LEGACY PHOSPHORUS IN AGRICULTURE: ROLE OF PAST MANAGEMENT AND PERSPECTIVES FOR THE FUTURE

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LEGACY PHOSPHORUS IN AGRICULTURE: ROLE OF PAST MANAGEMENT AND PERSPECTIVES FOR THE FUTURE

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Editorial: Legacy Phosphorus in Agriculture: Role of Past Management and Perspectives for the Future

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Editorial on the Research Topic

Legacy Phosphorus in Agriculture: Role of Past Management and Perspectives for the Future

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INTRODUCTION

Phosphorus (P) is an essential nutrient for higher plants and the primary source of this nutrient is from soil. However, many soils globally are P deficient and phosphate fertilizer input is necessary for agricultural production. Part of applied P is accumulated in the soil in non-labile forms due to its chemical high-affinity reactions and occlusion to soil minerals and organic matter, known as "legacy P." Continued fertilizer applications beyond plant requirements have led to a build-up of legacy P in soils. Unfortunately, this imbalanced P input: output ratio is necessary in most cases to maintain satisfactory yields in conventional agricultural systems, ultimately promoting environmental concerns such as eutrophication, if transferred to water bodies. Moreover, as economically viable phosphate rock is finite and non-renewable, agricultural systems need to be redesigned for a better balance (input = output) of P and postpone a future scarcity scenario.

In this Research Topic we have collected contributions from North America, South America and Australasia, highlighting the impacts of legacy P on agricultural and environmental sustainability. Moreover, some agronomic approaches are described which have the potential to enable the use of legacy P more effectively and allow more accurate predictions on the ability to reduce legacy P on the environment, while maintaining sustainable production of crops.

IMPACT OF LEGACY-P AS SOURCE OF P TO CROPS

In a long-term (45 years) land use assessment, Zhang et al. (2020a) demonstrate that cropping systems, even with and without fertilization, lead to increased conversion of organic legacy P into inorganic forms compared to native land-use. Cropping without fertilization reduced legacy P significantly, while cropping with fertilization maintained or increased legacy P, particularly in pastures. This work suggests that fertilization leads to soil P accumulation and increase risk of environmental degradation. This was also confirmed in Zhang et al. (2020b) where after 11 years of cultivation, in which the yield and P uptake by the maize-soybean crops was not affected by withdrawal of P fertilizer down to the critical level, legacy P was reduced by 3.3 mg kg^{-1} yr^{-1}. In fact the reliance on legacy P improved farmers' economic margins and reduced the soil test P levels to safe levels for surrounding catchments.

IMPACTS OF AGRONOMIC MANAGEMENT ON LEGACY P

Soil Management Impacts

The impact of cover crops and fertilizer source on legacy P was investigated by Soltanghesi et al. (2020), demonstrating that cover crops are able to improve soil P availability for the following cash crop, but the effectiveness was dependent on the species and the source of P added, with rock phosphate being more effective compared to soluble P source. Nunes et al. (2020) assessed a long-term (17 years) tillage trial and demonstrated that the combination of fertilizer source and placement had an impact on the distribution and accumulation of legacy P. Under no-till, P tended to accumulate at the surface, in both labile and mod-labile forms, while conventional tillage tended to dilute P down the profile, with a more pronounced effect on legacy P for rock phosphate. This has impacts on both the availability of P to the crop and the potential transfer to the wider environment by erosion or runoff.

Fertilization With Alternative Nutrient Sources

Addition of sewage sludge to forestry can both reduce the amount of mineral fertiliser needed and increase labile P relative to non-labile P, suggesting an improved availability and use of legacy P, as proved by Abreu-Junior et al. (2020). While, Zhang X et al. (2020) demonstrated that papermill biosolids (PB) could be used as an effective alternative P-fertiliser, as its addition increased the labile P fractions in soil. However, only around a third of the total P added in the PB was recovered in the labile pool, suggesting a substantial addition to the legacy P as well. Taken together these findings suggest that alternative bulky organic waste products may be useful fertilisers. However, little is known about their short and long-term impact on P cycling and legacy.

Addition of Biologicals

The addition of P solubilizing bacteria (PSB) showed increase in PUE by maize, although some PSB's were more effective than others, as presented by Pereira et al. (2020). Similarly, Rosa et al. (2020) demonstrate that PSB increased PUE of sugarcane and led to more efficient use of fertilisers. In addition, both papers confirm that combinations of bacteria may be even more effective than single species, suggesting that PSBs may reduce the soil legacy P by increasing PUE from fertilizer, improving long-term efficiency in the production system.

IMPACTS OF REDUCING FERTILIZATION ON LEGACY P

One of the key ways to reduce legacy P is to reduce fertiliser input. While this practice may reduce environmental risk, it needs to sustain cropping production. Messiga et al. (2020) demonstrate that P added as a "starter" in maize production systems can be reduced by up to 75% without effecting yield, as crops rely more on legacy P in over-fertilized soils. Changing this agronomic practice will reduce the risk of P transfer to watercourses. This approach is investigated more in Tyson et al. (2020) who describe a model which is able to predict the impact of reducing P fertilization rates on achieving agronomic optimum P concentrations in pasture systems and demonstrates that it is likely to take decades to bring the legacy P down in over-fertilized pasture soils. Likewise, McDowell et al. (2020) demonstrates that it is possible to reduce legacy P by reducing P fertiliser inputs, being possible to achieve the optimal agronomic targets in a relatively short time, one year for most soils in New Zealand. However, they highlight that the ability to reach the environmental target for reduced pollution of water can take several decades and may not compliment the aim of maintaining agricultural output.

PROSPECTS FOR FUTURE MANAGEMENT OF LEGACY P

Papers in this Research Topic expose a dichotomy in the concept of legacy P. In over-fertilized young soils where soils storage capacity is saturated, reducing inputs of P and changing the agronomic practices to utilize legacy P are environmentally beneficial, with no impact on the sustainability of the production system. However, in old highly weathered soils the accumulation of P to available pools at the expense of large legacy P pools should not be the focus. In the latter case a number of agronomic interventions need to be better understood to allow legacy P exploration and avoid the unsustainable accumulation of legacy P. Future research should generate understanding on the mechanisms involved in the dynamics of legacy P and how this can be manipulated and allow us to deploy the interventions, some of which are highlighted here, to maximum effect.

AUTHOR CONTRIBUTIONS

LG, GB, PP, and TG contributed equally to defining the scope for this research topic. They worked outreaching the call for submissions, and they edited the manuscripts submitted to this research topic.

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The Ability to Reduce Soil Legacy Phosphorus at a Country Scale

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The build-up of soil phosphorus (P) beyond plant requirements can lead to a long-term legacy of P losses that could impair surface water quality. Using a database of ∼4,50,000 samples collected from 2001–2015 we report the level of soil P enrichment by soil type, land use and region and the time it would take for Olsen P to decline to agronomic targets (20–0 mg L^{-1}) if P fertilizer was stopped. We also modeled the time it would take for water extractable P (WEP), an indicator of P losses in surface runoff, to decline to an environmental target (0.02 mg L^{-1}). Some 63% of the samples were enriched beyond agronomic targets. The area-weighted median time to reach the agronomic target was predicted to occur within a year for 75% of samples but varied up to 11.8 years in some land uses. However, the area-weighted time to reach an environmental target was 26–55 years for the 50th and 75th percentile of areas. This indicates that while an agronomic target can be easily met, additional strategies other than stopping P fertilizer inputs are required to meet an environmental target.

Keywords: Olsen phosphorus, soil test phosphorus, surface runoff, water extractable phosphorus, leaching

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INTRODUCTION

The loss of phosphorus (P) from land can impair surface water quality (Carpenter, 2008). Much (30–90%) of the total P lost from a grassland or cropping farms originates from the loss of soil particles (McDowell et al., 2007). Other sources of P loss include dung or manure from grazing animals, fertilizer and plant residues (Hart et al., 2004; Dougherty et al., 2008). The quantity of P loss reaching surface water increases linearly or curvilinearly with increasing soil P concentrations but is also influenced by the soil's sorption capacity (McDowell and Condron, 2004; Nair, 2014). For instance, a soil with a high anion storage capacity (ASC; 0–100%), a measure of the soil aluminum (Al) and iron (Fe) oxide concentration, will have a greater P sorption capacity than a low ASC soil. If two soils have the same soil test P (STP) concentration, the low ASC soil will release more P into solution than the high ASC soil. Due to this variation in the STP (e.g., Olsen P) and ASC relationship, the ability to decrease P losses to a concentration not likely to impair surface water quality varies widely. This dissolved P could be lost to surface runoff or leaching or mopped up by the soil biota or plant roots (Dodd et al., 2014b). However, the amount of P taken up by plants and soil biota decreases beyond agronomic optimum STP concentrations where production becomes limited by other factors (Zhang et al., 2017).

The addition of fertilizer-P to soils aims to establish optimal STP concentrations. Due to differences in ASC or other measures of soil P sorption, recommended fertilizer rates to reach agronomic optima vary by soil and crop type. However, it has been common practice to apply a little extra P when cash is available as an insurance policy against poor years when little P may be applied (van Dijk et al., 2016). Furthermore, in confined animal feeding operations, there is often an imbalance between the amount of P brought in as feed and the amount of land that can receive the resulting manure-P (van Dijk et al., 2016). The over application of fertilizer and manure results in STP concentrations that are enriched beyond agronomic optima (Sharpley and Tunney, 2000). Enriched STP concentrations therefore represent a source of P that can result in a long legacy of P losses even if the balance is made negative to decrease STP concentrations (Sharpley et al., 2013).

The most extreme version of a negative P balance involves no application or returns of P to the soil. Research has shown that in grassland systems with little erosion and a similar climate this no-P scenario results in a rate of decline that reflects the initial STP concentration and ASC (Dodd et al., 2013; Coad et al., 2014). Other management strategies are available to decrease P losses while improving the chances of being profitable. Such strategies include the addition of nitrogen (N) fertilizer to enhance P-limitation and the uptake of P by pasture plants, or tillage to redistribute and dilute enriched topsoil P concentrations throughout the plough layer (Dodd et al., 2014a,b). However, these strategies cannot be used everywhere. For example, applying too much N will enhance N-leaching and impair surface and groundwater quality, while conventional tillage will promote mineralization and the loss of carbon and N and increases the risk of soil erosion (Shepherd et al., 1996; Bilotta et al., 2007).

The drive for profit is a common factor in rapid land use change. In New Zealand, the number of dairy cows increased from 2.9 M in 1996 to 5.0 M in 2016, much of this has been onto land traditionally used for drystock sheep and beef grazing (DairyNZ, 2016; McDowell et al., 2017). In reflecting greater stocking rates, most dairy-farmed land will be maintained at a greater STP concentration than land used for sheep and beef farming. At the beginning of the dairy expansion in 2001, Wheeler et al. (2004) noted that depending on the region, 30–65% of samples from dairy farms had Olsen P concentrations greater than that required for optimal pasture production. A preliminary analysis of Olsen P data from 2001–2015 suggested that increases in Olsen P were still happening in some regions, but the magnitude of enrichment was unclear (McDowell et al., 2019).

The objective of our paper was to determine: (1) the state as of 2016 and any trends over the last 15 years in soil Olsen P concentrations, and (2) to determine the time it would take soils enriched with P to decrease to an agronomic or environmental optimum for their land use and soil group.

MATERIALS AND METHODS

Soil Database

Data were obtained for soil Olsen P concentrations from the three major commercial laboratories in New Zealand (ARL, Eurofins, Hills Labs). This data comprises soil test results for various commercial agricultural enterprises undertaken to assess soil fertility and fertilizer requirements. The database covered the years 2001–2015 and contained ∼4,50,000 data points. Each laboratory has international standards (ISO 9000) relevant to the collection, analysis and recording of Olsen P data. All dairy and drystock samples were from the 0–7.5 cm depth, while cropping samples were from the 0–15 cm depth.

Each sample had metadata describing region (16 local authorities) and land use [dairy, drystock (sheep, sheep and beef, and red deer), cropping or horticulture]. The Eurofins and Hills Labs data (c. 2,30,000 samples) had data for soil group (ash, peat, pumice, and sedimentary). These groups corresponded to New Zealand soil orders (Hewitt, 2010) of Organic (Peat), Pumice (Pumice), Allophanic (Volcanic), and all other soil orders (Sedimentary), their regional distribution can be found in **Supplementary Table 1**. Equivalent classifications in United States Soil Taxonomy are: Fibrists, Hemists or Saprists (Organic); Vitraquands, Vitrands or Vitricryands (Pumice); Aquands, Cryands, and Udands (Allophanic); and with all other classes combined within the Sedimentary group (Hewitt, 2010). The soil groups combine soil orders with a similar relationship between Olsen P and production, and provide the basis for fertilizer management recommendations in New Zealand (Sparling et al., 2008). The ARL samples (c. 2,20,000) did not have soil group data but were identifiable at a finer spatial scale by post code and sometimes the name of the enterprise. To attribute post code to soil group we intersected the location of a sample's post code with the New Zealand Fundamental Soil Layers (Newsome et al., 2008).

We estimated the water extractable P (WEP) concentration (mg L^{-1}) of each sample using the equation of McDowell and Condron (2004). WEP estimates dissolved reactive P (DRP) concentrations in surface runoff from New Zealand soils (McDowell and Condron, 2004). The equation (Eq. 1: $r^2 = 0.83$; $P < 0.001$) used Olsen P and ASC (%) (Saunders, 1965) to predict WEP:

$$\text{WEP (mg L}^{-1}) = 0.03\left(\text{Olsen P/ASC}\right) + 0.03 \qquad (1)$$

As input data we combined median region-by-land use-by-soil group-specific Olsen P concentrations with location specific ASC data mapped nationally at a 1:50,000 scale within the New Zealand fundamental soil layer (Newsome et al., 2008).

Targets

We used targets for soil Olsen P concentrations that corresponded to an agronomically optimum level of production and a WEP concentration set as an environmental target unlikely to induce excessive levels of periphyton biomass in streams and rivers.

Agronomic targets were set for different land uses within each soil group (Nicholls et al., 2009; Roberts and Morton, 2009; Morton and Roberts, 2016). Targets for pasture production on drystock farms were taken as the 95th percentile of maximum pasture growth from Morton and Roberts (2016) and Edmeades et al. (2006). These concentrations were 20 and 22 mg L^{-1}, respectively in Sedimentary and Volcanic soils, and 38 mg L^{-1} for Pumice and Peat soils. The Olsen P corresponding to the 95th percentile was also used as a target dairy farms using data from Roberts and Morton (2009) and O'Connor et al. (2001). Targets for dairy farms were set at 25 mg L^{-1} for Volcanic and Sedimentary soils and 40 mg L^{-1} for Peat and Pumice soils. After considering the Olsen P requirements of the main arable and horticultural crops in New Zealand, targets were set at 18 mg L^{-1} for Volcanic and Sedimentary soils (Nicholls et al., 2009) and 25 mg L^{-1} for Pumice and Peat soils (Sparling et al., 2008). We note that agronomic targets for vegetable production tend to be high (Reid and Morton, 2019), but the area under vegetable production only represented a third of horticultural land use and was therefore not considered widespread enough to have its own target. We set a WEP target of 0.02 mg L^{-1} as the environmental target. This target captured all default guideline values for DRP concentrations (also called filterable reactive P) across lowland rivers or lakes of warm or cool climates set by the Australian and New Zealand Governments (2018). A slightly more restrictive target (0.018 mg L^{-1}) is currently being considered as part of freshwater policy (Ministry for the Environment, 2019).

Analyses

Median concentrations of soil Olsen P and WEP were derived for the 2010–2015 years. This range was chosen as an indicator, unlikely to be influenced by trends, of the present state of these parameters in each region by land use by soil group combination. Because the data were skewed, concentrations for each region, soil group and land use, and their interactions, were compared as medians using an analysis of variance of ranked data. Data from 2001–2015 for each region by land use by soil group combination with >500 data values were analyzed for trends in the annual enrichment or depletion of median Olsen P concentrations using a non-parametric Mann-Kendall test within the Time Trends software package (Jowett, 2009). We chose 500 data points as a cut-off to increase the likelihood that combinations would have good spatial and temporal representativeness. If the associated P value of the Mann-Kendall test was small (<0.05), the null hypothesis can be rejected; in other words, the observed trend, either upward or downward, is most unlikely to have arisen by chance. We presented significant trends as a relative change in median concentrations by dividing the Sen Slope estimate by the median. This allowed for the direct comparison between region by soil group by land use combinations measured as the percentage change per year. A positive change indicates an overall increasing trend, while a negative change indicates an overall decreasing trend.

To estimate the time to reach an agronomic and environmental target we used the equations of Dodd et al. (2013). We recognize that these equations were derived for grassland. We therefore advise caution when interpreting predictions for cropping or horticultural land use. However, due to small area in New Zealand under cropping or horticultural land use (<5% of productive land) this was not thought to influence estimates at a national scale.

We calculated the time (t, in years) that it would take for P-enriched soils for each region by land use by soil group combination to decrease to a target Olsen P concentration (Olsen P_t in mg L^{-1}) as:

$$t = 1/(0.032 - 2.81 \times \mathrm{WEP}_i) \times \left(\ln \text{Olsen } \mathrm{P}_t - \ln \text{Olsen } \mathrm{P}_i\right) \quad (2)$$

where WEP_i is the initial WEP concentration calculated via Eq. 1 and Olsen P_i is the initial median Olsen P for the between 2010–2015.

We calculated the time (t, in years) that it would take for P-enriched soils to decrease to a target WEP concentration (WEP_t), set at 0.02 mgP L^{-1} for each region by land use by soil group combination as:

$$t = 1/(-0.035 \times \ln \text{Olsen } \mathrm{P}_i/\mathrm{ASC} - 0.0455) \times \left(\ln \mathrm{WEP}_t - \ln \mathrm{WEP}_i\right) \quad (3)$$

Equations 1–3 were calibrated by Dodd et al. (2012) to soils of Olsen $P > 15$ and high ASC (<72). Concentrations of WEP are likely to be below the environmental target if Olsen $P < 15$ and ASC is >50% (McDowell and Condron, 2004), therefore t was set to zero in these situations.

Data for the time to deplete the median region by soil group by land use combination for soil Olsen P or WEP to their respective agronomic or environmental target in each combination or at the land use, soil group or national level were also calculated as area-weighted medians. Areas for each combination are given in **Supplementary Table 1**.

RESULTS

State and Trend of Olsen P Concentrations

Within the database, Peat, Pumice, Sedimentary and Volcanic soils had 7,561, 16,109, 3,42,537, and 54,749 samples in them, respectively. Most soils were sampled under dairy land (2,21,035), followed by drystock (1,41,775), cropping (47,398) and horticulture (10,748). The median number of samples within a region by land use by soil group combination was 380 while the mean was 3,028. The number of samples measured per year was slightly greater in later years.

Median Olsen P concentrations across region by land use by soil group combinations are given in the **Table 1**. These concentrations range from a low of 6 mg L^{-1} for drystock Peat soils in the Northland region to 106 mg L^{-1} for horticultural Volcanic soils in the Taranaki region. An analysis of variance using ranked data indicated that there were significant differences ($P < 0.001$) between regions, land uses, soil groups and for the interactions between these terms. Pumice soils were more enriched (44 mg L^{-1}) than Peat (40 mg L^{-1}), Volcanic (37 mg L^{-1}) or Sedimentary (26 mg L^{-1}) soils, while Horticulture was more enriched (39 mg L^{-1}) than dairy (32 mg L^{-1}), cropping (22 mg L^{-1}) or drystock (20 mg L^{-1}) land uses. Across the regions, the Bay of Plenty had the greatest

TABLE 1 | Median Olsen P concentrations (mg L^{-1}) in each region by soil group by land use (Hort = horticulture) combination.

Region	Peat				Pumice				Sedimentary				Volcanic			
	Cropping	Dairy	Drystock	Hort	Cropping	Dairy	Drystock	Hort	Cropping	Dairy	Drystock	Hort	Cropping	Dairy	Drystock	Hort
Auckland	31	52	34	–	–	–	–	–	40	38	22	26	30	40	19	9
Bay of Plenty	23	43	29	43	47	47	25	48	36	39	18	49	48	41	22	40
Canterbury	–[1]	28	13	–	–	–	–	–	22	28	21	24	–	–	–	–
Gisborne	–	–	–	–	–	19	–	–	34	25	19	41	–	–	–	–
Hawkes Bay	–	–	–	–	19	22	17	34	25	29	20	36	13	32	20	29
Manawatu/ Wanganui	–	24	–	–	–	22	7	–	25	31	19	94	20	38	18	22
Marlborough	–	–	–	–	–	–	–	–	25	28	20	22	–	–	–	–
Nelson	–	–	–	–	–	–	–	–	–	29	23	–	–	–	–	–
Northland	33	42	6	–	–	–	–	–	29	37	24	38	32	32	34	34
Otago	–	26	9	–	–	–	–	–	21	28	19	21	–	–	–	–
Southland	20	18	17	–	–	–	–	–	20	27	21	24	–	–	–	–
Taranaki	–	38	–	–	–	–	–	–	29	32	20	–	24	41	26	106
Tasman	–	–	–	–	–	–	–	–	24	29	21	52	–	–	–	–
Waikato	33	43	23	21	44	48	25	33	31	43	21	34	26	40	20	29
Wellington	–	–	–	–	–	–	–	–	25	32	19	23	–	–	–	–
West Coast	–	–	–	–	–	–	–	–	11	29	20	–	–	–	–	–

Note that using an analysis of variance of ranks, significant differences were noted for median concentrations of region, soil group, land use and for all their interactions. [1] *Combination does not exist or insufficient data available to calculate.*

TABLE 2 | Mean annual percentage change in the median concentration of Olsen P for each region by soil group by land use (Hort = horticulture) combination over 2001–2015.

Region	Peat				Pumice				Sedimentary				Volcanic			
	Cropping	Dairy	Drystock	Hort	Cropping	Dairy	Drystock	Hort	Cropping	Dairy	Drystock	Hort	Cropping	Dairy	Drystock	Hort
Auckland	ns[1]	2.5%	ns	–	–	–	–	–	6.6%	−0.9%	ns	ns	ns	−0.9%	ns	ns
Bay of Plenty	ns	ns	ns	ns	ns	ns	−0.6%	ns	ns	ns	−5.2%	ns	ns	−1.8%	ns	1.4%
Canterbury	–[2]	ns	ns	–	–	–	–	–	ns	ns	ns	ns	–	–	–	–
Gisborne	–		–	–	–	ns	–	–	ns	ns	ns	ns	–	–	–	–
Hawkes Bay	–		–	–	ns	ns	−4.0%	ns	5.3%	ns	0.1%	3.4%	ns	ns	ns	ns
Manawatu/ Wanganui	–	ns	–	–	–	ns	ns	–	2.1%	ns	3.0%	ns	ns	1.3%	−2.1%	16.2%
Marlborough	–	–	–	–	–	–	–	–	ns	−3.2%	1.3%	ns	–	–	–	–
Nelson	–	–	–	–	–	–	–	–	ns	ns	ns	–	–	–	–	–
Northland	ns	1.3%	ns	–	–	–	–	–	ns	−0.5%	1.7%	4.2%	ns	−1.6%	5.3%	ns
Otago		ns	ns	–	–	–	–	–	ns	−1.1%	ns	ns	–	–	–	–
Southland	ns	ns	ns	–	–	–	–	–	1.3%	ns	ns	ns	–	–	–	–
Taranaki	–	ns	–	–	–	–	–	–	ns	ns	ns	–	ns	ns	ns	ns
Tasman	–	–	–	–	–	–	–	–	ns	ns	ns	5.2%	–	–	–	–
Waikato	ns	ns	ns	ns	ns	0.8%	ns	ns	ns	ns	−1.6%	ns	−1.8%	−0.3%	ns	ns
Wellington	–	–	–	–	–	–	–	–	ns	ns	ns	ns	–	–	–	–
West Coast	–	–	–	–	–	–	–	–	ns	ns	ns	–	–	–	–	–

[1] *No significant trend was detected.* [2] *Combination does not exist or insufficient data available to calculate.*

concentration while Otago had the least, presumably due to the prevalence of horticulture or dairying in the Bay of Plenty and drystock in Otago.

Of the 124 combinations, 32 showed significant trends in concentrations from 2001 to 2015 (**Table 2**). Of these, 18 were increases that ranged from 0.8% per year under dairy Pumice soils in the Waikato to 16.2% under horticultural Volcanic soils in the Manawatu-Wanganui region. Decreases ranged from −0.5% under dairy Sedimentary soils in Northland to −5.2% per annum under drystock Sedimentary soils in the Bay of Plenty region. Across New Zealand there was a mean rate of increase in the median concentration of 1.2% per annum. Although modest, this trend tallies with an increase in the number of soils that exceeded an agronomic target set by Wheeler et al. (2004) for dairy and drystock land uses sampled between 1988 and 2001 (**Figure 1**). On an area basis those soils that were above the agronomic target represented 7.1 M ha (63%) or 11.3 M ha of productive land.

