vetch promoted the highest accumulation of organic P on the surface, while under RP application, this cover crop showed the lowest organic P level and ryegrass was responsible for the highest level on the soil surface layer. Nziguheba et al. (2000) and Sugito et al. (2001) observed an increase in organic P when plant residues were added to soil, while Tiecher et al. (2012) did not observe the effect of different winter crops on organic P measured by ignition method. Although not evaluated in this study, it seems likely that acid phosphatase enzyme activity under common vetch and RP was much higher than the other cover crops as organic P was less than half of the average of the other cover crops. Tiecher et al. (2012) detected the highest activity of this enzyme under vetch cultivation in comparison with oat, radish, lupin, wheat, and fallow. Tarafdar and Jungk (1987) also reported the depletion of organic P in the rhizosphere of wheat due to enhanced phosphatase activity. Common vetch is a non-mycorrhizal plant species which exudes higher amounts of acid phosphatase to compensate the lack of mycorrhizal fungi related to mycorrhizal plant species (Dalla Costa and Lovato, 2004; Kunze et al., 2011). Hallama et al. (2019) in a meta-analysis also showed that extracellular phosphatase activity was enhanced by 20% with cover cropping, being Fabaceae as one of the most effective plant families. Some studies have shown that besides inorganic P, organic P increases with continuous inorganic P application due to higher P uptake and consequently P addition to soil by cover crops and higher synthesis of organic P by soil microbiota (Stewart and Tiessen, 1987; George et al., 2007). This was observed in our study only when SSP was applied. Under RP application, organic P was reduced compared to nil-P. Averaged among cover crops, 38% of P was accumulated as P_{HCl} fraction under RP application (**Supplementary Table S2**). This P fraction is stable in acid soils (Tiessen and Stewart, 1985; Beck and Sanchez, 1994). Black oat was capable to change this P fraction as the amount of P_{HCl} reduced to half of the other cover crops in soil surface layer. Better RP utilization by black oat is also evidenced by its higher P balance efficiency (27%) compared to the others. Rapeseed was previously introduced as a crop which can efficiently utilize RP in calcareous soils (Habib et al., 1999; Chien et al., 2003), while we recommend black oat for clayey Oxisols; however, it is noteworthy that the interaction between P sources and cover crops is complex and site-specific recommendations are needed (Romanyà and Rovira, 2009; Hallama et al., 2019). The possible mechanism adapted by these crops to utilize RP is the higher proton release by their roots to improve the dissolution of RP in rhizosphere, as reported by many scientists (van Diest, 1981; Bekele et al., 1983; Ruiz and Arvieu, 1990; Hinsinger and Gilkes, 1995; Zoysa et al., 1998).

Averaged among cover crops, the amount of labile P in soil surface layer under phosphate sources was two times higher than nil-P which indicates that the adsorption sites have been saturated after 7 years of P application regardless of P source, resulting in weakening the binding energy of P. This was also observed by other studies in soil subsurface layer in no-tillage system (Rheinheimer et al., 2000; Tiecher et al., 2012; Wyngaard et al., 2012). Under SSP application, $Pi_{HID\text{-}0.1}$ and $P_{residual}$ were sinks of applied P, while under RP application, mostly P_{HCl} and to some extent Pi_{HID} (sum of $Pi_{HID\text{-}0.1}$ and $Pi_{HID\text{-}0.5}$) played this role (**Supplementary Table S2**). After 18 years of continuous P application in tropical Ultisols, Beck and Sanchez (1994) found out that $Pi_{HID\text{-}0.1}$ acts as the sink for fertilizer P added to soil. Stewart et al. (1987) and Dobermann et al. (2002) observed that the excess inorganic P fertilizer entered mostly to Pi_{HID} in Ultisols and Oxisols. Otherwise, in less weathered soils, Po_{HID}, P_{HCl} and $P_{residual}$ were found as the primary sink of applied P (Schmidt et al., 1996; Zhang and MacKenzie, 1997).