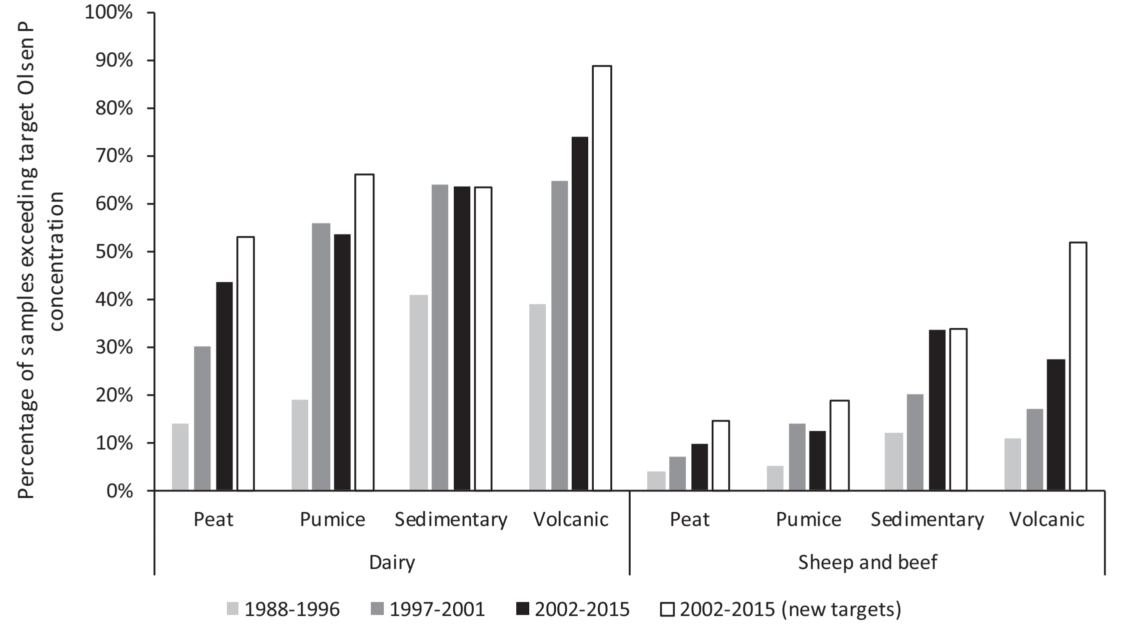

FIGURE 1 | The percentage of samples collected in 1988–1996, 1996–2001, and 2002–2015 exceeding target ranges in Olsen P for each soil group in dairy or sheep and beef land use. Data for 1988–2001 and targets (45, 45, 25, and 30 mg L^{-1} for Peat, Pumice, Sedimentary, and Volcanic soils, respectively) were taken from Wheeler et al. (2004). Targets used in this paper are referred as "new targets."

Time to Deplete Samples to Their Agronomic or Environmental Target

Median Olsen P concentrations were above their respective soil group-by-land use-specific agronomic target in 82 of the 124 combinations (**Table 3**). Across all combinations the national area-weighted median time to deplete samples to the agronomic target was <1 year but was as high as 11 years in horticultural Volcanic soils in the Taranaki region (**Figure 2**). This is similar to the 8–10 years reported by McCollum (1991) for a mid-Atlantic sandy loam soil that was 30–40% above an agronomic target for corn, but less than the 30–40+ years estimated in areas of the United Kingdom, United States, and Finland for soils that were where often >100% above agronomic targets (Withers et al., 2019).

Across all soil groups, the longest area-weighted time to reach the agronomic target was in the Volcanic soils (3.3 years), followed by the Pumice (2.3 years), Peat and Sedimentary (<1 year) soils. Amongst land uses, horticultural soils were estimated to take the longest time to reach the target (3.3 years) followed by dairy, cropping and drystock soils at 2.3, 1.9, and <1 year, respectively. The area-weighted time for the 75th percentile was 1 year and the corresponding time to reach the Olsen P target was 3.7, 2.6, 1.2, and 1.2 years for Volcanic, Pumice, Sedimentary and Peat soils, respectively.

Compared to the time to reach an agronomic target, the median time to reach the environmental target was much greater: the national area-weighted time was 26 years and ranged from 0 years for land uses of Peat soils in Southland to >100 years for six land uses of Volcanic soils (**Table 4** and **Figure 2**). Volcanic soils took longer to reach the environmental target (36.8 years), followed by Pumice soils (36.7 years), Sedimentary soils (30.7 years), and Peat soils (27.3). There was little time separating land uses with drystock (32.0 years) taking the longest to reach the target, followed by dairy, cropping and horticulture taking 30.0, 28.5, and 26.0 years, respectively. The area-weighted time for the 75th was 39 years and the corresponding time to reach the WEP target was 55, 42, 37, and 36 years for Volcanic, Pumice, Sedimentary and Peat soils, respectively.

DISCUSSION

Bias and Representativeness

Some care is needed when interpreting our dataset. There are questions over bias, representativeness, and the repeatability of measurements. Although we have isolated soil Olsen P concentrations to the post code level, no information was available to determine if the samples submitted represented the range of Olsen P concentrations present within a post code or within a land use by soil group combination. We also had no information to determine if a disproportionate number of samples were being sent from enterprises with low or high Olsen P concentrations or if multiple samples were coming from the same enterprises over time. However, analysis of data from one of the providers, ARL, enabled the enterprise to be identified in about 20,000 samples. Of these samples, <5% of enterprises were represented more than once on a sampling date or had multiple samples over the 15-year period of record.

Bias is also possible due to errors in recording a sample's location and assignment to the appropriate soil group. Related to

TABLE 3 | Median and the 75th percentile (in parentheses) of the time (years) taken for Olsen P to decrease to agronomic targets in each region by soil group by land use (Crop = Cropping, Hort = horticulture) combination if fertilizer P was withheld.

Region	Peat				Pumice				Sedimentary				Volcanic			
	Crop	Dairy	Drystock	Hort	Crop	Dairy	Drystock	Hort	Crop	Dairy	Drystock	Hort	Crop	Dairy	Drystock	Hort
Auckland	2.4 (2.6)	1.7 (2.6)	0 (0)	–	–	–	–	–	6.3 (8.1)	3.3 (3.3)	0.6 (1.0)	3.6 (4.4)	6.1 (6.1)	4.9 (4.9)	0 (0)	0 (0)
Bay of Plenty	0 (0)[1]	0.5 (0.6)	0 (0)	4.1 (5.3)	4.5 (5.5)	1.2 (1.2)	0 (0)	4.6 (4.6)	5.8 (6.4)	3.6 (3.9)	0 (0)	7.0 (7.0)	6.9 (9.0)	4.6 (4.9)	0 (0)	8.1 (8.1)
Canterbury	–[2]	0 (0)	0 (0)	–	–	–	–	–	1.4 (1.4)	0.7 (0.7)	0.3 (0.5)	1.8 (1.8)	–	–	–	–
Gisborne	–	–	–	–	–	0 (0)	–	–	3.9 (5.5)	0 (0)	0 (0)	4.4 (6.4)	–	–	–	–
Hawkes Bay	–	–	–	–	0 (0)	0 (0)	0 (0)	2.3 (2.7)	2.1 (2.1)	0.9 (1.4)	0 (0)	3.4 (3.4)	–	3 (3)	0 (0)	–
Manawatu/ Wanganui	–	0 (0)	–	–	–	0 (0)	0 (0)	–	2.5 (3.3)	1.2 (1.9)	0 (0)	3.7 (7.2)	1.4 (1.4)	4.4 (4.9)	0 (0)	2.8 (2.9)
Marlborough	–	–	–	–	–	–	–	–	2.1 (2.1)	1.1 (1.1)	0 (0)	1.4 (1.4)	–	–	–	–
Nelson	–	–	–	–	–	–	–	–	–	1.4 (1.5)	1.5 (1.6)	0 (0)	–	–	–	–
Northland	0.4 (2.4)	0.2 (0.3)	0 (0)	–	–	–	–	–	2.7 (5.3)	1.9 (3.2)	1.2 (1.9)	6.1 (7.7)	6.3 (6.3)	2.9 (3.2)	4.7 (5.3)	7.0 (7.8)
Otago	–	0 (0)	0 (0)	–	–	–	–	–	0.8 (1.1)	0.6 (0.7)	0	0.4 (0.8)	–	–	–	–
Southland	0 (0)	0 (0)	0 (0)	–	–	–	–	–	1.4 (1.4)	0.5 (0.7)	0.3 (0.4)	3 (3)	–	–	–	–
Taranaki	–	0 (0)	–	–	–	–	–	–	5.2 (5.5)	2.6 (2.9)	0 (0)	0 (0)	3.8 (3.9)	5.4 (5.5)	2.2 (2.2)	11.5 (11.5)
Tasman	–	–	–	–	–	–	–	–	1.9 (3.2)	1.4 (1.4)	0.5 (0.5)	3.9 (4.8)	–	–	–	–
Waikato	2.7 (3.4)	0.6 (0.8)	0 (0)	0 (0)	4.2 (4.2)	1.3 (1.3)	0 (0)	2.5 (2.5)	4.8 (6.0)	4.1 (5.3)	0.5 (0.6)	5.4 (6.8)	4.6 (4.6)	5.2 (5.2)	0 (0)	6.1 (6.1)
Wellington	–	–	–	–	–	–	–	–	2.1 (3.1)	1.3 (2.2)	0 (0)	1.7 (2.6)	–	–	–	–
West Coast	–	–	–	–	–	–	–	–	0 (0)	1.1 (1.2)	0 (0)	–	–	–	–	–

[1] *Already at or below the agronomic target Olsen P concentration.* [2] *Combination does not exist or insufficient data available to calculate.*

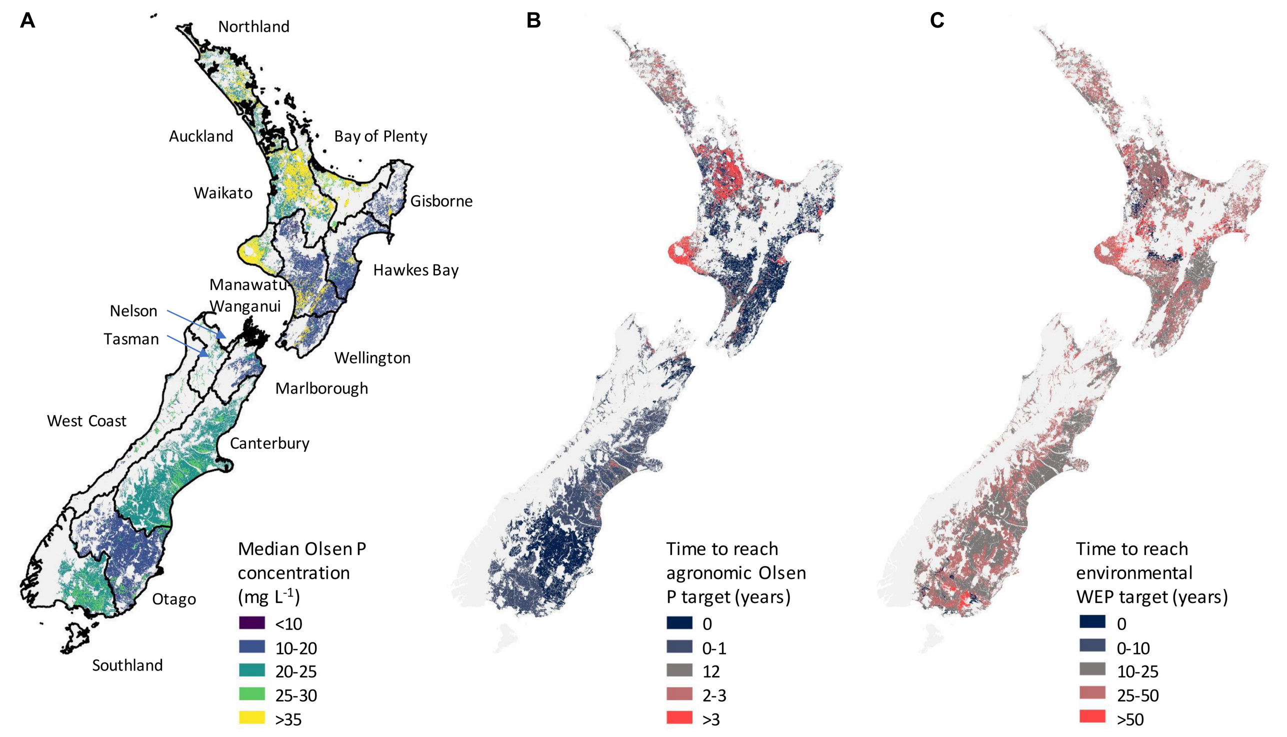

FIGURE 2 | Maps showing the distribution of median topsoil Olsen P (mg L^{-1}) concentrations **(A)**, and the number of years it would take areas to decrease their soil median soil Olsen P and water extractable P concentrations (mg L^{-1}) to agronomic **(B)** and environmental **(C)** targets, respectively.

this, the calculation of timeframes to deplete soil P to agronomic and environmental targets is subject to errors associated with the use of default ASC values for specific soil types. The original equations generated by Dodd et al. (2012) utilized ASC derived analytically for each specific soil using the method of Saunders (1965). This error will be exacerbated using ASC to estimate WEP concentrations for Olsen P in equation 1. However, to minimize this bias, we cross-checked locations with their soil group as per the New Zealand Fundamental Soil Layer (Newsome et al., 2008) and removed those that were in the wrong location. Fewer than 1% of samples met this criterion. Little bias was found in the number of samples within each Soil group, 2% (Peat), 4% (Pumice), 80% (Sedimentary) and 13% (Volcanic) which were very similar to the aerial coverage in these groups at 1, 5, 85, and 9%, respectively.

State and Trends in Olsen P Concentrations

The majority of region by soil by land use by soil group combinations had median concentrations that were close to their agronomic target. However, of those soils with an Olsen P concentration above the agronomic target a large proportion were in Volcanic dairy soils (**Figure 1**). Soils used for horticulture, specifically vegetable production, can have high Olsen P concentrations (>100 mg L^{-1}) (Cavanagh et al., 2019). However, this was only occasionally detected in regions. Before 2003, the recommended Olsen P concentration for Volcanic dairy soils were based on a single trial in the Taranaki region which showed production benefits to Olsen P concentrations >50 mg L^{-1} (Morton et al., 2003). The target has subsequently been refined to sit at 20–30 mg L^{-1} (note we chose a mid-point representing a 95th percentile of pasture production as 25 mg L^{-1}), but it will take time for recommendations to be reflected in fertilizer application rates and decreased soil Olsen P concentrations.

There were few trends in Olsen P concentrations. This is despite significant land use change in some regions. For instance, dairy cattle numbers in Canterbury, Otago, and Southland increased from 5,76,632 to 1,767,763 from 2001 to 2015 (Livestock Improvement, 2001; DairyNZ, 2016). Land use was from sheep and beef farming, which traditionally maintained a lower Olsen P than dairying. The absence of trends could have been caused by a decrease in the number of sheep and beef samples with an Olsen P above the target. However, no negative trends were found in areas with large increases in dairy cattle numbers. Furthermore, the proportion of samples above the agronomic target had not decreased compared to 1997–2001 when dairy cattle numbers were just starting to increase (**Figure 1**). A more likely reason for the absence of trends were that a small proportion of samples that came from the same farm in the dataset (<5%). Coupled with on-farm spatial variation in P, which can be as high as 100% in many grassland farms (McDonald et al., 2019), the likelihood of detecting a change associated with repeated measures is probably low. Trends within a land use such as dairy farms may not occur as New Zealand dairy farms are largely able to balance P inputs and outputs. For

TABLE 4 | Median and the 75th percentile (in parentheses) of the time (years) taken for water extractable P (WEP) to decrease to environmental targets in each region by soil group by land use (Crop = Cropping, Hort = horticulture) combination if fertilizer P was withheld.

Region	Peat				Pumice				Sedimentary				Volcanic			
	Crop	Dairy	Drystock	Hort	Crop	Dairy	Drystock	Hort	Crop	Dairy	Drystock	Hort	Crop	Dairy	Drystock	Hort
Auckland	48 (100)	23 (34)	23 (27)						25 (34)	26 (26)	24 (39)	33 (72)	64 (64)	39 (39)	0 (23)	0 (0)
Bay of Plenty	37 (37)	25 (29)	30 (54)	25 (32)	24 (28)	24 (24)	34 (34)	24 (24)	26 (29)	25 (28)	25 (52)	24 (24)	24 (29)	30 (33)	40 (89)	34 (34)
Canterbury		24 (24)	31 (31)						23 (23)	22 (23)	24 (42)	23 (23)				
Gisborne						76 (76)			23 (27)	24 (34)	28 (35)	22 (25)				
Hawkes Bay					36 (52)	39 (100)	64 (64)	24 (27)	23 (23)	22 (30)	24 (33)	22 (22)		88 (88)	100 (100)	
Manawatu/ Wanganui		23 (36)				40 (40)	77 (77)		24 (34)	22 (29)	25 (35)	22 (23)	100 (100)	36 (52)	0 (55)	100 (100)
Marlborough									23 (23)	31 (31)	24 (45)	23 (23)				
Nelson										30 (33)	37 (49)	47 (72)				
Northland	28 (28)	22 (25)	18 (74)						22 (44)	22 (26)	23 (35)	26 (36)	46 (46)	44 (82)	41 (69)	40 (67)
Otago		23 (23)	18 (22)						23 (24)	22 (24)	24 (34)	21 (23)				
Southland	0 (24)	0 (21)	0 (21)						24 (44)	23 (32)	24 (43)	35 (35)				
Taranaki		40 (52)							44 (59)	38 (55)	27 (100)	0 (18)	100 (100)	42 (46)	100 (100)	23 (23)
Tasman									23 (45)	30 (30)	42 (42)	21 (22)				
Waikato	32 (70)	24 (35)	23 (37)	24 (24)	24 (24)	24 (24)	34 (34)	28 (28)	29 (49)	25 (32)	24 (42)	27 (42)	100 (100)	49 (49)	0 (45)	100 (100)
Wellington									23 (28)	22 (28)	25 (35)	23 (37)				
West Coast									43 (66)	26 (30)	27 (45)					

A gap indicates that the combination does not exist or insufficient data is available to calculate.

instance, the land areas available for the application of farm dairy effluent is sufficiently large enough to avoid soil P-enrichment.

Variation in the Rate of Olsen P and WEP Decline

Following the cessation of fertilizer-P additions the rate of decline in Olsen P has been shown to vary according to soil type and the initial STP concentration (Dodd et al., 2012). Soils with a greater initial STP concentration decrease at a faster rate than those with a lower STP concentration. For instance, Ma et al. (2009) found that stopping the application of P fertilizer for 14–15 years to five Cambisols growing winter wheat and maize in China resulted in a faster rates of Olsen P decline in the soil with the highest initial Olsen P concentration (27 mg kg^{-1}), and the slowest rate of decline in the soil with the least Olsen P concentration (4 mg kg^{-1}). A faster decrease at more enriched Olsen P concentrations is probably due to a greater proportion of P that is loosely bound and available either for uptake by plant roots or loss in leachate or surface runoff than in soils with lower Olsen P concentrations where the P is likely bound in more recalcitrant perhaps organic forms (Toor and Sims, 2015; Boitt et al., 2018). In soils of different soil P sorption, P in soil solution tends to be lower where soil P sorption strength or capacity is high (Barrow, 2000). In our study, Volcanic soils were predicted to take the longest time to decrease to an agronomic target and Peat or Sedimentary soils, the least. The ASC, a proxy for soil P sorption, of Volcanic soils tends to be >70%, but <10% for most Peat soils (without containing allophane) and 20–50% for Sedimentary soils (Sparling and Schipper, 2002).

Much like Olsen P, the rate of decline in WEP is a function of the initial STP concentration of the soil and soil P sorption. For example, in addition to predicting the rate of decline in WEP was a function of Olsen P and ASC, Dodd et al. (2013) investigated further using ^{33}P to show that the rate of decline in WEP was associated with P that was immediately exchangeable, while more strongly sorbed P only exchangeable over months was not. In measuring $CaCl_2$-P in six Australian soils of varying soil P sorption capacity, Coad et al. (2014) found that the rate of decline in $CaCl_2$-P over 4 years was inversely related to soil P sorption capacity, measured as the P buffering index (Burkitt et al., 2006). Calcium chloride extractable P was used in this study a proxy to estimate the potential for P loss in leachate (McDowell and Condron, 2004).

Meeting Agronomic Targets

On those soils where Olsen P is above the agronomic targets, Olsen P concentrations must reduce. The first step in making reductions is to ensure that the on-farm P balance is not in surplus thereby avoiding soil P enrichment. While livestock systems with confinement will likely have P a surplus (Reid et al., 2019), systems that get most of their feed from forage grown on-farm are likely to be balanced, but could support a negative P balance. A negative, but still profitable on farm P balance can be achieved by shifting producers from nutrient regimes that focus on minimizing P inputs and augmenting production with N (Dodd et al., 2014b; Sadeghpour et al., 2017).

While a P balance is a good method of decreasing Olsen P toward a target, it can take many years to do so. There are methods that can achieve reductions in Olsen P faster. For instance, plowing to reduce the soil P stratification can decrease topsoil P concentrations by half – overnight, abating surface runoff losses (Sharpley, 2003). However, while plowing can disrupt macropores and P leaching, Dodd et al. (2014a) found that earthworm activity restored macropore function and P leaching within 18 months. Other researchers have focused on altering crop rotations to phytoextract available (and leachable) P from topsoil. Of 12 different crops, Delorme et al. (2000) measured annual P uptake rates of 114 kg P ha^{-1} for maize and 108 kg P ha^{-1} for Indian mustard (*Brassica juncea* L.).

In pasture systems, Sistani et al. (2003) and Gotcher et al. (2014) measured uptake rates for Red River crabgrass (*Digitaria ciliaris* Retz. Koel.) of 34–49 kg P ha^{-1} $year^{-1}$. However, by using pastures like *Lucerne* sp. to fix nitrogen for subsequent rotations of maize and wheat-soybean, STP concentrations were reduced by half compared to grain crops alone Fiorellino et al. (2017). The enhanced extraction is likely caused by N-fixation that shifted the crops into P-limitation thereby extract more P from the soil.

Grain crops and grass forage rotations were able to deplete topsoil STP by 11 – 36% over 7–16 years in Swedish lysimeter trials. However, translocation of P to the subsoil was noted in three of four soil types with associated increases in P leaching (Svanbäck et al., 2015). The degree of subsoil enrichment was associated with the P saturation index, suggesting that care is needed when mobilizing legacy P stores in soils with limited P sorption capacity, either through inherent soil properties or saturation of sorption sites, to protect water quality.

Meeting Environmental Targets

We predicted that the area-weighted time for median and 75th percentile of Olsen P concentration across New Zealand to decrease to its agronomic target was within a year. However, the equivalent area-weighted time for WEP was 26–55 years, inferring that to reach targets for WEP would require the cessation of P fertilizers over generational timeframes. Pursuing low WEP concentrations may also result in Olsen P concentrations lower than the agronomic target in many soils, impairing production. For instance, in a sheep grazed trial in Winchmore, Canterbury, New Zealand withholding fertilizer for 6 years resulted in a 34–53% decrease in pasture production (McBride et al., 1990). In plots that had received superphosphate at rates of 34 and 51 kg P ha^{-1} $year^{-1}$ this decreased Olsen P from 28 and 24 mg L^{-1} to <20 mg L^{-1}, respectively. However, Dodd et al. (2012) estimated that it would take ∼22–24 years of withholding fertilizer in these soils to reach an environmental target of 0.02 mg WEP L^{-1} and an Olsen $P < 10$ mg L^{-1}. This reduction in Olsen P concentration would likely result in stocking rates falling from 18 to 6 stock units ha^{-1}, equivalent to an unfertilized control (Fraser et al., 2012). The consequences of not reducing stocking rates are poor liveweight gain and wool growth. In a trial, again withholding fertilizer for 6 years to a sedimentary soil, O'Connor et al. (1990) found that Olsen P reduced from

14 to 8 mg L^{-1} causing pasture production to decrease by 30%. Maintaining the same stocking rate throughout, winter spring wool growth decreased by 28%. In contrast to pasture, other studies have found little or no decrease in maize (*Zea mays* L.) production as STP concentrations and WEP trend toward their respective targets (Zhang et al., 2004; Ribey and O'Halloran, 2016). This is presumably due to the ability of maize to explore a much greater depth and volume of soil than pasture and therefore access greater P reserves.

Other strategies are therefore required to augment or replace the cessation of P fertilizer to soils to reduce WEP concentrations. Such strategies include, but are not limited to: the application of Al-, Fe- or Ca-P-sorbing compounds like alum, bauxite or crushed shells to reduce the dissolution of P into soil solution (Vlahos et al., 1989; Ballantine and Tanner, 2010); altering soil pH to a range where P is less soluble (e.g., <5.5 or >7.5) but still available to plants (McDowell et al., 2003); or the application of sparingly soluble fertilizer P forms. In addition, it is important to understand that 10–80% of P losses can come from other parts of the farm such as recent fertilizer or manure (and dung) applications or unutilized forage (McDowell et al., 2007). Strategies are also available to reduce losses from these sources such as the application of sparingly soluble P fertilizers (Nash et al., 2019) or avoiding the grazing of wet areas that are well-connected to nearby streams (Monaghan et al., 2017). Each strategy varies in its cost and effectiveness and while cost-effectiveness can be improved by targeting strategies to the sources of P loss, this may not be sufficient to enable WEP losses to meet the target nor do so without significantly reducing farm profitability (McDowell and Nash, 2012). This may force land use change to systems that can produce well from very low Olsen P concentrations. Although traditionally this may have been restricted to forestry, new pasture cultivars are becoming available that can access recalcitrant P forms negating the need to maintain Olsen P concentrations at current targets (Nichols and Crush, 2016; Sandral et al., 2018). Some evidence also suggests that production can be enhanced by adding silicon which solubilizes P at low STP concentrations (Qin and Shober, 2018).

CONCLUSION

Analysis of the P status of New Zealand agricultural soils revealed that 63% of the productive land area had Olsen P concentrations more than the agronomic optimum. Accumulation of soil P was found to be related to both the soil order and farming system with dairy operations on volcanic soils, with high ASC values, exhibiting the greatest exceedances. If fertilizer application was halted, we predicted that the median Olsen P concentration could be reduced to the agronomic optimum within 1 year. In contrast, reducing the median WEP to an environmental target would require 36–55 years for most soil orders and farming systems, but greater than 100 years for volcanic soils with the greatest P enrichment. To safeguard water quality, strategies such as phytomining are required to accelerate the decline in WEP from the most enriched soils along with mitigation strategies to reduce P solubility. The discrepancy between the timescales to reach these two targets highlights the difficulty in maintaining high agronomic productivity while ensuring good water quality status. Revision of the agronomic targets should be undertaken to account for increases in P use efficiency in modern farming systems and could go some way to closing this gap. However, our results suggest that new land management strategies will be required to develop agronomically and environmentally sustainable farming systems which are productive at a lower Olsen P status.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

RM wrote the manuscript and analyzed the data along with AN. RD contributed text to the manuscript. PP developed the maps and generated spatial data.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2020.00006/full#supplementary-material

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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

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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 ($\sim$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):

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

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.

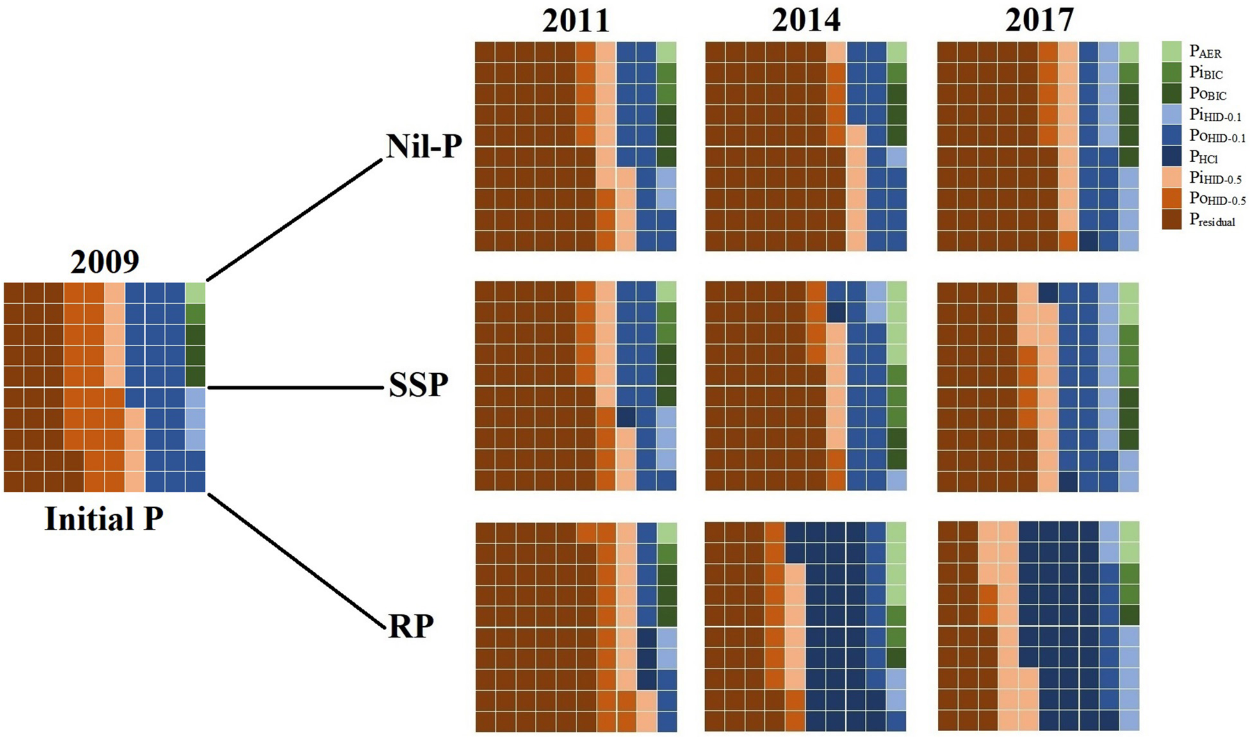

FIGURE 1 | Proportions of each P fraction under phosphate sources in 0–5 cm soil layer from 2009 to 2017. Each small square represents 1% of total P. Labile, mod-labile, and non-labile P fractions were shown with the shades of green, blue, and brown colors, respectively. 2009 and 2011 data were obtained 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.

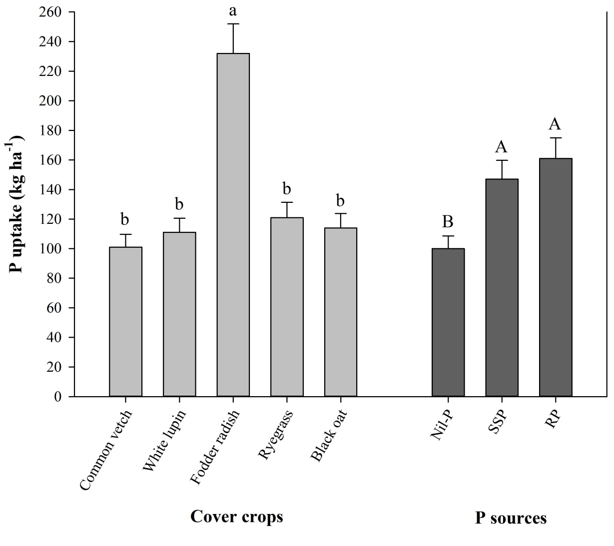

FIGURE 2 | Cumulative shoot P uptake by winter cover crops from 2009 to 2017 affected by cover crops and P sources. Mean values followed by the same tiny letter in cover crops and capital letter in P sources do not differ by *t*-test (LSD, $P < 0.05$).

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.

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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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Inoculation With Growth-Promoting Bacteria Associated With the Reduction of Phosphate Fertilization in Sugarcane

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Sugarcane holds the prominent position in global economy. Extensive research is needed to enhance the efficiency of phosphorous fertilizers which increase the cost of sugarcane production more specifically in the less fertile soils which are weathered and phosphorous deficit as tropical region soils. We are currently living in a transitional period called the "Green Micro-revolution," in which the use of plant growth-promoting bacteria (PGPB) is justified and although bacteria such as *Azospirillum brasilense*, *Bacillus,* and *Pseudomonas fluorescens* in other crops are recognized for increasing production however, little is known about the effects of these microorganisms on sugarcane. The current study aimed to evaluate the effect of inoculation with three species of plant growth promoting bacteria (*A. brasilense, Bacillus subtilis,* and *P. fluorescens*) without or with combine application of reduced doses of phosphate fertilizer, in the P soil available, P leaf concentration, shoot yield and P accumulation in sugarcane (cane-plant) at the end of the cycle. The experiment was carried out with a sugarcane crop in a Hapludox Rhodic with low available P content, in a randomized block design with 8 × 5 factorial scheme, being eight inoculations and five P doses (0, 45, 90, 135, and 180 kg ha^{-1} P_2O_5) as triple superphosphate. The bacterial inoculations influenced the leaf P content, so that inoculation with *B. subtilis* + *P. fluorescens* provided the highest concentration of phosphorus in the sugarcane leaf. Both the layers of the soil were differently influenced by inoculation and P doses whereas inoculation with *A. brasilense* + *P. fluorescens* at 135 kg P_2O_5 ha^{-1} provided the highest soil P content in the soil layer of 0–0.25 m. Although, maximum stalk yield was obtained with inoculation of *B. subtilis* + *P. fluorescens* at the dose of 135 kg ha^{-1} P_2O_5. The inoculation of *A. brasilense* + *B. subtilis* with application of 45 kg P_2O_5 ha^{-1} improved dry matter, total P accumulation and stalk production by 38% in sugarcane variety (RB92579) and reduced P fertilization by 75% for the same variety grown in low-P soil.

Keywords: ***Azospirillum brasilense*****,** ***Bacillus subtilis*****,** ***Pseudomonas fluorescens*****, grass inoculation, phosphate solubilization, phosphorus concentration**

INTRODUCTION

Brazil has the title of world's largest producer of sugarcane, followed by India and China and accounts for 28% of ethanol and 16% of sugar consumed worldwide (RFA, 2019; USDA, 2019). This follow-up only drives the country's agribusiness forward, as the global demand for ethanol is growing every day and the soil and climatic conditions of the region are very favorable to the cultivation of this of cane crop, along with the availability of extensive agricultural areas (CONAB, 2019). In the last harvest of sugarcane (2018/2019) with cultivated of 8.5 million hectare, the country hit production of 633.3 million tons with an average yield of 72.2 t ha^{-1}. The state of São Paulo was occupying 51% of the sugarcane cultivated area and is responsible for 53% of national production (CONAB, 2019).

One of the main limitations is the poor and inadequate fertile soils which fail to meet the nutritional and growth requirements and hence unable to achieve high production. Phosphorus (P) is the most critical element with high interaction with soil (Raij, 2011), required in a small quantity by sugarcane in comparison of N and K but still plays an essential role in the development of tillering and root system (Kingston, 2014) and greatly influence the longevity. Most of Brazilian soils (tropical and weathered) are highly deficient in phosphorus. Even though the soils are efficient with total P but still a tiny fraction of P is available to plants (Sampaio, 2011). The low availability of phosphorus is due to low P in the source material, clay adsorption and its precipitation with oxides and hydroxides of Fe and Al (Caione et al., 2015). Therefore, a huge quantity of P fertilizers is applied which significantly adds to the cost of production of cane crop. In addition, phosphate fertilizers are produced by phosphate rock and therefore, Morocco being responsible for 85% of the known active mining reserves (Campos et al., 2018) and it is estimated that it will be diminish among 200 to 300 years (Sattari et al., 2012). Around 10 to 30% of the phosphorus fertilizer applied in the first year is absorbed by roots of cane crop whereas an extensive amount accumulates in the soil as fixed P, not available to plants (Syers et al., 2008). It is necessary to find alternatives to reduce the use of phosphate fertilizers. Since every day looking to a more sustainable agriculture combined with increase in productivity and economically viable.

The use of plant growth promoting bacteria (PGPB) is a promising alternative with low environmental impact to increase the efficiency of use of mineral fertilizers, including phosphate, providing high cost-effective yields (Spolaor et al., 2016). The PGPB promote plant growth due to various mechanisms such as biosynthesis of phytohormones and secondary metabolites (Duca et al., 2014; Tahir et al., 2017), biological nitrogen fixation (Li et al., 2017), induction of resistance to biotic stresses (phytopathogen biocontrol) and abiotic stresses (drought and salinity) (Yan et al., 2016; Takishita et al., 2018) production of siderophores metal accumulator (Ali et al., 2014) and soil nutrient solubilization such as phosphorus and potassium (Gupta et al., 2015; Shen et al., 2016; Patel and Archana, 2017). Bacteria with combination of these mechanisms, promote and increase the productivity of several crops (Kumar et al., 2014; Smith et al., 2015; Galindo et al., 2016).

Some of these microorganisms are being studied together to enhance P utilization which is unavailable/immobilized in the soil. Hereby theses microbes try to make P available to the plants and reduce the application of P which certainly decrease the hazardous environmental impact caused by the application of these fertilizers. Phosphate solubilization or increased inorganic P availability by PGBP *in vitro* is observed by proton production, organic acid binders and phytate (organic P) mobilization probably due to phytase production mobilization phytate (organic P) probably by the production of phytase (Jorquera et al., 2008; Hinsinger et al., 2011) provided by these microorganisms.

Several field and greenhouse researches with phosphate fertilizer in sugar cane (Calheiros et al., 2012; Caione et al., 2015; Albuquerque et al., 2016; Borges et al., 2019) however, they did not work with inoculation of phosphate-solubilizing bacteria (alone or in combination). Plant growth-promoting bacteria (PGPB) are playing a key role in plant health and growth and can be promisingly applied in agricultural production to reduce the use of chemical fertilizers. This manuscript evaluated the effect of inoculation with three PGPB species and five P doses in sugarcane. Inoculation can play a fundamental role in cultivation, generating great benefits to the crop and saving fertilizers cost for the producers. The results revealed a combination of *Azospirillum brasilense* and *Bacillus subtilis* allied to the low dose of P_2O_5 was the best fertilizer management in the sugarcane, which is meaningful for production practice of sugarcane. Seeking for further study with PGPB and its ability to solubilize inorganic phosphate, this study aimed to assess the effect of inoculation with three species of plant growth-promoting or phosphate solubilizing bacteria associated with or without reduced phosphate fertilizer application on soil P content, leaf P concentration, stalk yield (kg ha^{-1}), dry matter and P accumulation in sugarcane at the end of cycle.

MATERIALS AND METHODS

Location of the Experimental Area

The research was conducted in the field with sugar cane crop (crop year 2017/18) in Ilha Solteira in area belonging to one of sugar and ethanol industry, located at the northwest state of São Paulo—Brazil. Geographically, the area is located at the latitude 20° 21′ S, longitude 51° 04′ W and an altitude of 371 meters. The area was being cultivated with *Urochloa brizantha* for the last 10 years which was grazed and pasture degraded. The soil of the area is a Dystrophic Red Latosol with a medium to coarse texture, classified according to criteria established by Brazilian Society of Soil Science (Santos et al., 2018), and as a Rhodic Hapludox according to the Soil Survey Staff (2014), with a particle size of 777, 98, 125 and 747, 88, 165 g kg^{-1} of sand, silt and clay at depths of 0.25-0.50 m and from 0.00 to 0.25, respectively. The soil was chemically analyzed before experiment implantation according to the methodology proposed by van Raij et al. (2001) and described in **Table 1**.

According to Koppen, the climate type of the region is classified as Aw which was defined as humid tropical with the rainy season in summer and dry in winter. Climatological data were recorded throughout the experimental period (**Figure 1**).

Experimental Design and Treatments

The experimental design was a randomized block with three replicates, arranged in 8 × 5 factorial scheme, comprised of eight inoculations (1) Without inoculation—control (W.I.), (2) Inoculation with *Azospirillum brasilense* (Azo), (3) Inoculation with *Bacillus subtilis* (Bac), (4) Inoculation with *Pseudomonas fluorescens* (Pseud), (5) Inoculation with *A. brasilense* + *B. subtilis* (Azo + Bac), (6) Inoculation with *A. brasilense* + *P. fluorescens* (Azo + Pseud), (7) Inoculation with *B. subtilis* + *P. fluorescens* (Bac + Pseud), (8) Inoculation with *A. brasilense* + *B. subtilis* + *P. fluorescens* (Azo + Bac + Pseud), and five phosphorus doses at planting (0, 45, 90, 135, and 180 kg ha^{-1} P$_2$O$_5$) as a source of triple superphosphate, corresponding to 0, 25, 50, 75, and 100% of the recommended dose for sugarcane crop according to Raij and Cantarella (1997).

TABLE 1 | Pre-experiment soil chemical characterizations in the soil layers of 0.00–0.25 and 0.25–0.50 m.

Layer	P	S-SO$_4$	OM	pH	K	Ca	Mg	H+Al	Al	SB
(m)	mg dm^{-3}		g dm^{-3}	CaCl$_2$	mmol$_c$ dm^{-3}					
0.00-0.25	2	3	13	4.7	2.6	8	6	20	1	16.6
0.25-0.50	2	2	12	4.8	2.4	9	7	20	2	18.4

Layer	B^a	Cu^b	Fe^b	Mn^b	Zn^b	CEC	V	m
(m)	mg dm^{-3}					mmol$_c$ dm^{-3}	%	%
0.00–0.25	0.22	0.8	14	16.2	0.6	36.6	45	6
0.25–0.50	0.22	1.0	7	8.3	0.3	38.4	48	10

OM, organic matter; CEC, cation exchange capacity; SB, sum of bases; V, bases saturation; m, Al saturation.

[a] *Determined in DTPA (diethylenetriaminepentaacetic acid).*

[b] *Determined in hot water.*

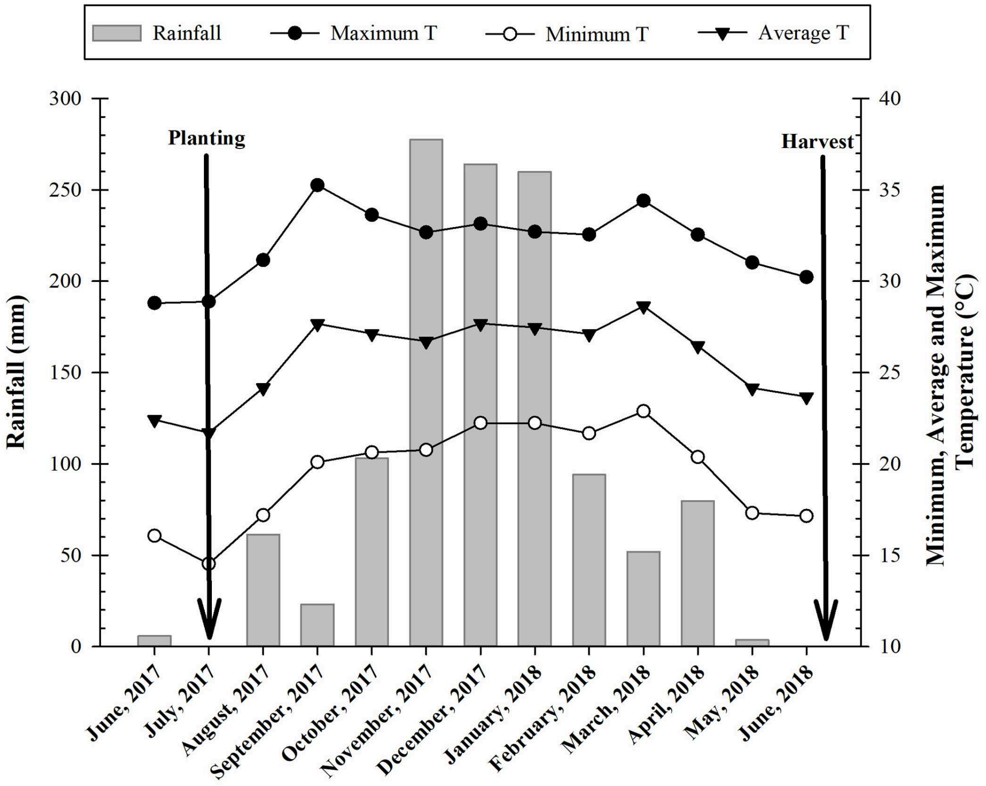

FIGURE 1 | Monthly averages of precipitation (mm), maximum, average, and minimum temperatures recorded during the period in which the experiment was conducted. Ilha Solteira, state of São Paulo, Brazil, 2017/2018.

Establishment and Management of the Experiment

After pre-experiment soil chemical characterization and 15 days before planting, soil profile prepared with application of 1 t ha^{-1} of limestone (PRNT 85%) in order to obtain 60% saturation of bases and 1 t ha^{-1} of gypsum to increase the sulfur (S), as recommended by Raij and Cantarella (1997) for sugarcane crop. Three gradings and subsoiling were carried out, after which the soil was furrowed at 0.40 m depth and insecticide fipronil (180 g ha^{-1} of the active ingredient—ai) + fungicide pyraclostrobin (125 g ha^{-1} of ai) were applied in the planting furrow. The sugarcane variety RB92579 was grown on 11th July, 2017 by adapting manual planting system. One-meter long channel of cane was having around 22 buds, cut and placed within the planting furrows. The plots were 5 m long with five rows, spaced 1.5 m, whereas central three rows were used for data collection.

The following doses of liquid inoculants were used for bacterial inoculations: *A. brasilense* [strains Ab-V5 and Ab-V6 with guarantee of 2×10^8 colony forming units (CFU) ml^{-1}] was applied at the dose of 1.0 L ha^{-1}; *B. subtilis* (strain CCTB04, with guarantee of 1×10^8 CFU ml^{-1}) and *P. fluorescens* (strain CCTB03, with guarantee of 2×10^8 CFU mL^{-1}) were applied at the dose of 0.5 L ha^{-1}, based on the manufacturer's recommendation. The spray volume was 200 L ha^{-1} (for three bacterial inoculations) applied by backpack sprayer pump spraying in the furrow of sugarcane plant. The spray was applied in the late afternoon to prolong the influence of bacterial inoculation with lower temperature.

The recommended doses of nitrogen and potassium was applied to all the treatment beyond the levels of phosphorus at the time of sowing. Nitrogen was applied at the rate of 30 kg N ha^{-1} from a source of ammonium nitrate and potassium was applied at the rate of 120 kg K_2O ha^{-1} from the source of potassium chloride based on soil analysis and utilization of fertilizer by the plant. The experiment was irrigated with sprinkler irrigation system (30 mm slide) soon after 5 days of sugarcane sowing due to low precipitation period. The plots were applied with 5 kg Zn ha^{-1} from zinc sulfate at the time of tillering. This application was done due to the reasons that experimental soil was deficient with micronutrients and phosphate was used as main factor which in excess can lead to zinc deficiency due to antagonistic effect, in which phosphate react with Zn and make it less soluble for absorption by plants.

Throughout cane life cycle different chemicals were applied for the control weeds, pests, and diseases: pre-emergence (24 days after planting—DAP) the hexazinone herbicide (320 g ai ha^{-1}) + 2.4 D (967 g ai ha^{-1}) + tebuthiuron (1,000 g ai ha^{-1}). At 110 DAP, in the lion breaking 2.4 D (967 g ai ha^{-1}) + Amicarbazone (840 g ai ha^{-1}) + S-metolachlor (2,400 g ai ha^{-1}) were applied. At 101 and 199 DAP, insecticides chlorantraniliprole (10 g ai ha^{-1}) + lambda-cyhalothrin (5 g ai ha^{-1}) were applied. Biological control of the sugarcane borer (*Diatraea saccharalis*) was used through the release of *Trichogramma galloi* (at 142, 148, 213, and 220 DAP, respectively) and *Cotesia flavipes* (at 192 DAP).

In order to induce the sugarcane to accumulate sucrose, application of maturing agent, trinexapaque-ethyl (275 g ai ha^{-1}) was performed at 301 DAP. The crop was manually harvested after 349 days of planting on 25th June, 2018.

Assessments

To determine the leaf P concentration (Malavolta et al., 1997), at the stage of higher vegetative development of the crop, at 195 DAP, were collected in the morning, the middle third 15 flag leaves (leaf diagnose- highest with visible leaf collar) per share, excluding the central rib, as recommended by Raij and Cantarella (1997).

Five representative plants from each plot were harvested 349 DAP. Its parts were properly separated into stalks s and leaves, dried in air forced desiccator at 65°C until obtaining a constant weight. Each sample was then quantified for dry mass of stalks, leaves, and total dry mass (entire plant), which were converted into kg ha^{-1}.

Then, the same samples were crushed and grinded separately and subjected to analysis for the determination of P concentration, according to the methodology proposed by Malavolta et al. (1997). For subsequent calculation of this macronutrient accumulation in leaves, stalks s and total (entire plant) at the end of the cycle, in kg ha^{-1}.

At the time of harvest (349 DAP), the stalks collected from all the treatment of five lines were weighed for the calculation of sugarcane yield (kg ha^{-1}).

The soil available P content for each treatment in two layers (0–0.25 and 0.25–0.50 m) of the soil was determined after harvesting of sugarcane. The soil was collected from four different planting locations and forming a composite sample, which was subsequently sent to the laboratory for analysis according to the methodology described by van Raij et al. (2001).

Statistical Analysis

The results were submitted to analysis of variance (F test) and Scott-Knott test with probability ($p \leq 0.05$) for means comparison of inoculation treatments. Regression equations were adjusted for the effect of P_2O_5 doses. The collected data were subjected to SISVAR statistical software for analysis interpretation and Sigma-Plot 12.5 software for plotting the graphics.

RESULTS

Phosphorus Content in Soil and Cane Leaf

The overall average available soil P concentrations were 8.11 and 3.70 mg dm^{-3} in the layers of 0–0.25 and 0.25–0.50 m, respectively (**Table 2**) which were obviously higher than that of P content obtained in pre-experiment analysis (**Table 1**). There were significant interactions between phosphorus doses and inoculations with PGPB for available P content in the two soil layers analyzed (**Table 2**). The highest P available contents in both soil layers were observed with inoculation of *A. brasilense* + *P. fluorescens* at a dose of 135 kg P_2O_5 ha^{-1} of in the layer of 0–0.25m and *A. brasilense* inoculation with 180 kg P_2O_5 ha^{-1} in the 0.25–0.50 m layer (**Table 3**).