Higher P uptake by some cover crops like sorghum, oat, rye, and vetch was previously observed by Karasawa and Takahashi (2015). Higher P uptake by fodder radish in comparison with other cover crops in our research is in accordance with Teles et al. (2017) and Soltangheisi et al. (2018) in a 3-years and 6-years data evaluation of this experiment, respectively. Hallama et al. (2019) also observed high P concentrations in Brassicaceae, like radish. According to Calegari et al. (2013), it is expected that cover crops with higher P uptake (black oat and blue lupin in their research) cycle a higher amount of P, resulting in the higher content of labile and mod-labile P fractions, while we did not observe it in the soil layers under fodder radish cultivation; however, its P uptake was 2.1 times higher than the average of the other cover crops after 9 years. It can be stated that fodder radish is a suitable cover crop when soluble P fertilizers are applied, as it accumulates high amount of P in its tissues and protect P from losses via erosion or leaching, without reducing available P for the summer cash crop. Other studies in Brazilian Oxisols showed that radish enhanced malic acid and soil P availability in comparison with other cover crops (Carvalho et al., 2008; Pavinato et al., 2008). The higher P uptake capacity of fodder radish can be the result of its different P acquisition strategy and needs more investigation. Under limited P condition, the uptake and consequently P release by cover crops was reduced significantly compared to high P condition, where the plots were fertilized. This was previously observed by Blair and Boland (1978) and many other studies. When soil P is restrictive, P released by cover crops would be immobilized by microbial biomass (Bünemann et al., 2012) which reinforces the need for P application for the subsequent crop.

Focusing on organic fractions, Po_{BIC}:Po_{HID} ratio under nil-P reached to 25% (**Supplementary Table S2**) which shows that Po is an important source of plant available P in the absence of P inputs (Kuo et al., 2005), while it was 14 and 15% under SSP and RP, respectively. Under nil-P, Po_{HID} (sum of $Po_{HID\text{-}0.1}$ and $Po_{HID\text{-}0.5}$) was depleted by 31%, from 51% in 2009 to 20% in 2017, which indicates that these organic P fractions are acting as a source of available P over the time. As mentioned by some authors, without P fertilization, organic P contribution to supply P for plant uptake is higher than inorganic P (Daroub et al., 2001; Soltangheisi et al., 2019).

As expected, the higher P balance efficiency of the system was achieved under SSP in comparison with RP application but it seems that cover crops are more effective at improving the efficiency under RP as this improvement was not observed under SSP application considering the differences between fallow and cover crops. Simpson et al. (2011) presented phase farming as one of the management options to improve the P balance efficiency of farming systems; however, they mentioned that more field studies are required as the majority of the evidences came from glasshouse experiments. Our long-term field experiment confirmed their hypothesis.

CONCLUSION

Cover crops evaluated in our study were able to take up P from less labile P fractions in subsurface layers and accumulate it on the surface after the decomposition of their residues. Among them, black oat and common vetch cycled more labile P under SSP and RP, respectively. Organic P accumulation on the soil surface was also enhanced with common vetch and ryegrass cultivation under SSP and RP, respectively.

Under RP effect, black oat was capable to remobilize calcium-P to more labile P fractions as the amount of it reduced to half of the other cover crops in soil surface layer. Better RP utilization by black oat is also evidenced by its higher P balance efficiency compared to the other cover crops in our study in clayey Oxisol. Fodder radish took up the highest amount of P in comparison with other cover crops, being suitable when soluble P fertilizers are applied as it accumulates high amounts of P in its tissues and protect it from runoff/erosion losses.

The highest P balance efficiency was achieved under SSP compared to RP application. Otherwise, cover crops were more effective at improving the efficiency under RP considering the balance between fallow and cover crops.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

AS conceived the data analysis and finalized all text, figures, and tables. LS collected plant and soil samples. PP and LS set up the experiment. AT guided the laboratory work. All authors contributed to the main text.

FUNDING

The authors are grateful to São Paulo Research Foundation (FAPESP), which supported the scholarship to AS (Grant Nos. 2017/11332-5 and 2019/21532-7).

ACKNOWLEDGMENTS

We thank the National Council for Scientific and Technological Development – CNPq for funding this research under project 574950/2008-7. We also thank the Federal Technology University of Paraná, Dois Vizinhos (UTFPR), and College of Agriculture "Luiz de Queiroz" – ESALQ/USP for providing the experimental and laboratory facilities and necessary materials for the research, development and analysis.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2020.00013/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.