In the 0–0.25 m soil layer, the highest P available contents were observed with lowest soil applied phosphate (45 kg P_2O_5

TABLE 2 | Available P contents in soil after cane harvest as influenced by inoculation of PGPB and P doses in sugarcane.

	P soil (mg dm^{-3})	
	Layer 0–0.25 m	**Layer 0.25–0.50 m**
P_2O_5 rates (kg ha^{-1})		
0	3.78	1.44
45	6.56	3.00
90	7.69	4.38
135	11.81	4.06
180	10.69	5.63
Inoculation		
W.I.	5.50	2.80
Azo	9.90	4.30
Bac	6.80	4.80
Pseud	6.10	4.30
Azo + Bac	10.70	2.90
Azo + Pseud	12.40	3.20
Bac + Pseud	4.75	3.30
Azo + Bac + Pseud	8.70	4.00
F test		
P_2O_5 rates (R)	**	**
Inoculation (I)	**	**
R × I	**	**
Overall average	8.11	3.70
Standard error	0.20	0.23
CV (%)	9.32	23.94

***, * and ns: significant at 1 and 5% at $p < 0.01$, $p < 0.01$, < 0.05, and not significant, respectively.*
CV, coefficient of variation.
W.I., Without inoculation.
Azo, Azospirillum brasilense.
Bac, Bacillus subtilis.
Pseud, Pseudomonas fluorescens.
Azo + Bac, A. brasilense + B. subtilis.
Azo + Pseud, A. brasilense + P. fluorescens.
Bac + Pseud, B. subtilis + P. fluorescens.
Azo + Bac + Pseud, A. brasilense + B. subtilis + P. fluorescens.

TABLE 3 | Interaction between bacterial inoculations and P_2O_5 doses for soil available P in the layers of 0-0.25 m and 0.25-0.50 m.

	P soil (mg dm^{-3}) layer 0-0.25 m				
	P_2O_5 rates (kg ha^{-1})				
Inoculation	**0**	**45**	**90**	**135**	**180**
W.I.	4.00 b	4.00 c	4.00 c	7.50 e	8.00 d
Azo	4.50 b	5.50 b	6.50 b	17.00 b	16.00 b
Bac	5.50 a	6.00 b	6.50 b	7.00 f	9.00 d
Pseud	3.50 c	4.00 c	5.00 c	11.50 c	6.50 e
Azo + Bac	3.50 c	17.00 a	14.00 a	10.00 d	9.00 d
Azo + Pseud	2.50 c	6.50 b	14.00 a	27.00 a	12.00 c
Bac + Pseud	2.77 c	3.50 c	4.50 c	6.00 f	7.00 e
Azo + Bac + Pseud	4.00 b	6.00 b	7.00 b	8.50 e	18.00 a
Standard error			0.44		

	P soil (mg dm^{-3}) layer 0.25–0.50 m				
	P_2O_5 rates (kg ha^{-1})				
Inoculation	**0**	**45**	**90**	**135**	**180**
W.I.	1.50 a	1.50 c	2.00 c	5.50 a	3.50 c
Azo	1.50 a	3.00 b	2.50 c	3.00 b	11.50 a
Bac	1.50 a	3.50 b	4.00 b	6.00 a	9.00 b
Pseud	1.50 a	2.00 c	4.00 b	5.00 a	9.00 b
Azo + Bac	1.50 a	7.00 a	2.50 c	2.00 b	1.50 d
Azo + Pseud	1.50 a	2.00 c	6.50 a	4.00 a	2.00 d
Bac + Pseud	1.50 a	2.00 c	7.00 a	2.00 b	4.00 c
Azo + Bac + Pseud	1.00 a	3.00 b	6.50 a	5.00 a	4.50 c
Standard error			0.51		

Averages followed by the same letter in the column do not differ statistically by the Scott-Knott test at 5% probability.
W.I., Without inoculation.
Azo, Azospirillum brasilense.
Bac, Bacillus subtilis.
Pseud, Pseudomonas fluorescens.
Azo + Bac, A. brasilense + B. subtilis.
Azo + Pseud, A. brasilense + P. fluorescens.
Bac + Pseud, B. subtilis + P. fluorescens.
Azo + Bac + Pseud, A. brasilense + B. subtilis + P. fluorescens.

ha^{-1}), which was statistically similar to that of the highest applied dose (180 kg P_2O_5 ha^{-1} of P_2O_5 dose commonly used by plant). However, in the 0–0.25 m soil layer, the combined inoculation of *A. brasilense* + *B. subtilis* resulted in highest available P contents with the lowest doses of phosphate applied fertilizer (45 and 90 kg ha^{-1} P_2O_5) (**Figure 2A**).

In case of interaction PGPB and phosphorus doses available P content in soil layer of 0–0.25 m, the graph trend indicated a linear increase for no-inoculation, *A. brasilense*, *B. subtilis*, *B. subtilis* + *P. fluorescens,* and *A. brasilense* + *B. subtilis* + *P. fluorescens* (**Figure 2A**). Quadratic regression for the treatments described that available P contents were improved with inoculation of *A. brasilense* + *B. subtilis* up to the dose of 91 kg P_2O_5 ha^{-1} and inoculation of *A. brasilense* + *P. fluorescens* up to the dose of 134 kg P_2O_5 ha^{-1}. Similarly, the graph trend for 0.25–0.50 m soil layer indicated that there an increasing linear trend for no-inoculation, *A. brasilense*, *B. subtilis,* and *P. fluorescens* and quadratic adjustments for *A. brasilense* + *P. fluorescens,* and *A. brasilense* + *B. subtilis* + *P. fluorescens* up to the dose of 104 kg ha^{-1} P_2O_5 (**Figure 2B**). For leaf P concentration, there was a unique influence of inoculation on leaf P concentrations regardless of applied phosphorus doses. Inoculation with *P. fluorescens* and other bacteria in combination (except *A. brasilense* + *P. fluorescens*) resulted in higher cane leaf P concentration during nutrient analysis whereas the interaction between inoculations and phosphorus doses was non-significant (**Table 4**).

Dry Matter Partitioning

Different phosphorus (P) doses and bacterial inoculations significantly influenced leaf, stalk and total plant dry matter. Stalk and total dry mass were significantly increased with inoculation of *A. brasilense* + *P. fluorescens* and soil applied P doses (90 and

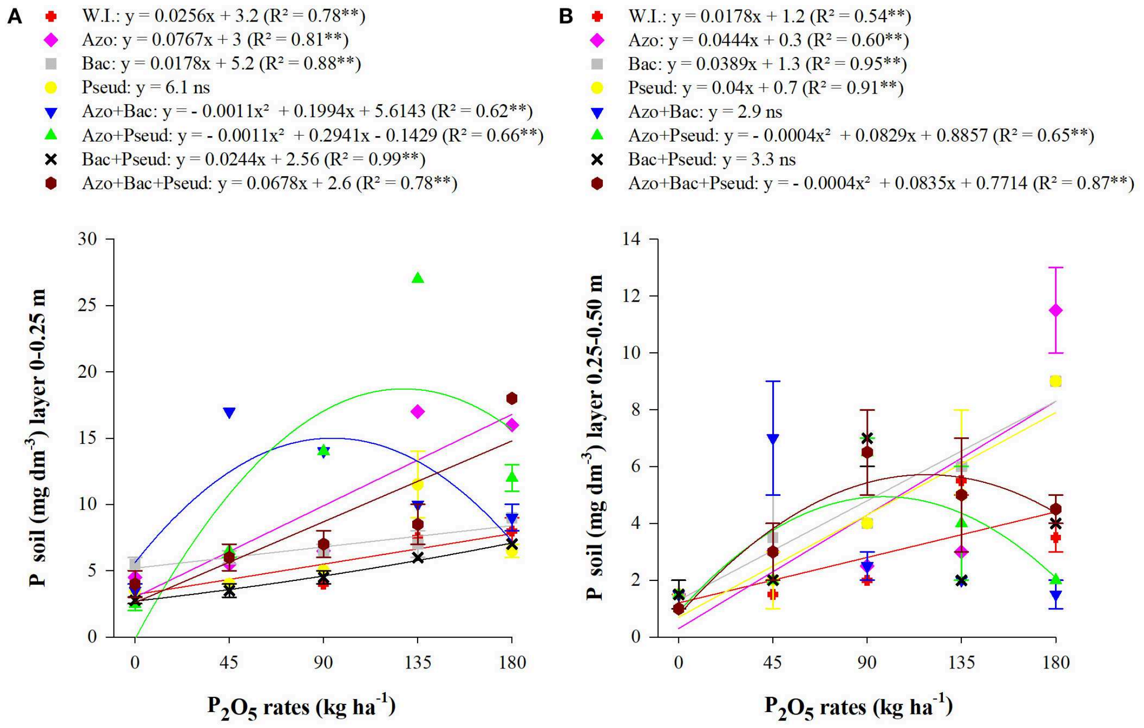

FIGURE 2 | Interactions between bacterial inoculations and P_2O_5 doses for soil available P in the 0–0.25 m **(A)** and 0.25–0.50 m layers **(B)**. (Mean values ± standard deviation). ** and ns: significant at 1% at $p < 0.01$ and not significant, respectively. W.I., without inoculation; Azo, *Azospirillum brasilense*; Bac, *Bacillus subtilis*; Pseud, *Pseudomonas fluorescens*; Azo + Bac, *A. brasilense* + *B. subtilis*; Azo + Pseud, *A. brasilense* + *P. fluorescens*; Bac + Pseud, *B. subtilis* + *P. fluorescens*; Azo + Bac + Pseud, *A. brasilense* + *B. subtilis* + *P. fluorescens*.

135 kg P_2O_5 ha^{-1}) which were statistically similar. Leaf dry mass was also increased with *A. brasilense* + *P. fluorescens* inoculation P application at the rate of 135 kg P_2O_5 ha^{-1} (**Table 6**).

The inoculation of *A. brasilense* + *B. subtilis* combined with the dose of 45 kg P_2O_5 ha^{-1} increased the stalk and total dry mass in sugarcane in comparison to other inoculations at the same P dose (**Table 6**). Inoculation of *B. subtilis* + *P. fluorescens* resulted in maximum dry mass for different parts of cane plant in the absence of phosphate fertilizer (**Table 6**). In general, the influence of P doses (45 and 90 kg ha^{-1} P_2O_5) along with inoculation of PGPB were observed more prominent in dry weight of the sugarcane (**Table 6**).

The interactions between different P doses and bacterial inoculations for leaf, stalk and total dry mass were also found significant (**Table 5**). The graph trend for leaf dry mass set increasing linear functions in *A. brasilense* and *A. brasilense* + *P. fluorescens* inoculations and a decreasing linear function in inoculations of *A. brasilense* + *B. subtilis* and *B. subtilis* + *P. fluorescens* whereas the quadratic adjustment in un-inoculated treatments up to the dose of 105 kg P_2O_5 ha^{-1} (**Figure 3A**). Similarly, the stalks dry mass set a decreasing linear function for *B. subtilis*, *P. fluorescens* and *B. subtilis* + *P. fluorescens* (**Figure 3B**). The stalks dry mass was set to quadratic functions for *A. brasilense* and *A. brasilense* + *P. fluorescens* up to 120 and 113 kg P_2O_5 ha^{-1}, respectively, whereas for no-inoculation up to the dose of 115 kg P_2O_5 ha^{-1}. Hence, by utilizing the above P doses, the estimated stalk dry mass yield could be 60,709, 87,406, and 60,811 kg ha^{-1} respectively. Furthermore, the graph trend of total dry mass also set decreasing functions for *B. subtilis*, *P. fluorescens* and *B. subtilis* + *P. fluorescens* (**Figure 3C**). The graph trend of quadratic functions was set for A. *brasilense* up to P dose of 128 kg P_2O_5 ha^{-1}, *A. brasilense* + *P. fluorescens* up to the dose of 122 kg P_2O_5 ha^{-1} and no-inoculation up to a dose of 111 kg P_2O_5 ha^{-1}.

Phosphorus Accumulation in Leaf, Stalk, and Entire Plant

The leaf phosphorus (P) accumulation was enhanced with inoculation of *B. subtilis* + *P. fluorescens* with soil applied phosphate at the doses of 0.0, 45, 90, and 135 kg P_2O_5 ha^{-1}.The inoculation of *P. fluorescens* with at 45 P_2O_5 kg ha^{-1} whereas *A. brasilense* + *B. subtilis* + *P. fluorescens* in the absence of phosphate fertilizer improved leaf P accumulation (**Table 8**). The highest P accumulation in leaves of cane variety (RB92579) was observed at a dose of 180 kg P_2O_5 ha^{-1} with inoculation of *P. fluorescens*. The interaction of inoculation and P doses was also found significant for P accumulation in the cane leaves (**Table 7**). The graph trend set a linear increment for P leaf accumulation with inoculation of *A. brasilense*, *B. subtilis*, *A. brasilense* + *B. subtilis,* and *A. brasilense* + *P. fluorescens* (**Figure 4A**) whereas non-inoculated treatments set to a quadratic function up to a dose of 105 kg P_2O_5 ha^{-1}.

TABLE 4 | Leaf P contents as influenced by different P_2O_5 doses and bacterial inoculations.

	P leaf (g kg^{-1})
P_2O_5 rates (kg ha^{-1})	
0	2.32
45	2.34
90	2.37
135	2.31
180	2.36
Inoculation	
W.I.	2.25 b
Azo	2.22 b
Bac	2.32 b
Pseud	2.41 a
Azo + Bac	2.37 a
Azo + Pseud	2.31 b
Bac + Pseud	2.48 a
Azo + Bac + Pseud	2.35 a
F test	
P_2O_5 rates (R)	ns
Inoculation (I)	*
R × I	ns
Overall average	2.34
Standard error	0.05
CV (%)	6.44

***, * and ns: significant at 1 and 5% at p <0.01, p < 0.01 < 0.05, and not significant respectively. Means followed by the same letter in the column are not statistically different by the Scott-Knott test at 5% probability.*
CV, coefficient of variation.
W.I., Without inoculation.
Azo, Azospirillum brasilense.
Bac, Bacillus subtilis.
Pseud, Pseudomonas fluorescens.
Azo + Bac, A. brasilense + B. subtilis.
Azo + Pseud, A. brasilense + P. fluorescens.
Bac + Pseud, B. subtilis + P. fluorescens.
Azo + Bac + Pseud, A. brasilense + B. subtilis + P. fluorescens.

TABLE 5 | Leaf dry mass, stalk dry mass and total dry mass (entire plant) of sugarcane as influenced by different bacterial inoculations and P_2O_5 doses.

	Leaf dry mass	Stalk dry mass	Total dry mass
		kg ha^{-1}	
P_2O_5 rates (kg ha^{-1})			
0	15,800	57,961	73,761
45	15,637	58,898	74,535
90	14,758	59,500	74,258
135	14,429	59,969	74,397
180	15,444	55,579	71,023
Inoculation			
W.I.	12,961	54,432	67,394
Azo	13,183	54,759	67,942
Bac	15,588	54,126	69,714
Pseud	16,445	60,439	76,884
Azo + Bac	14,813	59,684	74,497
Azo + Pseud	16,656	65,966	82,621
Bac + Pseud	18,018	62,774	80,792
Azo + Bac + Pseud	14,044	54,870	68,913
F test			
P_2O_5 rates (R)	**	**	**
Inoculation (I)	**	**	**
R × I	**	**	**
Overall average	15,213	58,381	73,595
Standard error	326.18	670.52	766.64
CV (%)	6.78	3.63	3.29

***, * and ns: significant at 1% and 5% at p <0.01, p < 0.01 <0.05, and not significant respectively.*
CV: coefficient of variation.
W.I., Without inoculation.
Azo, Azospirillum brasilense.
Bac, Bacillus subtilis.
Pseud, Pseudomonas fluorescens.
Azo + Bac, A. brasilense + B. subtilis.
Azo + Pseud, A. brasilense + P. fluorescens.
Bac + Pseud, B. subtilis + P. fluorescens.
Azo + Bac + Pseud, A. brasilense + B. subtilis + P. fluorescens.

The accumulation of P in the stalks of the sugarcane was significantly influenced by the higher doses of soil applied P (135 and 180 kg P_2O_5 ha^{-1}) (**Table 8**). However, it was improved with inoculation of *A. brasilense* + *P. fluorescens* at a dose of 90 kg P_2O_5 ha^{-1} and *B. subtilis* + *P. fluorescens* inoculation without phosphorus application. The highest accumulation of P in the stalks was recorded with *A. brasilense* + *B. subtilis* inoculation in combination with a dose of 45 kg P_2O_5 ha^{-1}. The interaction of inoculation and P doses was also found significant for P accumulation in the cane stalks (**Table 7**). The graph trend indicated that P content in stalks was improved and set a linear function for no-inoculation, *A. brasilense* and *B. subtilis* whereas inoculation of *B. subtilis* + *P. fluorescens* was set to a decreasing linear function (**Figure 4B**). Quadratic functions were set by inoculation with Pseud up to the dose of 61 kg ha^{-1} P_2O_5 and inoculation with *A. brasilense* + *P. fluorescens* up to the dose of 102 kg ha^{-1} P_2O_5.

The total P accumulation in the entire plant was also positively influenced by bacterial inoculations and phosphate doses. The inoculation of *B. subtilis* + *P. fluorescens* at 0 kg P_2O_5 ha^{-1} increased P total in cane plant in comparison to other treatments for the same P dose whereas the highest P total accumulation was observed with inoculation of *A. brasilense* + *B. subtilis* treated with 45 kg P_2O_5 ha^{-1} (**Table 8**). Similarly, total P accumulation was prominently influenced by all the inoculations at 135 kg P_2O_5 ha^{-1} in comparison to non-inoculated plots whereas inoculation of *A. brasilense* and triple (*A. brasilense* + *B. subtilis* + *P. fluorescens*) were statistically similar to that of control. The interaction of inoculation and P doses was also found significant for P accumulation in the cane stalks (**Table 7**). The total P accumulation in the entire plant set an increasing linear function for *A. brasilense* and *B. subtilis* inoculations and decreasing linear function for *B. subtilis* + *P. fluorescens* (**Figure 4C**). *A. brasilense* + *P. fluorescens* and no-inoculation were set up to quadratic functions for the doses of 112 and 125 kg P_2O_5 ha^{-1}, respectively.

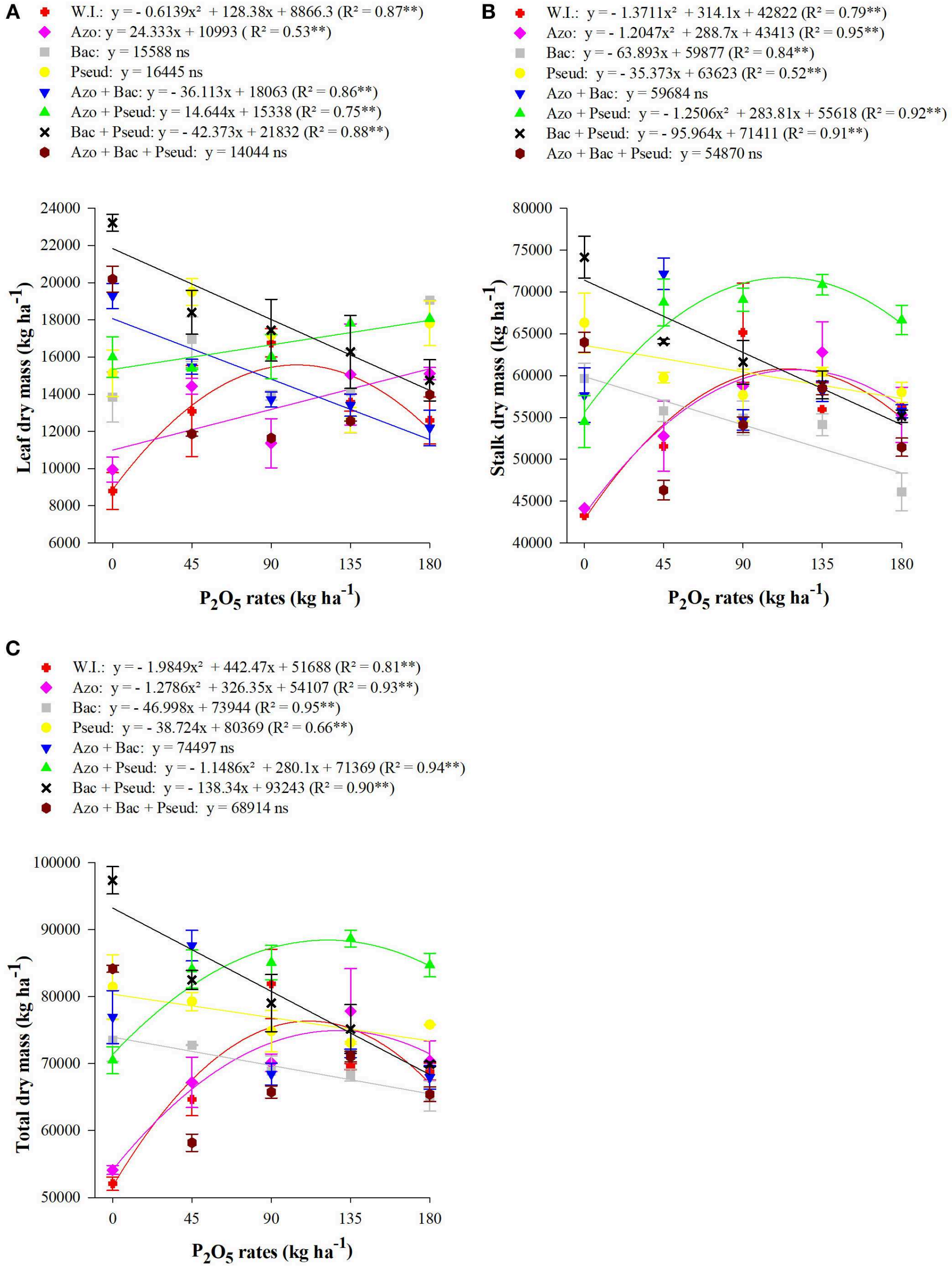

FIGURE 3 | Interactions between different bacterial inoculations and P_2O_5 doses for leaf dry mass **(A)**, stalk dry mass **(B),** and total dry mass **(C)** at the end of the plant-cane cycle, RB92579 variety (Mean values ± standard deviation). ** and ns: significant at 1% at $p < 0.01$ and not significant, respectively. W.I., without inoculation; Azo, *Azospirillum brasilense*; Bac, *Bacillus subtilis*; Pseud, *Pseudomonas fluorescens*; Azo + Bac, *A. brasilense* + *B. subtilis*; Azo + Pseud, *A. brasilense* + *P. fluorescens*; Bac + Pseud, *B. subtilis* + *P. fluorescens*; Azo + Bac + Pseud, *A. brasilense* + *B. subtilis* + *P. fluorescens*.

Sugarcane Yield

The cane yield was positively influenced by inoculations and phosphorus (P) doses (**Table 9**). Among P doses, the highest stalk yield was observed at 135 kg P_2O_5 ha^{-1} with inoculation of *B. subtilis* + *P. fluorescens* whereas P applied at the dose of 45 kg P_2O_5 ha^{-1} with inoculation of *A. brasilense* + *B. subtilis* also resulted in high stalk yield (**Table 10**). The lower and higher applied doses did not significantly influence stalk yield. P applied at the rates of 45, 90 and 135 kg ha^{-1} P_2O_5 with triple inoculation (*A. brasilense* + *B. subtilis* + *P. fluorescens*) resulted in statistically similar yield to control (no-inoculation) (**Table 10**).

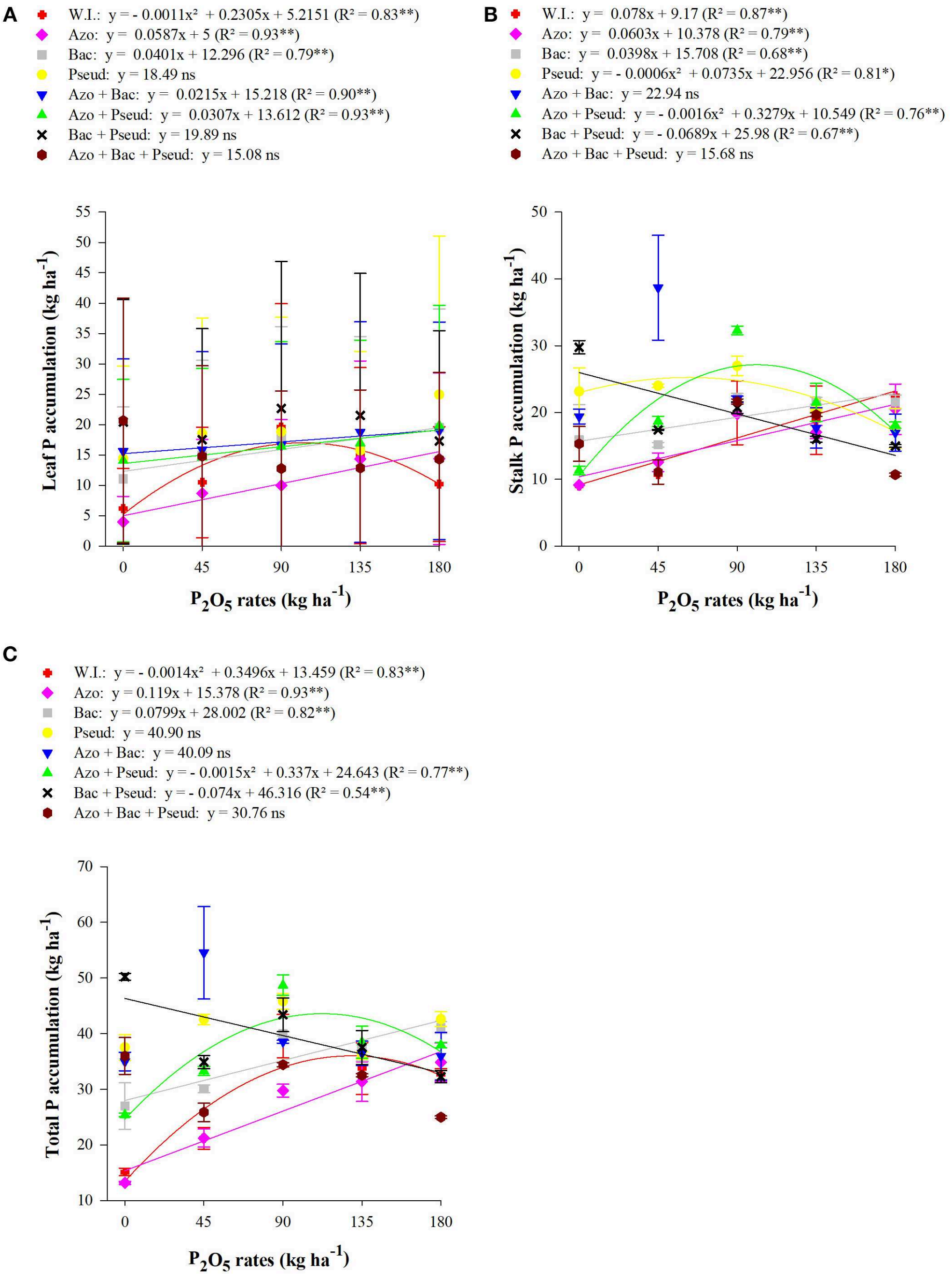

FIGURE 4 | Interactions between different bacterial inoculations and P_2O_5 doses for leaf P accumulation **(A)**, stalk P accumulation **(B)** and total P accumulation **(C)**, at the end cycle of the plant-cane, RB92579 variety (Mean values ± standard deviation). **, * and ns: significant at 1 and 5% at $p < 0.01$, $0.01 < p < 0.05$, and not significant, respectively. W.I., without inoculation; Azo, *Azospirillum brasilense*; Bac, *Bacillus subtilis*; Pseud, *Pseudomonas fluorescens*; Azo + Bac, *A. brasilense* + *B. subtilis*; Azo + Pseud, *A. brasilense* + *P. fluorescens*; Bac + Pseud, *B. subtilis* + *P. fluorescens*; Azo + Bac + Pseud, *A. brasilense* + *B. subtilis* + *P. fluorescens*.

Interaction between P_2O_5 doses and inoculation with PGPB was observed for the yield of sugarcane stalks (**Table 9**). The treatments with no-inoculation, *P. fluorescens* and *B. subtilis* + *P. fluorescens* inoculations set to increasing linear functions (**Figure 5**). The inoculation of *B. subtilis* up to a dose of 97 kg P_2O_5 ha^{-1} was set to quadratic functions, therefore using this dose, the estimated maximum stalk yield would be 188,502 kg ha^{-1}. The inoculation of *A. brasilense* + *P. fluorescens* to the dose of 110 kg P_2O_5 ha^{-1} estimated with maximum stalk yield of 202,126 kg ha^{-1}.

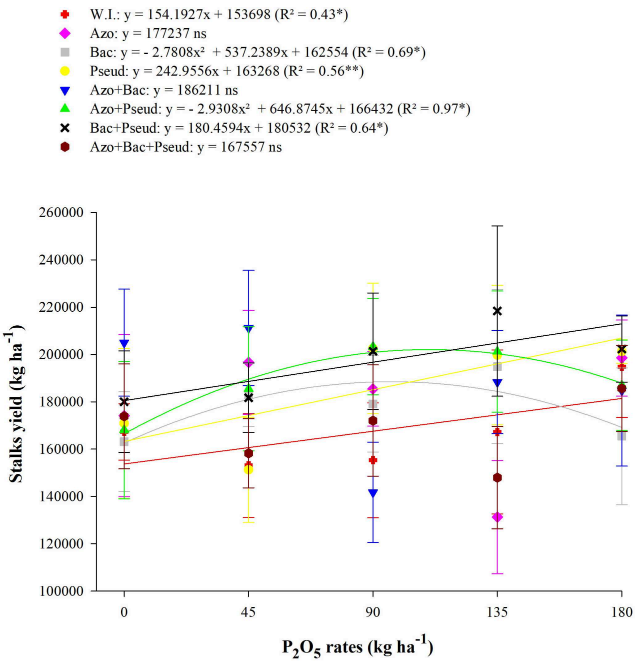

FIGURE 5 | Interactions between different bacterial inoculations and P_2O_5 doses for stalks yield at the end of the plant-cane, RB92579 variety (Mean values ± standard deviation). **, * and ns: significant at 1 and 5% at $p < 0.01$, $0.01 < p < 0.05$, and not significant, respectively. W.I., without inoculation; Azo, *Azospirillum brasilense*; Bac, *Bacillus subtilis*; Pseud, *Pseudomonas fluorescens*; Azo + Bac, *A. brasilense* + *B. subtilis*; Azo + Pseud, *A. brasilense* + *P. fluorescens*; Bac + Pseud, *B. subtilis* + *P. fluorescens*; Azo + Bac + Pseud, *A. brasilense* + *B. subtilis* + *P. fluorescens*.

DISCUSSION

Phosphorus Content in Soil and Cane Leaf

Agricultural soil holds huge phosphorus reserves but all this phosphorus is not readily available to the plants, especially in acidic and highly weathered soils. In case of application of fertilizer containing phosphate can bind to Fe and Al oxides forming insoluble compounds. Phosphate in alkaline soils can bind with Ca and Mg making it unavailable for the plants to accumulate due to the high reactivity (Santos et al., 2012). Thus, a tiny fraction of P is available to plants for absorption.

The P available content in the soil of experimental area was improved with P fertilization however, there were no much differences among the un-inoculated treatments undergo different P doses (0, 45, and 90 kg P_2O_5 ha^{-1}) (**Table 3**). The inoculation of PGPB with different P doses reflected higher variation for available P content. It might indicate that these microorganisms through their mechanisms of solubilizing inorganic P and making it available for the use of plants (Granada et al., 2018).

The soil layer of 0–0.25 m was analyzed after the inoculation of *B. subtilis* + *A. brasilense* along with application of 45 kg P_2O_5 ha^{-1} resulted in 17 mg dm^{-3} P. therefore, it was observed that this inoculation improved available P soil content more than four times in comparison of un-inoculated treatments. Thus, in both of soil depths, it is possible to observe that this inoculation increased the available soil P contents more than 4 times in comparison to control treatments. Caione et al. (2015) analyzed a Dystrophic Red Latosol soil submitted to the application of 360 kg P_2O_5 ha^{-1} from superphosphate and cultivated with sugarcane for six months cycle. It was reported that the P available content increased to 57 mg dm^{-3} in 0–0.20 m layer of soil. The P content can be vary depending on the amount of clay and organic matter present in each soil. The phosphorus dynamics in the soil is complex, especially in tropical soils. The lower availability of this nutrient in the soil may be due to the low content of organic matter in the soil. It is also known that soils with low clay content tend to have low levels of organic matter, and consequently, the available phosphorus content is altered by these soil attributes. Which also interfere with the activity of the enzyme phosphatase and microorganisms involved in the solubilization of unavailable phosphate.

Granada et al. (2018) utilized efficient P solubilizing bacterial strains that resulted in an average reduction of 33% P fertilization during the crop cycle. There must be considered an interaction between plant and bacteria, especially the relevance of these microorganisms with studied genotype. Lira-Cadete et al. (2012) studied three sugarcane varieties (RB92579, RB867515, and RB863129) and observed that RB92579 indicated positive interaction with PGPB (high phosphate solubilization rate in the laboratory). Borges et al. (2019) reported that the P use efficiency and dry matter production of sugarcane may increase in weathered soils when organic and inorganic P sources combinedly applied to the soil to meet the demand for this nutrient.

Leaf is the key to the development of the plant. Phosphorous plays an important role in the synthesis of different compounds of cells, phosphate-sugar catalyzes, photosynthesis and respiration, as well as help in the synthesis of cell membrane components (phospholipids), nucleotides (ATP), DNA and RNA (Taiz et al., 2017). Leaf analysis is one of the most commonly used methods to evaluate the demand of nutrients in sugarcane crop and contribution in fertilizers management (Moura Filho et al., 2014). The leaf P concentrations of all treatments (**Table 4**) are within the range (1.5–3.0 g kg^{-1}), considered appropriate P content for the crop growth according to Raij and Cantarella (1997). Caione et al. (2015) studied P doses from different sources including triple superphosphate and indicated that cane leaf P content after 4 and 8 months of sprouting was significantly influenced by P doses. Hence, regardless of P sources, the obtained leaf P contents (1.4 to 1.8 g kg^{-1}) were slightly lower than present research. Lima (2011) investigated an average P concentration of 1.7 g kg^{-1} in leaf analysis of sugarcane (variety RB867515) after five-month cycle. Cane variety (SP92 4221) was evaluated after 4 months of plantation resulted in foliar P contents ranging between 0.4 and 0.8 g kg^{-1} (Nobile et al., 2010). These differences may occur due to the characteristic of each variety and sampling time of each study.

Nobile et al. (2010) conducted a study with medium texture dystrophic Red Latossol soil and indicated that increasing P content in the soil enhances the absorption capacity of the plant and high P content was accumulated in leaf. The results of the present study were not inline to that of the previous studies and indicated that the P content in soil (**Table 2**) didn't correlate with the P content in leaf (**Table 4**). The treatments with higher levels of P in soil were not correlated to those with the highest concentration of P in leaf. The bacterial inoculation had differently functioned in the soil phosphate solubilization and hence, inoculation (*B. subtilis* + *P. fluorescens*) with the highest P content in leaf had the lowest content of P in soil. Here is the fact that combinations of certain microbes are activated and enabled in result of metabolites and phytohormones production that help in expanding the sugarcane root system and allowing them to achieve greater volume of soil and reach the more distant P, as it is a poorly mobile nutrient (Granada et al., 2018). Solubilization of soil fixed P through microbes making it available for increased plants absorption and its greater expression in leaf concentration.

The plants under soluble phosphate limiting conditions are when inoculated *P. fluorescens fluorescens* strains producing high amounts of gluconic acid and improving growth capacity due to uptake of solubilized P (Oteino et al., 2015). The primary mechanism for mineral P solubilization is the production of organic acids and acid phosphatases (Illmer and Schinner, 1995). Gluconic acid appears to be the most frequent acid in inorganic phosphate solubilization (Rodriguez et al., 2006). According to some authors, the inoculation with *Bacillus* sp. (Vardharajula et al., 2011) or *A. brasilense* (García et al., 2017) improved proline content in plants and make them more resistant to drought stress. The *Arabidopsis* plants when inoculated with *A. brasilense* resulted in the production of high level of abscisic acid. This abscisic acid positively influenced *Arabidopsis* growth by modulating their root architecture with lateral increment, stimulating photosynthetic pigments and reducing water loss through slow stomatal conductance during the drought stress (Cohen et al., 2015).

Dry Matter Partitioning

To satisfy the environmental, agronomic, social and economic issues, manual harvest of sugarcane crop with previous burning was replaced by mechanical harvesting and hereby the leaves and pointers (meristem / apex in development) are currently left on soil surface with amount of 10 to 20 tons ha^{-1} y^{-1} of dry biomass (Trivelin et al., 2013). The green sugarcane harvesting system in association with other precise farming equipment and management practices changed the nutritional requirements of the most modern sugarcane varieties due to large amount of straw remaining on the soil of cultivated area (Leite et al., 2016).

Leite et al. (2016) studied three varieties of sugarcane at three different locations and obtained an average leaf dry mass of 9,000 kg ha^{-1} and stalk dry mass of 37,000 kg ha^{-1}. The present experiment resulted in more leaf dry mass of 15,213 kg ha^{-1} and stalk dry mass of 58,381 kg ha^{-1} (**Table 5**) which are far better than previous experiment. Calheiros et al. (2012) studied the same variety (RB92579) of the present study and found a mean shoot (leaves + stem) dry mass of 35,253 kg ha^{-1} however the present study resulted in an average shoot dry mass of 73,595 kg ha^{-1} (**Table 5**).

The combined dry mass of cane leaf and stalk resulted in maximum total dry mass (entire plant) and likewise happened in most of the inoculated treatments (**Table 6**). This might be due to the reason that these microorganisms utilize and adapt certain mechanisms which directly contribute and improve plant

TABLE 6 | Interactions between different bacterial inoculations and P_2O_5 doses for leaf dry mass, stalk dry mass and total dry mass in sugarcane crop.

	Leaf dry mass (kg ha^{-1})				
	P_2O_5 rates (kg ha^{-1})				
Inoculation	**0**	**45**	**90**	**135**	**180**
W.I.	8,800 d	13,094 c	16,764 a	13,560 c	12,589 c
Azo	9,950 d	14,432 b	11,365 c	15,058 b	15,112 b
Bac	13,847 c	16,956 a	13,941 b	14,140 c	19,056 a
Pseud	15,125 c	19,499 a	17,172 a	12,617 c	17,813 a
Azo + Bac	19,280 b	15,476 b	13,713 b	13,409 c	12,188 c
Azo + Pseud	15,995 c	15,375 b	16,024 a	17,817 a	18,069 a
Bac + Pseud	23,222 a	18,401 a	17,443 a	16,275 b	14,751 b
Azo + Bac + Pseud	20,185 b	11,865 c	11,642 c	12,553 c	13,973 b
Standard error			729		

	Stalk dry mass (kg ha^{-1})				
	P_2O_5 rates (kg ha^{-1})				
Inoculation	**0**	**45**	**90**	**135**	**180**
W.I.	43,270 e	51,564 d	65,142 b	55,978 c	56,208 b
Azo	44,157 e	52,768 d	58,764 c	62,795 b	55,312 b
Bac	59,663 c	55,796 c	54,918 c	54,144 c	46,113 d
Pseud	66,306 b	59,762 c	57,667 c	60,468 b	57,994 b
Azo + Bac	57,662 c	72,157 a	54,723 c	58,123 b	55,756 b
Azo + Pseud	54,500 d	68,745 a	69,074 a	70,861 a	66,650 a
Bac + Pseud	74,147 a	64,080 b	61,603 b	58,892 b	55,149 b
Azo + Bac + Pseud	63,981 b	46,315 e	54,114 c	58,492 b	51,449 c
Standard error			1,499		

	Total dry mass (kg ha^{-1})				
	P_2O_5 rates (kg ha^{-1})				
Inoculation	**0**	**45**	**90**	**135**	**180**
W.I.	52,070 d	64,658 d	81,906 a	69,538 c	68,798 c
Azo	54,107 d	67,200 d	70,129 c	77,853 b	70,424 c
Bac	73,509 c	72,752 c	68,859 c	68,283 c	65,169 c
Pseud	81,431 b	79,260 b	74,839 b	73,084 c	75,806 b
Azo + Bac	76,941 c	87,632 a	68,436 c	71,532 c	67,944 c
Azo + Pseud	70,495 c	84,120 a	85,097 a	88,678 a	84,719 a
Bac + Pseud	97,369 a	82,480 b	79,046 a	75,167 c	69,900 c
Azo + Bac + Pseud	84,166 b	58,180 e	65,756 c	71,045 c	65,422 c
Standard error			1,714		

Means followed by the same letter in the column are not statistically different by the Scott-Knott test at 5% probability.
W.I., Without inoculation.
Azo, Azospirillum brasilense.
Bac, Bacillus subtilis.
Pseud, Pseudomonas fluorescens.
Azo + Bac, A. brasilense + B. subtilis.
Azo + Pseud, A. brasilense + P. fluorescens.
Bac + Pseud, B. subtilis + P. fluorescens.
Azo + Bac + Pseud, A. brasilense + B. subtilis + P. fluorescens.

growth and yield. Inoculatin *Vicia faba* plants with phosphate solubilizing bacteria, Khalafallah et al. (1982) indicated that phosphorus fertilization was reduced by 50% with inoculation. The plants applied with half dose of fertilizer resulted in similar dry mass to that plant applied with 100% of fertilizers. The combined inoculation of *B. subtilis* + *P. fluorescens* with no phosphorus application increased leaf, stalk and total dry masses by 164, 71, and 87% (2.6, 1.7, and 1.9 times) respectively (**Table 6**). The combined inoculation may result in synergistic effect which had described in the present study for double inoculation. The double bacterial inoculation had distinctive influence than that of individual or triple inoculations in dry mass of sugarcane. Khan and Zaidi (2007) indicated that combine inoculation of *Bacillus* sp. and *Azotobacter chroococcum* with arbuscular mycorrhizal fungi (*Glomus fasciculatum*) significantly increased dry mass by about 2.6 times in comparison to control treatments (uninoculated).

Díaz-Zorita et al. (2012) reported that combine inoculation of *A. brasilense* with *Sinorhizobium meliloti* resulted in double dry mass of alfalfa pasture (94% increment). Park et al. (2015) investigated that the effect of volatile compounds produced by *P. fluorescens* in tobacco plants increased the dry mass up to 9.5 times. Tahir et al. (2017) documented that inoculation of *Bacillus subtilis* increased the dry mass of tomato by 3.3 times and root-shoot length by 1.4 times each. The authors also reported that the rate of photosynthetic and auxin contents was also enhanced which may promote and assist plant growth. Oliver and Silva (2018) conducted a research on the combined inoculation of PGPB (containing *A. brasilense*) with N doses in ratoon sugarcane variety (RB92579) and observed that stalk and total dry mass were negatively influenced by bacterial inoculation associated with a maximum N dose (120 kg N ha^{-1}) However, the inoculation associated with intermediate doses of N (60 and 90 kg ha^{-1}) increased stalk and total dry mass reflecting that higher N doses may inhibit the activities of the microorganisms. The same results were observed in the present study for phosphorus application in association with inoculations of *B. subtilis* and *B. subtilis* + *P. fluorescens* (**Figures 3B,C**).

Analyzing several studies published in the literature, Cassán and Díaz-Zorita (2016) claimed that maize inoculated with *A. brasilense* in rainfed regions of South America resulted in greater vegetative growth with higher shoot dry mass (8%). However, the same results for wheat and barley crops were reported under different environmental conditions. Although these authors also conducted two experiments at semi-arid and sub-humid regions on Rye crop inoculated with *A. brasilense* and reported that plant dry matter was increase by 13% (500 kg ha^{-1}) in comparison of non-inoculated treatments. They also reported that such increase inn dry matter was only possible due to the association of microorganisms with N fertilizer and emphasizing the complementary need for both practices (inoculation and fertilization). Naiman et al. (2009) studied N with combined inoculation of *A. brasilense* or *P. fluorescens fluorescens* in wheat crop and observed that shoot dry mass did not increase with urea application however, it had positively influenced with bacterial inoculation. Santos et al. (2019) studied the rooting system and growth of pre-germinated seedlings in sugarcane variety (IACSP95-5000) inoculated with PGPB and reported that shoot dry biomass for all the tested strains including *A. brasilense* was improved.

Inoculation of *A. brasilense* + *B. subtilis* at the dose of 45 kg P_2O_5 ha^{-1} resulted an increment of 40 and 36% in the stalk and total dry mass, respectively, in comparison to non-inoculated (**Table 6**). The dry masses were also significantly increased with inoculation of *A. brasilense* + *P. fluorescens* in combination with 90 and 135 kg P_2O_5 ha^{-1}. The increments in dry mass were 31% for leaf, 27% for stalk dry mass and 28% for total dry mass of the sugarcane variety RB92579. This might be due to the reason that plant hormone is responsible for cell division, stomatal conductance and may control the amount of the apical meristem and leaf chlorophyll content in the leaves. *Azospirillum brasilense* has a role in the synthesis of cytokines which had positive influence on vegetative growth of the plant (Vacheron et al., 2013). *Bacillus subtilis* under dry soil conditions producing cytokinin which may increase shoot dry mass in lettuce and decreased its root / shoot ratio (Arkhipova et al., 2007). The corn inoculated with *B. subtilis* and *P. fluorescens* resulting in production of indole acetic acid (IAA) which in turn increased shoot-root biomass along with water uptake and hereby, ensuring the survival and development of plants during drought (Marulanda et al., 2009).

Phosphorus Accumulation in Leaf, Stalk, and Entire Plant

The mean P accumulation observed at the end of sugarcane cycle in leaves, stalks and total (entire plant) was 15.68, 19.06, and 34.74 kg ha^{-1} respectively (**Table 7**). Leite et al. (2016) reported that mean P accumulation in shoot (stalks + leaves) was 32 kg ha^{-1} which is very similar to that found in the present study. Oliveira et al. (2010) reported that P accumulation in shoot of several sugarcane varieties is ranging from 19 and 30 kg ha^{-1}. The range of P accumulation in shoot for the present study was 13.17 to 54.52 kg ha^{-1} (**Table 8**). In another study, Oliveira et al. (2010) studied a cane variety RB92579 and observed an average of 25 kg P ha^{-1} accumulation in shoots of sugarcane. Calheiros et al. (2012) also studied the same cane variety RB92579 for P accumulation in shoot dry mass and found an average P accumulation of 24.66 kg ha^{-1}.

Caione et al. (2015) studied a cane variety CTC 15 under the application of triple superphosphate and observed that P accumulated in stalks was 25.4 kg ha^{-1} and straw (leaf + pointer) was 15.3 kg ha^{-1} which therefore, resulting in total P accumulation of 40.7 kg ha^{-1}. Thus, it was observed that 38% of the plant P was accumulated in straw and 62% was accumulated in the stalks. It was also observed that 30% of P accumulated in the leaves of sugarcane (Trivelin et al., 2013). In case of present study, an average P accumulated in the leaves was 45% and in stalk was 55%.

The P accumulation in the sugarcane aerial parts was positively influenced by bacterial inoculations and P doses. Inoculation of *B. subtilis* + *P. fluorescens* had possible positive interaction with P doses (0, 45, 90, and 135 kg P_2O_5 ha^{-1}) and improved leaf P accumulation. The leaf P accumulation was

TABLE 7 | Phosphorus (P) accumulation in leaf, stalk, and total plant as influenced by different bacterial inoculations and P_2O_5 doses in sugarcane crop.

	Leaf P accumulation	Stalk P accumulation	Total P accumulation
		kg ha^{-1}	
P_2O_5 rates (kg ha^{-1})			
0	13.30	16.63	29.92
45	14.43	18.53	32.96
90	16.85	23.17	40.02
135	16.39	19.11	35.49
180	17.42	17.86	35.29
Inoculation			
W.I.	12.27	16.19	28.46
Azo	10.28	15.80	26.08
Bac	15.90	19.28	35.19
Pseud	18.49	22.41	40.90
Azo + Bac	17.15	22.94	40.09
Azo + Pseud	16.38	20.38	36.76
Bac + Pseud	19.88	19.78	39.66
Azo + Bac + Pseud	15.08	15.68	30.75
F test			
P_2O_5 rates (R)	**	**	**
Inoculation (I)	**	**	**
R x I	**	**	**
Overall Average	15.68	19.06	34.74
Standard error	0.35	0.75	0.79
CV (%)	7.02	12.52	7.22

***, * and ns: significant at 1% and 5% at p <0.01, p < 0.01 <0.05, and not significant respectively.*
CV: coefficient of variation.
W.I., Without inoculation.
Azo, Azospirillum brasilense.
Bac, Bacillus subtilis.
Pseud, Pseudomonas fluorescens.
Azo + Bac, A. brasilense + B. subtilis.
Azo + Pseud, A. brasilense + P. fluorescens.
Bac + Pseud, B. subtilis + P. fluorescens.
Azo + Bac + Pseud, A. brasilense + B. subtilis + P. fluorescens.

TABLE 8 | Interactions between bacterial inoculations and P_2O_5 doses for leaf P accumulation, stalk P accumulation and total P accumulation in sugarcane crop.

	Leaf P accumulation (kg ha^{-1})				
	P_2O_5 rates (kg ha^{-1})				
Inoculation	**0**	**45**	**90**	**135**	**180**
W.I.	6.16 d	10.48 c	19.64 b	14.88 d	10.19 d
Azo	4.00 d	8.69 c	10.00 d	14.33 d	14.38 c
Bac	11.08 c	14.92 b	17.91 b	16.10 c	19.51 b
Pseud	14.39 b	18.55 a	18.84 b	15.73 c	24.93 a
Azo + Bac	15.60 b	15.85 b	16.57 c	18.77 b	18.98 b
Azo + Pseud	14.07 b	14.63 b	16.41 c	16.95 c	19.82 b
Bac + Pseud	20.43 a	17.51 a	22.68 a	21.53 a	17.28 b
Azo + Bac + Pseud	20.67 a	14.79 b	12.77 d	12.86 d	14.31 c
Standard error			0.78		

	Stalk P accumulation (kg ha^{-1})				
	P_2O_5 rates (kg ha^{-1})				
Inoculation	**0**	**45**	**90**	**135**	**180**
W.I.	8.99 d	10.71 d	19.94 c	18.85 a	22.48 a
Azo	9.17 d	12.55 d	19.76 c	17.06 a	20.48 a
Bac	15.91 c	15.16 d	22.05 c	21.75 a	21.56 a
Pseud	23.13 b	24.00 b	26.96 b	20.33 a	17.65 a
Azo + Bac	19.39 b	38.68 a	21.98 c	17.69 a	16.96 a
Azo + Pseud	11.32 d	18.68 c	32.30 a	21.51 a	18.11 a
Bac + Pseud	29.78 a	17.41 c	20.72 c	16.00 a	14.98 b
Azo + Bac + Pseud	15.31 c	11.08 d	21.65 c	19.67 a	10.69 b
Standard error			1.69		

	Total P accumulation (kg ha^{-1})				
	P_2O_5 rates (kg ha^{-1})				
Inoculation	**0**	**45**	**90**	**135**	**180**
W.I.	15.15 d	21.19 d	39.57 b	33.72 b	32.67 c
Azo	13.17 d	21.24 d	29.76 c	31.39 b	34.86 b
Bac	26.99 c	30.08 c	39.96 b	37.84 a	41.08 a
Pseud	37.52 b	42.55 b	45.80 a	36.06 a	42.59 a
Azo + Bac	34.99 b	54.52 a	38.54 b	36.46 a	35.95 b
Azo + Pseud	25.40 c	33.30 c	48.70 a	38.46 a	37.93 b
Bac + Pseud	50.21 a	34.91 c	43.39 a	37.52 a	32.26 c
Azo + Bac + Pseud	35.98 b	25.87 d	34.42 c	32.52 b	24.99 d
Standard error			1.77		

Means followed by the same letter in the column are not statistically different by the Scott-Knott test at 5% probability.
W.I., Without inoculation.
Azo, Azospirillum brasilense.
Bac, Bacillus subtilis.
Pseud, Pseudomonas fluorescens.
Azo + Bac, A. brasilense + B. subtilis.
Azo + Pseud, A. brasilense + P. fluorescens.
Bac + Pseud, B. subtilis + P. fluorescens.
Azo + Bac + Pseud, A. brasilense + B. subtilis + P. fluorescens.

increased by 15 to 232% (1.2 to 3.3 times) when compared to non-inoculated (control) (**Table 8**). The same combination (*B. subtilis* + *P. fluorescens*) also increased P accumulation in the cane stalks with dose 1 kg P_2O_5 ha^{-1} which consequently resulted in the increment of total P accumulation (entire plant) by 231% (3.3 times) for both leaves and stalks in relation to non-inoculated treatment (**Table 8**). Inoculation of *A. brasilense* + *P. fluorescens* with the application of 90 kg P_2O_5 ha^{-1} increased P accumulation in the cane stalks by 62% (1.6 times) in comparison to non-inoculated (**Table 8**). *A. brasilense* + *B. subtilis* inoculation associated with 45 kg P_2O_5 ha^{-1} increased the P accumulation in the stalks by 261% (3.6 times) and the total P accumulation (entire plant) by 157% (2.6 times) in comparison to control (non-inoculated) (**Table 8**).

An interesting feature was evaluated that both stalk and total P accumulation was decreased with inoculation of *B. subtilis* + *P. fluorescens* and increasing doses of applied phosphate fertilizer (**Figure 4B**). It indicated that this combination of bacterial inoculation may solubilize the soil attached P because P applied from fertilizer wasn't available for the plant accumulation.

As the supply of phosphorus via input increased, bacteria were stagnating P solubilization and may have been harmed by high P doses. The ecto-rhizospheric strains (colonize outside of the roots) and rhizobium endosymbiotic strains (colonize interior of the plants) of *P. fluorescens* and *B. subtilis* have been described as the most effective phosphate solubilizers among soil bacterial communities (Igual et al., 2001). Inoculation of PGPB with phosphate solubilization capability directly to the soil may increase P availability to the plants by 15 times in the soil (Seneviratne and Jayasinghearachchi, 2005).

Soil bacteria increase the solubility of calcium phosphate because majority of these microorganisms have the ability to secrete organic acids (carboxylic acid) which help in the reduction of rhizosphere pH by decoupling the connection between calcium and phosphate (Jayakumar et al., 2019). Solubilizing bacteria may also assist in solubilization of aluminum and iron-bound phosphate and release carboxylic acids which promote direct dissolution of Al and Fe bound P by chelating ions associated with phosphate through a linker exchange mechanism (Tomar, 1998).

Sugarcane Yield

Sugarcane yield was prominently improved by phosphorus doses and bacterial inoculation. Mean comparison indicated that stalk yield (180,831 kg ha^{-1}) of cane variety (RB92579) was obtained with P doses (**Table 9**). Several studies have been conducted to highlight the role of phosphorous in improved production of sugarcane (Tsado et al., 2013; Caione et al., 2015). Albuquerque et al. (2016) described that application of P as triple superphosphate up to 100 kg P_2O_5 ha^{-1} in the furrow increases the yield of cane variety RB92579. Application of P in the furrow as triple superphosphate (at the rate of 100 kg P_2O_5 ha^{-1}) increased the yield of sugarcane by 34% in comparison of control (Caione et al., 2013). Calheiros et al. (2012) performed experiment with same variety (RB92579) under different phosphate fertilizer and observed that greater stalk yield (133,000 kg ha^{-1}) was recorded with application of 90 kg P_2O_5 ha^{-1}. Stalk yield of 232,000 kg ha^{-1} was obtained with application of 180 kg P_2O_5 ha^{-1} in cane variety CTC 15 (Caione et al., 2015).

Inoculation of *B. subtilis* + *P. fluorescens* with application of 135 kg P_2O_5 ha^{-1} increased cane yield by 51,163 kg ha^{-1}(31%) in comparison to non-inoculated treatments (**Table 10**). This might be due to the reason that this combination of bacteria and phosphorus also stood with highest leaf P concentration which possibly contribute to the most of cane yield for all treatments. Inoculation of different PGPB strains resulted an average stalk yield of 101,800 and 108,300 kg ha^{-1} for the sugarcane varieties RB72454 and RB867515 respectively (Schultz et al., 2014).

A. brasilense + *B. subtilis* in combination with 45 kg P_2O_5 ha^{-1} increased stalk yield by 58,152 kg ha^{-1} (38%). This combination of bacteria and P demonstrated that it is possible to decrease the amount of phosphate applied fertilizer. Certainly, this treatment resulted in highest stalk and total dry mass, highest stalk and total P accumulation for all treatments and also increase available P content in both soil layers (0–0.25 and 0.25–0.50 m) which in turn resulting into high stalk yield of sugarcane variety RB92579.

The activity of microorganisms may be harmful in combination with a high or with no dose of P. In case of present study, inoculation of PGPB with P doses of 0 and 180 kg P_2O_5 ha^{-1} were statistically similar to that of non-inoculated (**Table 10**). Oliver and Silva (2018) performed an experiment with certain diazotrophic bacteria including *A. brasilense* and observed that inoculation with combined application of N doses (60 and 90 kg N ha^{-1}) resulted in higher stalk yield in sugarcane variety (RB92579). Oliveira et al. (2017) efficiently inoculated crops (mainly cereals) with *A. brasilense* and reduced fertilizer application which may not have any negative influence on crop yield. They indicated that inoculation with combination of N fertilizers in wheat crop had greatly influenced the yield which was not statistically different than without N fertilization. *Azospirillum brasilense* was applied to corn in furrow planting at a dose of 200 ml h^{-1} in Cerrado region of Brazil resulted in higher grain yield (Morais et al., 2016).

TABLE 9 | Stalks yield of sugarcane as influenced by different bacterial inoculations and P_2O_5 doses.

	Stalks yield (kg ha^{-1})
P_2O_5 rates (kg ha^{-1})	
0	175,310
45	177,600
90	180,110
135	181,140
180	189,990
Inoculation	
W.I.	167,576
Azo	177,237
Bac	177,118
Pseud	185,134
Azo + Bac	186,211
Azo + Pseud	189,042
Bac + Pseud	196,774
Azo + Bac + Pseud	167,557
F test	
P_2O_5 rates (R)	ns
Inoculation (I)	**
R × I	**
Overall average	180,831
Standard error	5,301
CV (%)	13.11

***, * and ns: significant at 1 and 5% at p <0.01, p < 0.01 <0.05, and not significant respectively.*
CV, coefficient of variation.
W.I., Without inoculation.
Azo, Azospirillum brasilense.
Bac, Bacillus subtilis.
Pseud, Pseudomonas fluorescens.
Azo + Bac, A. brasilense + B. subtilis.
Azo + Pseud, A. brasilense + P. fluorescens.
Bac + Pseud, B. subtilis + P. fluorescens.
Azo + Bac + Pseud, A. brasilense + B. subtilis + P. fluorescens.

TABLE 10 | Interactions between different bacterial inoculations and P_2O_5 doses for stalks yield in sugarcane crop.

	Stalks yield (kg ha^{-1})				
	P_2O_5 rates (kg ha^{-1})				
Inoculation	**0**	**45**	**90**	**135**	**180**
W.I.	167,293 a	153,103 b	155,318 b	167,250 b	194,913 a
Azo	174,197 a	196,693 a	185,477 a	131,273 b	198,547 a
Bac	163,167 a	182,963 a	179,107 a	194,900 a	165,453 a
Pseud	170,860 a	151,223 b	202,580 a	199,740 a	201,267 a
Azo + Bac	205,017 a	211,255 a	141,667 b	188,343 a	184,773 a
Azo + Pseud	168,080 a	185,522 a	203,287 a	201,267 a	187,057 a
Bac + Pseud	180,023 a	181,733 a	201,412 a	218,413 a	202,287 a
Azo + Bac + Pseud	173,885 a	158,237 b	172,043 b	147,940 b	185,682 a
Standard error			11,852		

Means followed by the same letter in the column are not statistically different by the Scott-Knott test at 5% probability.
W.I., Without inoculation.
Azo, Azospirillum brasilense.
Bac, Bacillus subtilis.
Pseud, Pseudomonas fluorescens.
Azo + Bac, A. brasilense + B. subtilis.
Azo + Pseud, A. brasilense + P. fluorescens.
Bac + Pseud, B. subtilis + P. fluorescens.
Azo + Bac + Pseud, A. brasilense + B. subtilis + P. fluorescens.

According to Naiman et al. (2009) application of 45 kg ha^{-1} N with inoculation of *A. brasilense* and *P. fluorescens fluorescens* to wheat seeds resulted in an increment of 9 and 10% in the yield of wheat grain, respectively, in comparison to uninoculated treatments. The authors also claimed that farmers could use PGPB to achieve the same increase in wheat yield as achieved with application of 45 kg N ha^{-1} of urea. Sahandi et al. (2019) performed two field experiments with peppermint (*Mentha piperita* L.) using phosphorus doses in association with inoculation of phosphate solubilizing bacteria (PSB). They reported that PSB inoculation increased the efficiency of phosphate fertilizers which leads to reduce the consumption of phosphate fertilizers and also increased plant biomass and yield of essential oils. Several studies demonstrated that crop seed inoculation with phosphate solubilizing microorganisms have the potential to reduce phosphate fertilizer application up to 50% without significant changes in yields (Jilani et al., 2007; Yazdani et al., 2009).

Hussain et al. (2013) investigated some strains of *Bacillus* sp. (PS-12) and *P. fluorescens* sp. (PS-32 and PS-51) and observed that corn grain yield was increased by 11, 42, and 33%, respectively, in comparison to non-inoculated treatments. Rudresh et al. (2005) reported that chickpea inoculated with *Bacillus* sp. improved grain yield by twice and also improved grain P concentration.

Lavakush et al. (2014) reported that rice raised in greenhouse inoculated with P solubilizing bacteria (*A. brasilense* and *Pseudomonas* spp.) potentially reduced P fertilization by 50%. The inoculated plants showed statistically similar performance in plant height, panicle length, grains panicle^{-1} and grain yield to the plants fertilized with 30 and 60 kg P_2O_5 ha^{-1}. Similar results were observed in the present study, the stalk yield obtained with inoculation of *P. fluorescens*, *A. brasilense* + *P. fluorescens,* and *B. subtilis* + *P. fluorescens* and P dose (90 kg P_2O_5 ha^{-1}) was statistically similar to the yield obtained at 180 kg P_2O_5 ha^{-1} without inoculation. It was demonstrated that this inoculation combination if applied in planting furrow of sugarcane variety RB92579 could save half of the phosphorus applied in the current study.

Hassan et al. (2010) indicated that Bacillus strains (*B. subtillus* NH-100 and *Bacillus* sp. NH-217) had antagonistic interaction with fungus *Colletotrichum falcatum* to control red rot from sugarcane. The antagonistic strains of *Bacillus* sp. stand relatively efficient than other biocontrol agents because of their excessive sporulation, prolonged life, improved plant nutrition and potential in controlling plant diseases (Hassan et al., 2010; Patel et al., 2019). Zarei et al. (2019) studied *P. fluorescens* strains and different irrigation levels in sweet corn. It was reported that *P. fluorescens* inoculation increased growth and grain yield as well-mitigated the adverse impact of drought stress. It was exhibited that these strains have the ability to solubilize phosphate, increased siderophores production and availability of nutrients such as P and Fe to plants. Schultz et al. (2014) studied sugarcane varieties with five strains of PGPB (including *A. brasilense*) and observed that the productivity of sugarcane variety RB72454 in the first cane plant and ratoon cane was improved with bacterial inoculation which was statistically similar to the yield obtained with 120 kg N ha^{-1}. It is once again demonstrated that fertilizers could be reduced by utilization of PGPB inoculation.

According to Taulé et al. (2012), potentially N fixing bacteria were more effective in promoting sugarcane growth when they were also phosphate solubilizers and indole acetic acid producers. Phosphate solubilizing and indole acetic acid producing bacteria in sugarcane provided beneficial effect on plant-bacteria interaction (Beneduzi et al., 2013). The appearance of specific features of PGPB indicated that these organisms can promote plant growth by more than one mechanism. Thus, it is critical to characterize bacterial strains that adapted to sugarcane cultivation and are able to excrete substances that promote plant growth (Beneduzi et al., 2013). Hence, further field studies are needed which are carried out over long periods (five or six harvests) with existing strains to confirm whether or not the behavior obtained in this study, contribution of these bacteria in reducing application of phosphate fertilizers in sugarcane crop.

CONCLUSIONS

The bacterial inoculation and P doses prominently improved nutrient accumulation, dry matter and yield of sugarcane crop. Bacteria influenced P leaf concentration and higher values were obtained with *B. subtilis* + *P. fluorescens* inoculation. The inoculation with *Azospirillum brasilense* + *Pseudomonas fluorescens* at the dose 135 kg P_2O_5 ha^{-1} stood for the highest available P content in the 0–0.25 m soil layer.

The highest stalks yield, stalk and total dry matter, P accumulation in stalks and P total was obtained with inoculation of *A. brasilense* + *B. subtilis* allied to the dose of 45 kg P_2O_5 ha^{-1}. In addition, the P content available in the soil surface layer after the plant-cane harvesting in this treatment was still within the P average range recommended by the literature, indicating that such a bacterial combination solubilized unavailable part of phosphate in the soil and allowing plant to use it. Thus, the combination of these two bacteria associated with such a dose is recommended in the sugarcane planting in soil with low available P with an increase of 38% in stalk yield and providing 75% of reduction in the phosphate fertilization, enabling the producer to minimize the production costs of sugarcane.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

PR wrote the manuscript, with contributions from EM and AJ. MT, FG, AJ, and SB corrected and improved the manuscript. PR, EM, GF, and MB conducted the samplings and data collection. PR and EM did the analysis, with the support of MT, SB, FG, and PP.

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Corn Yield and Phosphorus Use Efficiency Response to Phosphorus Rates Associated With Plant Growth Promoting Bacteria

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The use of plant growth promoting bacteria (PGPB) that can solubilize phosphorus (P) has shown potential to improve nutrient availability in grass crops such as corn (*Zea mays* L.) This study was developed to investigate if inoculation with *Azospirillum brasilense*, *Bacillus subtilis* or *Pseudomonas fluorescens* associated with P_2O_5 rates can improve phosphorus use efficiency (PUE) reflecting on greater corn development and yield. The field trial was set up in a Rhodic Hapludox under no-till system under Savannah conditions, in a completely randomized block design with four replicates. Treatments were tested in a full factorial design and included: (i) five P_2O_5 rates (0 to 105 kg ha^{-1}) and (ii) four PGPB seed inoculation (Control–without inoculation, *A. brasilense*, *B. subtilis* or *P. fluorescens*). Inoculation was found to increase grain yield by 39.5, 29.1, and 15.9% when *B. subtilis* was inoculated in the absence of P_2O_5 rates and associated with 17.5 and 70 kg P_2O_5 ha^{-1} and by 34.7% when *A. brasilense* was inoculated with application of 105 kg P_2O_5 ha^{-1}. In addition, inoculation with *B. subtilis* and *A. brasilense* were found to increase P uptake, benefiting productive components development, leading to an improved PUE, and greater corn grain yield. The results of this study showed positive improvements in P uptake as a result of *B. subtilis* and *A. brasilense* inoculation, with an increase of 100.5 and 54.6% on PUE, respectively; while the *P. fluorescens* inoculation were less evident. Further research should be conducted under biotic or/and abiotic conditions such as attack of pathogens and insects, drought, salinity, water flooding, low and high temperature to better understand the role of PGPB, inoculated alone or in combination as the co-inoculated method.

Keywords: ***Azospirillum brasilense*, *Bacillus subtilis*, phosphorus fertilization management, *Pseudomonas fluorescens*, *Zea mays* L.**

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INTRODUCTION

Phosphorus (P) is one of the most limiting nutrients in agricultural cropping systems (Roberts and Johnston, 2015; Guignard et al., 2017; Khan et al., 2018). It is estimated that P deficiencies can be found in nearly 67% of world land designated for crop production (Dhillon et al., 2017). Also, P use efficiency (PUE) for cereal production in the world is too low, varying between 15 and 30% (Dhillon et al., 2017). Under tropical conditions, P can precipitate as minerals of Fe, and Al (Penn and Camberato, 2019). Both minerals decrease the availability of P for plant growth (Dhillon et al., 2017). Clay fractions such as amorphous hydrated oxides of Fe and Al, in addition to gibbsite, goethite, and kaolinite are responsible for the greatest P fixation (Dhillon et al., 2017). This has created a cascade of environmental problems (e.g., global warming, air pollution, and eutrophication) that threaten ecosystems and human health (Gu et al., 2015). Therefore, the P demand by crops must be considered. Plants of intense and short-cycle development, such as the corn plant (*Zea mays* L.), require higher amounts of P in solution and faster adsorbed-P replenishment than perennial crops (Lino et al., 2018). However, fertilizer application is one of the highest input costs for cereal crops and yet most of the P-fertilizer used to supplement crops is lost into the environment, due to the low PUE in cereal crops (Metson et al., 2016; Li et al., 2017).

The use of agroecological practices such as inoculation by plant growth-promoting bacteria (PGPB) can represent a sustainable alternative for increase nutrient use efficiency in tropical agriculture (Galindo et al., 2018a,b, 2019a,b; Martins et al., 2018). The use of these PGPB is growing, particularly in Latin America, for different crops (Souza et al., 2015; Martins et al., 2018; Galindo et al., 2019a). Several PGPB genera show association with different species of agricultural importance, such as *Azospirillum*, *Bacillus* and *Pseudomonas* (Zeffa et al., 2018). These bacteria can stimulate plant growth by a series of mechanisms, including but not restricted, the production of phytohormones, such as salicylic acid, gibberellins, cytokinins and indole-3-acetic acid (IAA) (Cassán and Diaz-Zorita, 2016; Fukami et al., 2017), phosphate solubilization (Ludueña et al., 2018; Qi et al., 2018), nutrient availability increase (Galindo et al., 2018b), production of indolic compounds and siderophores (Ambrosini and Passaglia, 2017), increase on 1—aminocyclopropane—1—carboxylate deaminase activity (Ambrosini and Passaglia, 2017), biological nitrogen fixation (BNF) (Pankievicz et al., 2015), biological control of plants, production of natural antibiotics and protective effect against secondary soil phytopathogens (Zhou et al., 2016; Mishra and Arora, 2018; Shameer and Prasad, 2018).

The *Azospirillum* spp. is considered one of the most studied plant growth promoter genera (Galindo et al., 2016, 2017). An analysis of field trials conducted worldwide for over 20 years, where various non-legume crops were inoculated with *Azospirillum* spp. under different weather and soil conditions, concluded that crop yield can increase up to 30% with inoculation (Fukami et al., 2016). Also, positive results in corn development and yield has been reported with *Azospirillum brasilense* inoculation (strains Ab-V5 and Ab-V6) under tropical conditions (Martins et al., 2018; Oliveira et al., 2018; Galindo et al., 2019b). However, greater responses with other PGPB can be achieved (Pankievicz et al., 2019). New research investigating *Bacillus* spp. and *Pseudomonas* spp. as beneficial PGPB are being conducted, especially for annual crops (Oliveira et al., 2019; Pankievicz et al., 2019; Tavanti et al., 2020). For example, under tropical conditions, *Bacillus subtilis* inoculation (strains Pant001 and QST713) associated with *Bradyrhizobium japonicum* has been reported to increase soybean [*Glycine max* (L.) Merr.] yield compared to single inoculation with *B. japonicum*, besides improving seed quality due to the increase in total storage proteins concentration, seedling emergence percentage and seed vigor (Tavanti et al., 2020). Traoré et al. (2016) reported improved corn seed germination, plant growth, plant production (increase yield by 42%) grain and shoot P biomass content of 34 and 64%, respectively with *B. subtilis* inoculation (strain DSM10). Lima et al. (2019) verified that *B. subtilis* (strains AP-3 and PRBS-1) promoted common bean (*Phaseolus vulgaris* L.) and corn growth, increasing the water use efficiency, leaf water content and the regulation of stomata, without damaging photosynthetic rates. Zarei et al. (2019) concluded that *Pseudomonas fluorescens* (P1, P3, P8, and P14 – prepared from the collection of Vali-e-Asr University of Rafsanjan) can improve plant water deficit stress tolerance, P solubilization and siderophore production, leading to an increased sweet corn (*Zea mays* L. var saccharata) growth and yield. Differently, Oliveira et al. (2019), studying six PGPB inoculation in soybean (*A. brasilense*, *B. amyloliquefacens*, *B. licheniformis*, *B. pumilus*, *B. subtilis* e *P. fluorescens*) associated with *B. japonicum* did not verified plant development and increased grain yield compared to single inoculation with *B. japonicum*.

Understanding the success or failure of inoculation requires understanding the complex interactions between the roots of inoculated plants, the specificity between hosts and PGPB, and the major microbial communities in the rhizosphere (Bashan et al., 2004; Florio et al., 2017, 2019). Therefore, studies with different PGPB inoculation such as *Bacillus* spp. and *Pseudomonas* spp. in tropical conditions should be performed, since new reports can be largely applicable to other important producing countries. Regardless of several benefits may be verified with PGPB inoculation, an increase in PUE and corn grain yield is not always the case (Bounaffaa et al., 2018). Further research with different PGPB inoculation associated with P_2O_5 rates are needed to determine how to maximize its benefits on PUE, corn development and yield. This study was based on the hypothesis of positive effect between the different PGPB inoculation and P utilization, providing greater nutrient use efficiency, reflecting on corn development and yield. The objective of this study was to evaluate the effect of *A. brasilense*, *B. subtilis*, and *P. fluorescens* inoculation and P_2O_5 application rates, on the nutritional, productive components PUE and corn grain yield under tropical conditions.

MATERIALS AND METHODS

The study was conducted under field conditions in Selvíria (Savannah region), state of Mato Grosso do Sul, Brazil [20°22′ south (S) and 51°22′ west (W), 335 m above sea level (a.s.l.)], during the crop year of 2016/17. The soil was classified as clayey Oxisol (Rhodic Hapludox) according to the Soil Survey Staff (2014). The experimental area was cultivated with annual crops (cereal and legume crops) for over 30 years, with the last 12 years using a no-tillage system. The last crop sequence prior to corn was wheat. The maximum and minimum temperatures, air relative

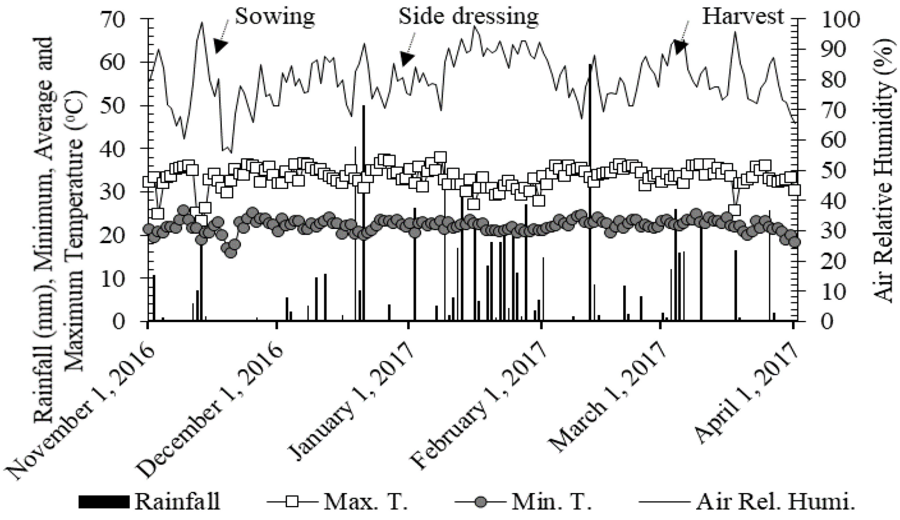

FIGURE 1 | Rainfall, air relative humidity, maximum and minimum temperatures obtained from the weather station located in the Education and Research Farm of FE/UNESP during the corn cultivation in the period November 2016 to April 2017.

humidity and the rainfall verified during the study are presented in **Figure 1**.

The experimental design was a completely randomized block design with four replicates arranged in a 4 × 5 factorial scheme: four PGBP seed inoculations (control–without inoculation, *A. brasilense*, *B. subtilis* and *P. fluorescens* and five P_2O_5 rates (0, 17.5, 35, 70, and 105 kg ha^{-1}) The experimental plots were composed of seven 3.5 m corn rows spaced at the distance of 0.45 m, with the useful area of the plot being the central three rows, with the exclusion of 0.5 m from each end.

Twenty soil samples were collected, mixed and a random sub-sample was used to determine soil chemical attributes before the beginning of field trial in the 0.0–0.2 m depth. This samples were collected with soil core sample type cup auger (0.4 m × 0.10 m–cup length and diameter, respectively), randomized in the entire experimental site, regardless of experimental blocks and plots. After samples be collected and mixed, the sub-sample was dried in the shade and soil chemical attributes were determined according to the Raij et al. (2001) methodology. The following results were verified in **Table 1**.

Weed control was performed with herbicides application of glyphosate [1800 g ha^{-1} of the active ingredient (a.i)] and 2,4-D (670 g ha^{-1} a.i). The mineral N and K fertilization was performed with 30 kg N ha^{-1} (urea) and 60 kg K_2O ha^{-1} (potassium chloride) at seedling and for all treatments, based on the soil analysis and corn crop requirements. Also, the application of P_2O_5 rates was performed at seedling based on soil analysis and crop requirements. The P_2O_5 rates (0, 17.5, 35, 70 e 105 kg ha^{-1}) corresponds to 0, 25, 50, 100, and 150% of the recommended rate, respectively (Cantarella et al., 1997). The source of P_2O_5 applied was triple superphosphate (18% of P_2O_5, 16% of Ca, and 8% of S).

The inoculation with *A. brasilense* strains Ab-V5 and Ab-V6 was performed at a dose of 100 mL of liquid inoculant per hectare [equivalent to 100 mL of inoculant per 73,400 corn seeds planted – guarantee of 2 × 10^8 CFU (colony forming unity) mL^{-1}]. The inoculation with *B. subtilis* strain CCTB04 was performed at a dose of 100 mL of liquid inoculant per hectare (equivalent to 100 mL of inoculant per 73,400 corn seeds planted – guarantee of 1 × 10^8 CFU mL^{-1}). The inoculation with *P. fluorescens* strain CCTB03 was performed at a dose of 100 mL of liquid inoculant per hectare (equivalent to 100 mL of inoculant per 73,400 corn seeds planted – guarantee of 1 × 10^8 CFU mL^{-1}). These are commercial strains used in Brazil [for both *A. brasilense* (brand name AzoTotal), *B. subtilis* (brand name Vult) and *P. fluorescens* (brand name Audax)]. Both inoculations (*A. brasilense*, *B. subtilis*, and *P. fluorescens*) were performed by coating and mixing the inoculants and corn seeds in plastic bags, manually. The seed inoculations were realized one hour before planting the corn crop and after seed treatment with fungicide and insecticide [the fungicides

TABLE 1 | Soil chemical attributes in 0–0.20 m layer before field trial beginning.

Soil chemical attributes	0–0.20 m layer
P (resin)	20 mg dm^{-3}
S (SO_4)	3 mg dm^{-3}
Organic matter	24 g dm^{-3}
pH ($CaCl_2$)	5.3
K	1.6 $mmol_c$ dm^{-3}
Ca	33.0 $mmol_c$ dm^{-3}
Mg	20.0 $mmol_c$ dm^{-3}
H + Al	28.0 $mmol_c$ dm^{-3}
B (hot water)	0.19 mg dm^{-3}
Cu (DTPA)	3.9 mg dm^{-3}
Fe (DTPA)	21.0 mg dm^{-3}
Mn (DTPA)	63.5 mg dm^{-3}
Zn (DTPA)	1.6 mg dm^{-3}
Base saturation	68%

n = 20.

thiophanate-methyl + pyraclostrobin (56 g + 6 g of a.i. per 100 kg of seed) and the insecticide fipronil (62 g of a.i. per 100 kg of seed) were used], when the seeds were completely dry. The control treatment did not receive inoculations, however, the chemical seed treatment with fungicide and insecticide was performed, similarly to inoculated treatments.

The corn simple hybrid used was DOW 2B710 PW and the planting took place on November 11, being planted 73,400 seeds per ha. Seedling emergence occurred 5 days after sowing, on November 16, 2016. When necessary, the corn crop was irrigated with supplementary irrigation, using a center pivot sprinkling system (water depth of 14 mm). Weed and insect control were performed according to crop demand.

Nitrogen fertilizer (side dress application) was spread on the soil surface without incorporation by placing the fertilizer in the middle of the rows when the plants were in the V4 stage (with four leaves completely unfolded) at the dose of 150 kg N ha^{-1} as urea source, for all plots. After N-fertilization, the area was irrigated (14 mm depth) at night to minimize losses by ammonia volatilization. The plants were harvested manually at 125 days after emergence (DAE) on March 21, 2017.

The following nutritional evaluations were performed: (a) P foliar concentration, in g kg^{-1} of dry matter, was determined by collecting the middle third of 20 leaves of the main ear insertion in each experimental plot in the female flowering stage, according to the methodology described in Cantarella et al. (1997). Also, the (b) P concentration in biomass and grains were determined, at harvest time, and the P uptake in biomass and grains were calculated, in kg ha^{-1}. P determinations in tissue and grains followed the methodology that was proposed in Malavolta et al. (1997). Five soil samples (depth of 0–0.20 m) per plot were collected at the harvest time to determine (c) P-resin according to Raij et al. (2001).

The following productive components measurements were performed: (d) plant height at maturity, defined as being at a distance (m) from the ground level to the apex of the tassel; (e) stem diameter in the second internode at corn maturation plant using a manual caliper. Ten corn spikes were collected at the harvest time to follow the evaluations: (f) spike diameter; (g) spike length, determined from the base of the spike to the apex; (h) number of rows per spike, obtained by counting the number of all rows in each spike; (i) number of grains per row of spike, determined by counting the number of grains in each row of the spike; (j) number of grains per spike, obtained by counting the number of grains in each spike; (k) mass of 100 grains, determined at 13% moisture (wet basis) by a 0.01 g precision scale; (l) phosphorus use efficiency (PUE) following the Eq. 1; and (m) grain yield, determined by the spike collection in the useful experimental area. After the mechanical track, the grains were quantified and corrected to 13% moisture (wet basis), in kg ha^{-1}.

$$\text{PUE, adapted of Moll et al. (1982)}:$$
$$[(\text{GYF} - \text{GYW}) \div (\text{amount of N applied})] \tag{1}$$

Where, GYF = Grain Yield with fertilizer and GYW = Grain Yield without fertilizer.

The data was analyzed by ANOVA in a 2-way factorial design with P_2O_5 application rates and inoculation and their interactions considered fixed effects in the model. Mean separation was done when significant factors or interactions were observed using the test Tukey. Regression analysis was used to discern whether there was a linear or non-linear response to P_2O_5 rates in R software (R Core Team, 2015).

RESULTS

Statistical analysis showed that leaf P concentration, biomass P uptake, grain P uptake, plant height, ear length, ear diameter, number of grains per row, and grains per ear, mass of 100 grains, PUE and grain yield were significantly affected by the interaction between P_2O_5 rates × PGPB inoculation (**Table 2**). Phosphorus resin in soil and stem diameter were significantly affected by the main effects of P_2O_5 rates and PGPB inoculation (**Table 2**). Number of rows per ear was not affected by P_2O_5 rates, PGPB inoculation or the interaction between the factors (**Table 2**).

Control plots associated with low and average P_2O_5 application rates (0, 17.5 and 35 kg ha^{-1}) resulted in greater leaf P concentration compared to *A. brasilense*, *P. fluorescens* and *B. subtilis* inoculated plots (**Figure 2A**). Also, control plots

TABLE 2 | F-values for leaf P concentration, biomass P uptake, grain P uptake, P-resin in soil, plant height, stem diameter, ear length, ear diameter, number of rows per ear, grains per row and grains per ear, mass of 100 grains, phosphorus use efficiency and corn grain yield 2016/17.

F-values	Leaf P concentration	Biomass P uptake	Grain P uptake	P-resin in soil	Plant height	Stem diameter	Ear length
P_2O_5 rates (R)	5.691^{**}	3.405^{*}	13.019^{**}	10.938^{**}	3.052^{*}	2.756^{*}	2.164^{ns}
PGBP inoculation (I)	75.765^{**}	0.828^{ns}	8.017^{**}	11.606^{**}	19.637^{**}	23.992^{**}	7.976^{**}
R x I	3.968^{**}	3.043^{**}	3.073^{**}	1.285^{ns}	3.685^{**}	1.642^{ns}	3.112^{**}

F-values	Ear diameter	Rows per ear	Grains per row	Grains per ear	Mass of 100 grains	Phosphorus use efficiency	Grain yield
P_2O_5 rates (R)	2.809^{*}	1.462^{ns}	1.740^{ns}	2.168^{ns}	1.635^{ns}	26.856^{**}	14.060^{**}
PGBP inoculation (I)	2.124^{ns}	1.815^{ns}	2.510^{ns}	3.085^{*}	1.795^{ns}	6.624^{**}	11.287^{**}
R x I	2.320^{*}	1.514^{ns}	3.452^{**}	1.957^{*}	2.553^{**}	2.946^{**}	3.471^{**}

**, * and ns: significant at $p < 0.01$, $p < 0.05$, and not significant, respectively.

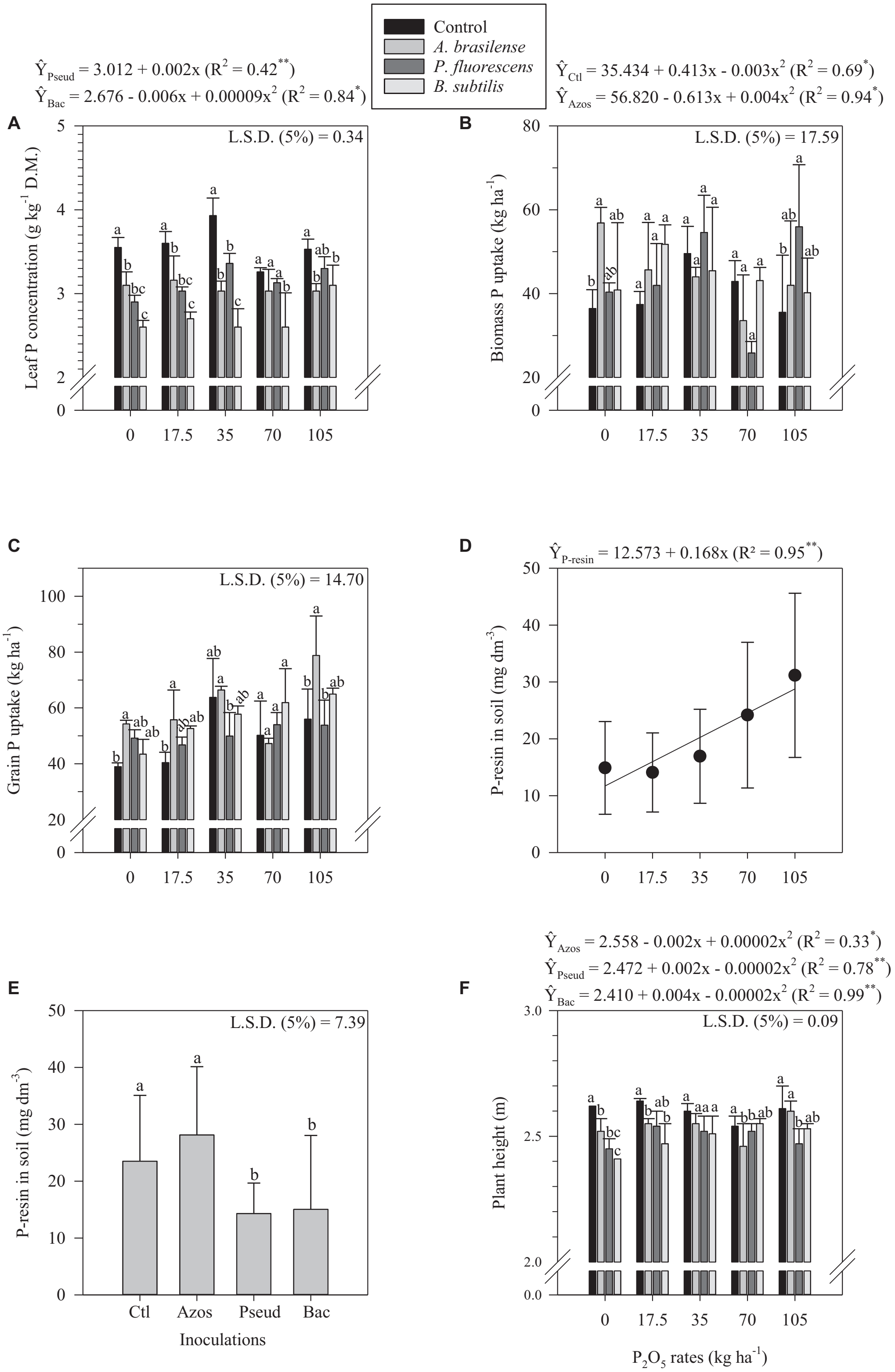

FIGURE 2 | Leaf P concentration **(A)**, Biomass P uptake **(B)**, Grain P uptake **(C)** as a function of the interaction between P_2O_5 rates and inoculations, P-resin in soil as a function of P_2O_5 rates **(D)** and inoculations **(E)** and plant height **(F)** as a function of the interaction between P_2O_5 rates and inoculations in corn crop. The letters correspond to a significant difference at 5% probability level ($p \leq 0.05$). Error bars indicate the standard deviation of the mean ($n = 4$). Ctl, Azos, Pseud e Bac refers to the control treatment and inoculations with *A. brasilense*, *P. fluorescens* and *B. subtilis*, respectively. L.S.D. (least significant difference), D.M. (dry matter).

associated with high P_2O_5 rates (105 kg ha^{-1}) resulted in greater leaf P concentration compared to *A. brasilense* and *B. subtilis* inoculated plots (**Figure 2A**). Leaf P concentration responded linearly to P_2O_5 application rates when *P. fluorescens* was inoculated (**Figure 2A**). Differently, leaf P concentration responded non-linearly to P_2O_5 application rates when *B. subtilis* was inoculated (**Figure 2A**).

In the absence of P_2O_5 application, control plots resulted in lower biomass P uptake compared to *A. brasilense* inoculated plots (**Figure 2B**). Also, control plots associated with high P_2O_5 application rates (105 kg ha^{-1}) resulted in lower biomass P uptake compared to *P. fluorescens* inoculated plots (**Figure 2B**). Biomass P uptake responded non-linearly to P_2O_5 application rates without inoculation and when *A. brasilense* was inoculated (**Figure 2B**). The *A. brasilense* inoculated plots associated with low and high P_2O_5 application rates (0, 17.5 and 105 kg ha^{-1}) resulted in greater grain P uptake compared to control plots (**Figure 2C**). In addition, *A. brasilense* inoculated plots associated with 35 kg P_2O_5 ha^{-1} resulted in greater grain P uptake compared to *P. fluorescens* inoculated plots (**Figure 2C**). Grain P uptake responded linearly to P_2O_5 application rates when *B. subtilis* was inoculated (**Figure 2C**). Differently, grain P uptake responded non-linearly to P_2O_5 application rates when control and *A. brasilense* was inoculated (**Figure 2C**). Phosphorus resin in soil responded linearly to P_2O_5 application rates (**Figure 2D**). In addition, control and *A. brasilense* inoculated plots resulted in greater P-resin in soil compared to *P. fluorescens* and *B. subtilis* inoculated plots (**Figure 2E**).

Control plots associated with low P_2O_5 application rates (0 and 17.5 kg ha^{-1}) resulted in greater plant height compared to *A. brasilense*, *P. fluorescens* and *B. subtilis* inoculated plots (**Figure 2F**). In addition, control plots associated with 105 kg P_2O_5 ha^{-1} resulted in greater plant height compared to *P. fluorescens* inoculated plots (**Figure 2F**). However, *B. subtilis* inoculated plots associated with 70 kg P_2O_5 ha^{-1} resulted in greater plant height compared to *A. brasilense* inoculated plots (**Figure 2F**). Plant height responded non-linearly to P_2O_5 application rates when *A. brasilense*, *P. fluorescens* and *B. subtilis* were inoculated (**Figure 2F**). Stem diameter responded linearly to P_2O_5 application rates (**Figure 3A**). In addition, *A. brasilense* inoculated plots resulted in greater stem diameter compared to control, *P. fluorescens* and *B. subtilis* inoculated plots (**Figure 3B**).

Ear length fluctuated throughout the P_2O_5 application rates; however, in general, control plots showed reduced ear length compared to *A. brasilense*, *P. fluorescens* and *B. subtilis* inoculated plots (**Figure 3C**). Ear length responded linearly to P_2O_5 application rates when *P. fluorescens* was inoculated (**Figure 3C**). Differently, ear length responded non-linearly to P_2O_5 application rates when *B. subtilis* was inoculated (**Figure 3C**). Control plots and *P. fluorescens* inoculated plots associated with average P_2O_5 rates (35 and 70 kg ha^{-1}) tended to result in lower ear diameter compared to *A. brasilense* and *B. subtilis* inoculated plots (**Figure 3D**). Ear diameter responded non-linearly to P_2O_5 application rates when control and *P. fluorescens* was inoculated (**Figure 3D**).

In the absence of of P_2O_5 application, *B. subtilis* inoculated plots resulted in greater number of grains per row compared to control, *A. brasilense* and *P. fluorescens* inoculated plots (**Figure 3E**). Grains per row responded non-linearly to P_2O_5 application rates when control and *B. subtilis* was inoculated (**Figure 3E**). Similarly, in the absence of of P_2O_5 application, *B. subtilis* inoculated plots resulted in greater number of grains per ear compared to control, *A. brasilense* and *P. fluorescens* inoculated plots (**Figure 3F**). Also, *A. brasilense* inoculated plots associated with 105 kg P_2O_5 ha^{-1} resulted in greater number of grains per ear compared to control plots (**Figure 3F**). Control plots and *P. fluorescens* inoculated plots associated with average and high P_2O_5 application rates (70 and 105 kg ha^{-1}) tended to result in lower mass of 100 grains compared to *A. brasilense* and *B. subtilis* inoculated plots (**Figure 4A**). Mass of 100 grains responded linearly to P_2O_5 application rates when *B. subtilis* was inoculated (**Figure 4A**). Differently, mass of 100 grains responded non-linearly to P_2O_5 application rates when control and *A. brasilense* and *P. fluorescens* were inoculated (**Figure 4A**).

The *B. subtilis* inoculated plots associated with 17.5 kg P_2O_5 ha^{-1} resulted in greater PUE compared to control, *A. brasilense* and *P. fluorescens* inoculated plots (**Figure 4B**). Phosphorus use efficiency responded linearly to P_2O_5 application rates regardless of inoculations (**Figure 4B**). The *B. subtilis* inoculation also benefited grain yield when associated with low P_2O_5 rates (0 and 17.5 kg ha^{-1}) as verified by greater grain yield compared to control (**Figure 4C**). The *A. brasilense* inoculated plots associated with 70 kg P_2O_5 ha^{-1} resulted in lower grain yield compared to *P. fluorescens* and *B. subtilis* inoculated plots (**Figure 4C**). However, *A. brasilense* inoculated plots associated with 105 kg P_2O_5 ha^{-1} resulted in greater grain yield compared to control and *P. fluorescens* inoculated plots (**Figure 4C**). Grain yield responded linearly to P_2O_5 application rates when *A. brasilense* was inoculated (**Figure 4C**). Differently, grain yield responded non-linearly to P_2O_5 application rates when control and *P. fluorescens* were inoculated (**Figure 4C**).

DISCUSSION

Phosphorus is the second nutrient that is most demanded by corn plants and directly affects crop development and yield (Dhillon et al., 2017). Thus, the higher P availability as a function of the P_2O_5 application probably favored initial root system development, reflecting on corn grain yield. The better growth of P_2O_5-fertilized plants can be attributed to the P readily available for absorption after being added to the soil as verified by the linear increasing response in P content in soil to P_2O_5 application rates. Phosphorus plays important roles in plant nutrition and development (Lollato et al., 2019), for example composition of adenosine triphosphate (ATP). In addition, P is responsible for the storage and transport of energy for endergonic processes, such as the synthesis of organic compounds and the active uptake of nutrients (Marschner, 2012). Also, P is related to root system development and plant growth (Sulieman and Tran, 2015; Fink et al., 2016; Zhang et al., 2016). There are several studies reporting the P fertilization benefits in corn crop (Wen et al., 2016; Li et al., 2017; Schlegel and Havlin, 2017; Ortas and Islam, 2018; Preston et al., 2019). However, plant nutritional demand

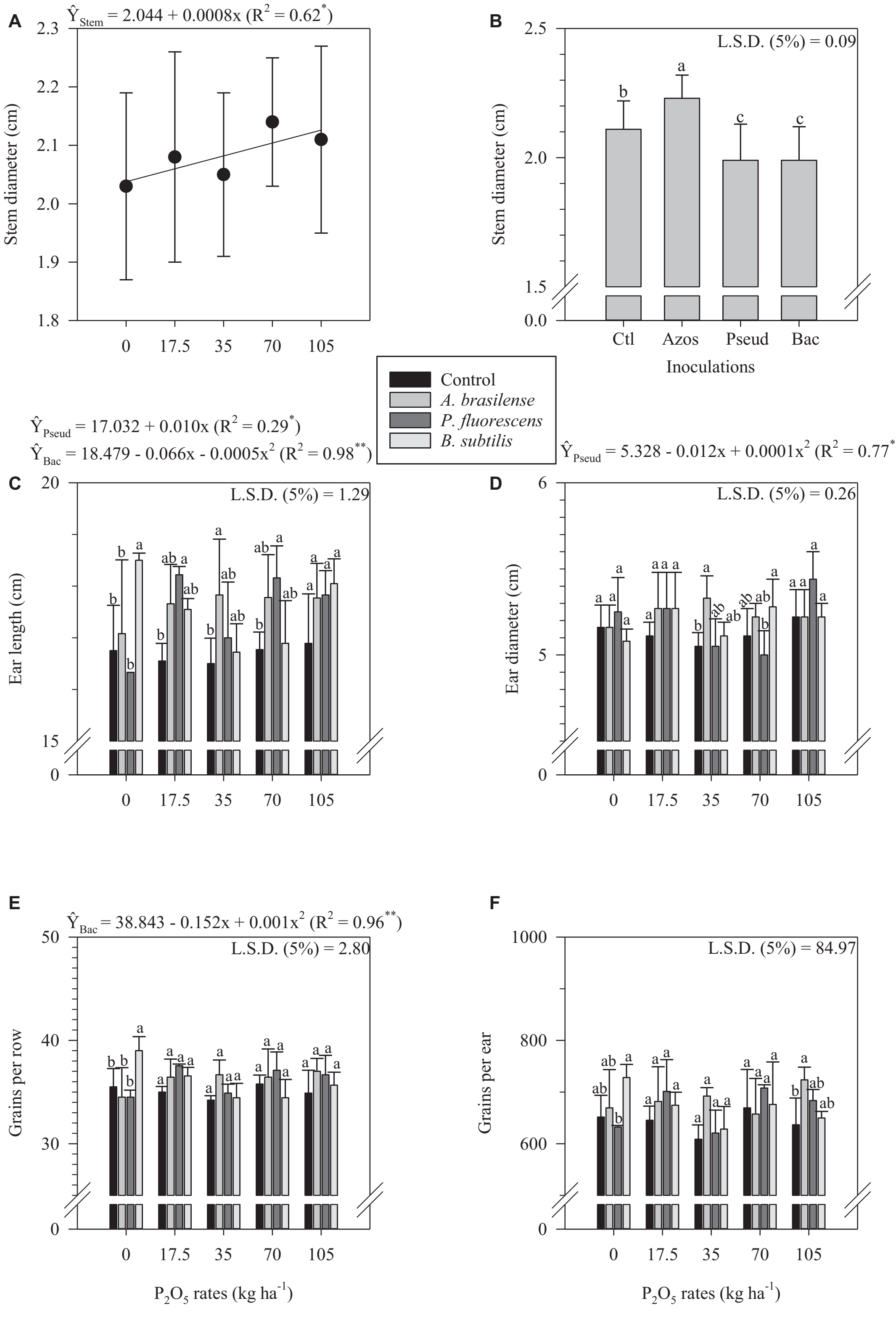

FIGURE 3 | Stem diameter as a function of P_2O_5 rates **(A)** and inoculations **(B)**, ear length **(C)**, ear diameter **(D)**, grains per row **(E)**, and grains per ear **(F)** as a function of the interaction between P_2O_5 rates and inoculations in corn crop. The letters correspond to a significant difference at 5% probability level ($p \leq 0.05$). Error bars indicate the standard deviation of the mean ($n = 4$). Ctl, Azos, Pseud e Bac refers to the control treatment and inoculations with *A. brasilense*, *P. fluorescens* and *B. subtilis*, respectively. L.S.D. (least significant difference).

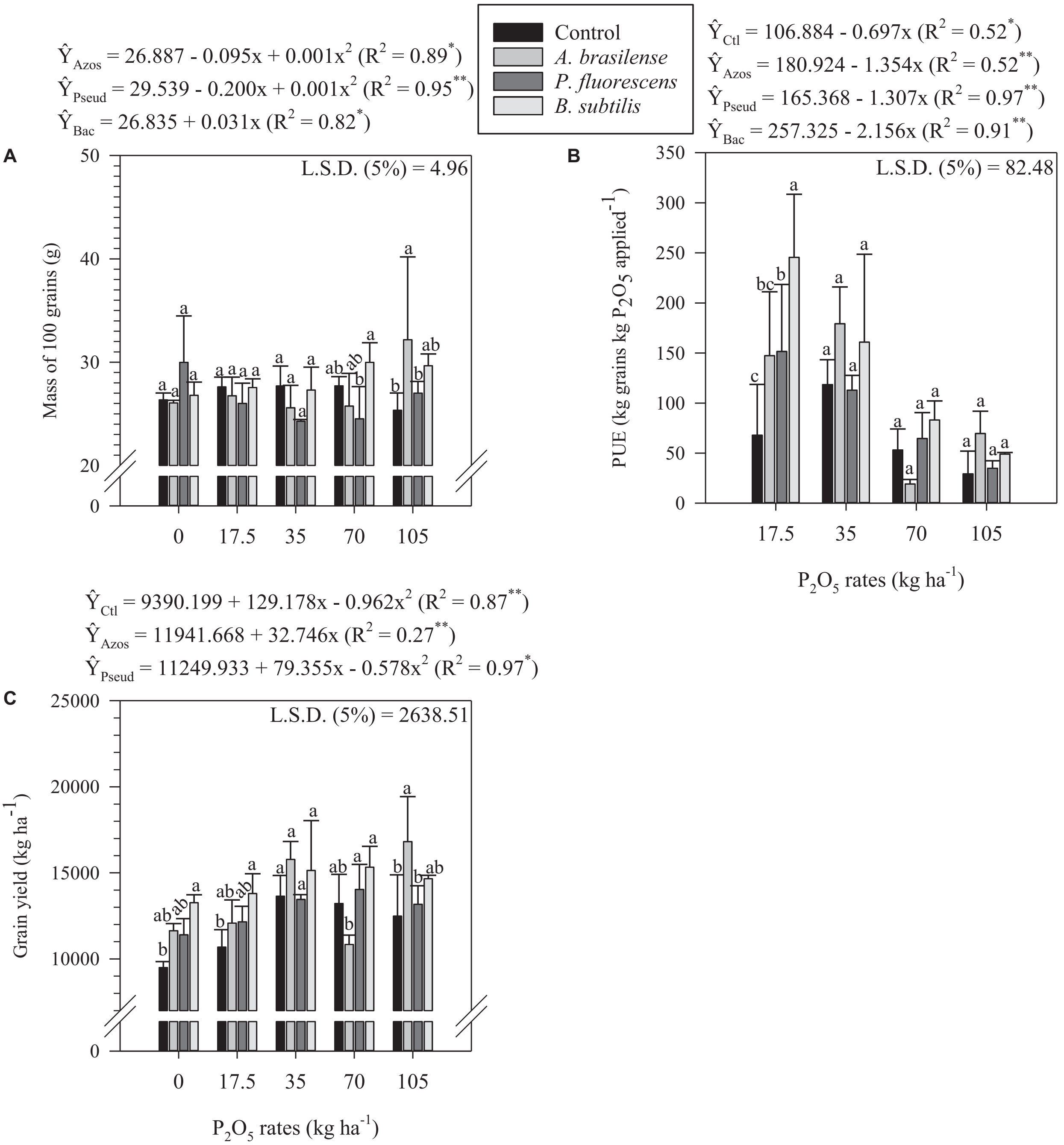

FIGURE 4 | Mass of 100 grains **(A)**, PUE **(B)**, and grain yield **(C)** as a function of the interaction between P_2O_5 rates and inoculations in corn crop. The letters correspond to a significant difference at 5% probability level ($p \leq 0.05$). Error bars indicate the standard deviation of the mean ($n = 4$). Ctl, Azos, Pseud e Bac refers to the control treatment and inoculations with *A. brasilense*, *P. fluorescens* and *B. subtilis*, respectively. L.S.D. (least significant difference), PUE (P use efficiency).

is limited as verified by the linear decreasing response in PUE to P_2O_5 application rates, regardless of PGPB inoculation. In addition, almost 80% of P content in soil can be fixed in forms unavailable to plants (White and Hammond, 2008; Zhang et al., 2016). Therefore, P application must be rational and optimized, since the increased P_2O_5 application rates results in increased losses and less utilization by cropping systems.

Although the response to PGPB inoculation under P_2O_5 application rates were different, the response to *B. subtilis* and *A. brasilense* inoculation associated with P_2O_5 rates were greater than control and *P. fluorescens* inoculation mainly when *P. fluorescens* inoculation was associated with P_2O_5 application rates. We verified this behavior on lower grain P uptake (associated with 35 and 105 kg P_2O_5 ha^{-1}), ear diameter (with

35 and 70 kg P_2O_5 ha^{-1}), mass of 100 grains (with 70 and 105 kg P_2O_5 ha^{-1}) and grain yield (with 105 kg P_2O_5 ha^{-1}) when *P. fluorescens* inoculation was performed. Positive responses to *B. subtilis* inoculation were verified mainly in the absence and application of 70 kg P_2O_5 ha^{-1}. In the absence of P_2O_5 application rates *B. subtilis* inoculation showed greater ear length, number of grains per row and grains per ear. With application of 70 kg P_2O_5 ha^{-1}, *B. subtilis* inoculation showed greater ear diameter and mass of 100 grains. In addition, when 17.5 kg P_2O_5 ha^{-1} was applied, *B. subtilis* inoculation showed greater PUE. Grain yield was also positive affected by *B. subtilis* inoculation in the absence of P_2O_5 application rates (increase of 39.5, 13.9, and 16.3%), 17.5 kg P_2O_5 ha^{-1} (increase of 29.1, 14.2, and 13.5%) and 70 kg P_2O_5 ha^{-1} (increase of 15.9, 41.3, and 9.2 compared to control, *A. brasilense* and *P. fluorescens*, respectively). Similarly, positive responses to *A. brasilense* inoculation were verified mainly with application of 35 and 105 kg P_2O_5 ha^{-1}. With application of 35 kg P_2O_5 ha^{-1}, *A. brasilense* inoculation showed greater ear length and ear diameter. When 105 kg P_2O_5 ha^{-1} was applied, *A. brasilense* showed greater mass of 100 grains and grain yield. Grain yield increased by 34.7, 27.7, and 14.8% compared to control, *P. fluorescens* and *B. subtilis* inoculated treatments, respectively, when *A. brasilense* was inoculated and 105 kg P_2O_5 $ha^{-1}$was applied. In addition, regardless of P_2O_5 application rates, *A. brasilense* inoculation showed greater P-resin in soil (increase of 19.7, 96.7, and 87.2%) and stem diameter (increase of 5.7, 12.1, and 12.1% compared to control, *P. fluorescens* and *B. subtilis*, respectively).

There was a dilution effect on P concentration as a function of *B. subtilis* and *A. brasilense* inoculation. It was observed that *B. subtilis* and *A. brasilense* inoculation promoted greater P uptake, optimizing the use of the absorbed P by corn plant. Our results showed that the inoculation with *B. subtilis* provided an increase of 9.7% for biomass P uptake (40.37 to 44.28 kg ha^{-1}, respectively) and 12.6% for grain P uptake (49.87 to 56.13 kg ha^{-1}, respectively) compared to control, regardless of P_2O_5 application rates. Also, the inoculation with *A. brasilense* provided an increase of increase of 10.% for biomass P uptake (40.37 to 44.42 kg ha^{-1}) and 21.3% for grain P uptake (49.87 to 60.50 kg ha^{-1}, respectively) compared to control, regardless of P_2O_5 application rates. The exact mechanisms underlying the *B. subtilis* and *A. brasilense* effect on corn growth was not evaluated in the present study, however, it is very likely that the improvement in P availability and uptake reflecting on improved PUE and grain yield by corn verified in this study is associated with the well-known ability of *A. brasilense* and *B. subtilis* to promote plant growth (Fukami et al., 2017; Jang et al., 2018; Martins et al., 2018; Posada et al., 2018; Salvo et al., 2018). The increase on PUE and corn yield as a function *B. subtilis* and *A. brasilense* inoculation compared to control was, on average, equivalent to 100.5 and 54.6% for PUE and 17.4 and 12.8% for grain yield, respectively, regardless of P_2O_5 application rates. Similar results with *B. subtilis* and *A. brasilense* inoculation were reported with greater corn yield between 3.8 to 34% in inoculated plants compared with non-inoculated plants (Kuan et al., 2016; Müller et al., 2016; Traoré et al., 2016; Ahmad et al., 2019; Galindo et al., 2019b).

The bacteria *B. subtilis* is well known to possess properties of plant growth promotion, phosphate solubilization, phytopathogen inhibition and heavy metal absorption (Traoré et al., 2016; Rekha et al., 2017; Muñoz-Moreno et al., 2018; Prakash and Arora, 2019). In addition, the strains Ab-V5 and Ab-V6 of *A. brasilense* share the genes related to the synthesis of auxins (Hungria et al., 2018). Also, *Azospirillum* spp. inoculation is related to nutrient availability increase (Galindo et al., 2018b) and BNF (Pankievicz et al., 2015). This growth promotion mechanisms might have improved the ability of the plants to more efficiently explore the soil and uptake P, as indicated in previous studies using *B. subtilis* and *A. brasilense* (Traoré et al., 2016; Martins et al., 2018; Prakash and Arora, 2019; Zeffa et al., 2019). According to Cormier et al. (2013), two strategies may be devised for nutrient use efficiency improvement: increasing the yield at a constant nutrient supply and/or maintaining high yield when reducing nutrient supply. Therefore, the results of our research show that inoculation with *B. subtilis* and *A. brasilense* may be a potential strategy to help improving PUE. It is not unusual that PGPB displays several different plant-beneficial properties (Bruto et al., 2014; Vacheron et al., 2016), which is thought to provide higher positive effects on the plant (Bashan and de-Bashan, 2010). This is expected to take place because (i) the effects of different modes of action may add-up quantitatively, or (ii) it could ensure that at least one mode of action is expressed in particular environmental conditions (Vacheron et al., 2016). Indeed, the most effective PGPB are typically multi-function strains (Almario et al., 2014).

The inoculation with *P. fluorescens* had little quantifiable effect on P uptake, corn development and grain yield. However, *P. fluorescens* has been reported to favor biological control of plants, production of natural antibiotics and protective effect against secondary soil phytopathogens (Garrido-Sanz et al., 2016), phosphate solubilization (Oteino et al., 2015) and N_2 fixation (Vacheron et al., 2016). Also, *P. fluorescens* resulted in 35.3 and 7.8% increase in PUE and corn grain yield compared to control treatment, respectively, regardless of P_2O_5 application rates. Evidently, there is still great divergence in the use of PGPB in corn and other grasses due to the variable results with inoculation. However, it is important to highlight the importance of research on the subject and the potential of using this technology, mainly because it is easy to apply, is low cost, and has a great potential to promote plant growth.

CONCLUSION

Inoculation with *B. subtilis* and *A. brasilense* associated with P_2O_5 application rates were found to increase P uptake, benefiting productive components development, leading to an improved PUE, and greater corn grain yield. Yield increased by 39.5, 29.1, and 15.9% when plants were inoculated with *B. subtilis* in the absence of P_2O_5 application rates, associated with 17.5 and 70 kg P_2O_5 ha^{-1}, respectively. Inoculation with *A. brasilense* increased grain yield by 34.7% when 105 kg P_2O_5 ha^{-1} was applied, showing the potential for improve PUE by the *B. subtilis* and *A. brasilense* inoculation, positively reflecting on corn grain yield. The inoculation with *P. fluorescens* had small effects on P uptake, plant development and grain yield, however, resulted in 35.3 and 7.8% increase on PUE and corn grain yield compared to control. Therefore, studies conducted under biotic or/and abiotic conditions are necessary to better understand the role of PGPB, inoculated alone or in combination as the co-inoculated method.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

FG and MT wrote the manuscript, with contributions from NP. MT, RG, and ED corrected and improved the manuscript. NP, FG, PR, and EM conducted the samplings and data collection. NP did the analysis, with the support of MT and FG.

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Combined Starter Phosphorus and Manure Applications on Silage Corn Yield and Phosphorus Uptake in Southern BC

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Phosphorus (P) management using mineral and manure P in excess of plant needs contributes to legacy soil P in coastal British Columbia, Canada. The objectives of this study were (a) to assess the effects of starter P at early growth stages and their carryover through corn maturity, and (b) to determine how corn yields relate to soil P saturation indicators. Eleven 1-year experiments were conducted on sites with contrasting legacy soil P in 2018 (three sites) and 2019 (eight sites). Six combinations of starter and manure P rates (T1: $0P_{starter} + 0P_{manure}$; T2: $0P_{starter} + 35P_{manure}$; T3: $5P_{starter} + 30P_{manure}$; T4: $10P_{starter} + 25P_{manure}$; T5: $15P_{starter} + 20P_{manure}$; T6: $20P_{starter} + 15P_{manure}$) were assigned to a RCBD with four replicates. In 2018, corn dry matter weight (DMY) at the 6-leaf stage was affected by starter P in all sites, and the response curve was described by a linear-plus-plateau model with critical starter P of 5.0 and 7.5 kg ha^{-1}. In 2019, corn DMY at early growth stages was not affected by starter P. Corn DMY at harvest varied between 15.2 Mg ha^{-1} in sites with low initial soil P and 27.2 Mg ha^{-1} in sites with excess P. Four sites exhibited a trend of high DMY with treatment comprising low starter P and high side-dressed manure P additions. The degree of P saturation (DPS, 14.0 to 38.0%) and P saturation index (PSI, 3.0 to 14.0%) were related to corn DMY by quadratic functions ($R^2 = 0.76–0.94$). From these results, we can conclude that the effect of starter P on young corn plants varies with year, but does not carryover through harvest. Moreover, current starter P (30–40 kg ha^{-1}) recommended for corn can be reduced by up to 75% (5.0–7.5 kg ha^{-1}) without affecting yield, thus reducing annual P inputs and farmers' production costs. Finally, PSI as a proxy of DPS is a possible agro-environmental indicator that, coupled with reduced starter P fertilizer, can further contribute to reducing the risk of P transfer from agricultural soils in coastal British Columbia.

Keywords: starter P fertilizer, manure P, silage corn yield, 3- and 6-leaf stage, legacy soil P, P saturation index

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INTRODUCTION

In coastal British Columbia (BC), Canada, agricultural soils receive large annual phosphorus (P) inputs including dairy and poultry manures as well as chemical fertilizers to support a diverse and intensive peri-urban agricultural sector. A provincial soil survey conducted in 2007 showed that 89% of fields have soil test P concentrations varying between 100 and 350 mg Mehlich-3 P (P_{M3})

kg^{-1}, which increases the risk of P transport by runoff (Kowalenko et al., 2007). A national study using the indicator of risk of water contamination by P showed that 35 to 75% of croplands in Abbotsford (largest city in Fraser Valley) have P source concentrations above the environmental threshold of 4 mg P kg^{-1} (van Bochove et al., 2012). Heavy rainfalls in these areas, mostly during the non-growing season, and the relatively dry summers also increase the risk of P loss by preferential flow. Reports have shown that the Fraser and Sumas Rivers fit in the Eutrophic class with total P concentrations between 30 and 100 mg L^{-1} (British Columbia Ministry of Environment, 2006). It is crucial to disentangle the mechanisms controlling P cycling (input, transformation, and output) in these soils with high legacy P concentrations.

Silage corn (8500 ha) and forage grass (13,670 ha) rotation is the main cropping system providing feed for dairy cows in BC. Phosphorus fertilization recommendations for the silage corn phase of the rotation averaged 90 kg P ha^{-1}, including 50 kg P ha^{-1} as dairy manure and 40 kg P ha^{-1} as starter fertilizer P for a crop that exports on average 35 kg P ha^{-1} (Bittman et al., 2006; Zhang et al., 2018). The annual P surplus for forage grass and silage corn rotation systems is 3030 Mg (50 kg P ha^{-1}) (Bittman et al., 2017), which contributes to buildup of legacy soil P. A better match of P applications to silage corn needs is necessary to maintain or drawdown legacy soil P.

Starter P is a small amount of P fertilizer placed in close proximity to the seed at planting to promote young plants' growth until the root system develops. Starter P fertilizer rates are 5 to 15 kg P ha^{-1} in Ontario and Quebec (Preston et al., 2019), and 5 to 13 kg P ha^{-1} in the US (Jokela, 1992). The placement or banding of P fertilizer near the seed reduces P fixation because soil-fertilizer contact is limited, making more P available to young plants soon after germination (Jokela, 1992). The benefits of starter fertilizer P on early growth of corn are well-recognized, particularly in low-P soils (Wolkowski, 2000; Vetsch and Randall, 2002; Preston et al., 2019). In areas where cool soil temperatures prevail early in the growing season, starter P can be beneficial even in high-P soils (Bittman et al., 2006). While some studies show the lasting effects of starter P through harvest (Bordoli and Mallarino, 1998; Malhi et al., 2001; Preston et al., 2019), other have highlighted the fact that early growth and nutrient uptake responses to starter P do not always translate into higher yield (Randall and Hoeft, 1988; Mallarino et al., 1999). The carryover of starter P effects through maturity was also observed primarily on a low-P soil (Bordoli and Mallarino, 1998). In Wisconsin, corn yield response to starter P was not observed on an excessively high-testing silt loam soil (Wolkowski, 2000).

Over the past two decades, extensive research has been conducted to develop or adapt agri-environmental P risk indicators to assess the risk of P loss from agricultural soils (Renneson et al., 2015). The P saturation index (PSI) as adaptation of the degree of P saturation (DPS) using routine agronomic soil test was developed in several agricultural regions of Canada and the United States. A PSI was adapted for the acidic soils of eastern Canada (Khiari et al., 2000; Pellerin et al., 2006; Benjannet et al., 2018) and the mid-Atlantic regions of the US (Sims et al., 2002) using P and Aluminum (Al) extracted by the Mehlich-3 method because P retention is correlated with Al in these soils. Benjannet et al. (2018) identified two critical values of PSI for Atlantic Canada above which P fertilization should be limited to crop requirements: 19.2% for very to extremely acidic soils (pH < 5.5) and 14.2% for slightly to moderately acidic soils (pH > 5.5). Pellerin et al. (2006) reported two critical PSI values for Quebec of 13.1 and 7.6% for coarse- and fine-textured soils, respectively. Given the differences observed on PSI values across areas and the influence of factors such as pH and soil textures, it is crucial to understand how these indicators of P risk relate to classical agronomic parameters before suggesting its use for the high legacy P soils of BC. The objectives of this study were (a) to assess the effects of starter fertilizers P at early growth stages (3- and 6-leaf stages) and their carryover through silage corn maturity, and (b) to determine how silage corn yields relate to soil P saturation indicators in high legacy soil P environments.

MATERIALS AND METHODS

Site Description

A 2-year study was conducted in 2018 and 2019 in Agassiz and Rosedale, located in south coastal BC, Canada. Three sites were selected in 2018 and eight sites in 2019, for a total of 11 1-year corn trials (site1–site11). Soils in the area include the Monroe series, characterized by a feeble profile development and coarse texture (Typic Dystroxerepts under the U.S. Soil Taxonomy; Soil Survey Staff, 2014). The minimum and maximum general soil properties across all sites were: soil pH: 4.9 and 6.0; total carbon: 0.94 and 11.35%; total nitrogen: 0.07 and 0.82%; and Mehlich-3 extractable P: 3.42 and 195.32 mg kg^{-1} (**Table 1**). The average daily temperature ranges from 3.2°C in December to 18.8°C in August. The local climate is moderate oceanic, characterized by warm, rainy winters and relatively cool, dry summers with annual rainfall of 1689 mm, 261.9 mm of which falls between May and July (**Table 2**).

TABLE 1 | Minimum and maximum values of general properties of soils (0–15 cm depth) at 11 studied sites in 2018 and 2019.

	2018		2019	
	Minimum	**Maximum**	**Minimum**	**Maximum**
Number of samples	12	12	28	28
pH_{H2O}	5.3	5.7	4.9	6.0
Total Carbon (%)	0.94	3.42	1.44	11.35
Total Nitrogen (%)	0.07	0.31	0.16	0.82
C to N ratio	9.65	13.10	8.84	14.28
Mehlich-3 P (mg kg^{-1})	51.47	204.09	10.09	172.68
Mehlich-3 Al (mg kg^{-1})	1413.93	1505.90	1103.66	3079.34
Mehlich-3 Fe (mg kg^{-1})	139.85	281.51	172.76	424.54
Oxalate P (mg L^{-1})	26.42	82.14	11.10	69.08
Oxalate Al (mg L^{-1})	132.53	294.19	116.62	562.08
Oxalate Fe (mg L^{-1})	132.35	281.76	129.27	823.77
Kelowna P (mg kg^{-1})	36.70	195.32	3.75	73.07

TABLE 2 | Monthly total precipitations and mean monthly air and soil temperatures at Agassiz – Rosedale, BC, in 2018 and 2019 in comparison with the 30-year (1981–2010) average.

	Precipitation (mm)			Air temperature (°C)			Soil temperature at 0–20 cm depth (°C)	
	2018	2019	30-year average	2018	2019	30-year average	2018	2019
May	23.3	41.0	103.0	16.5	16.0	13.6	19.9	18.2
June	89.4	72.4	92.3	16.3	17.1	16.2	19.6	20.7
July	30.3	64.4	66.6	20.7	18.8	18.5	23.8	22.8
August	18.5	55.2	58.2	19.5	19.6	18.7	22.2	22.7
September	134.9	172.2	87.6	15.4	16.0	15.9	17.7	17.6
Total	*296.4*	*405.2*	*407.7*					
Average				*17.7*	*17.5*	*16.6*	*20.6*	*20.4*

TABLE 3 | Description of the six combination of starter P and manure P treatments.

Treatment combinations	Starter P (kg ha^{-1})	Manure available P at 6-leaf stage (kg ha^{-1})[a]	Total available P (kg ha^{-1})	Total N applied (kg ha^{-1})[b]
$0P_{starter} + 0P_{manure}$	0	0	0	150
$0P_{starter} + 35P_{manure}$	0	35	35	150
$5P_{starter} + 30P_{manure}$	5	30	35	150
$10P_{starter} + 25P_{manure}$	10	25	35	150
$15P_{starter} + 20P_{manure}$	15	20	35	150
$20P_{starter} + 15P_{manure}$	20	15	35	150

[a] *Assumption: 35% of P is available during the year of application.* [b] *30 kg N ha^{-1} as urea at seeding and the rest was assigned at the 6-leaf stage using manure and urea.*

Experimental Design and Treatments

The 11 1-year corn experiments consisted of six treatments of combined starter fertilizer P and manure P rates. The six treatments were assigned to a randomized complete block design with four replicates for a total of 24 experimental units (10 m long and 4.5 m wide). Starter fertilizer P rates were 0, 0, 5, 10, 15, and 20 kg P ha^{-1} as triple super phosphate (TSP), and manure P rates were 0, 15, 20, 25, 30, and 35 kg available P ha^{-1}. The six combinations of starter P (mineral fertilizer) and manure P treatments were arranged to meet a total available P input of 35 kg P ha^{-1} as follows: T0: $0P_{starter} + 0P_{manure}$, T1: $0P_{starter} + 35P_{manure}$, T2: $5P_{starter} + 30P_{manure}$, T3: $10P_{starter} + 25P_{manure}$, T4: $15P_{starter} + 20P_{manure}$, T5: $20P_{starter} + 15P_{manure}$ (**Table 3**).

The fields selected for the trials were sprayed with Roundup [glyphosate (*N*)-(phosphonomethyl)glycine, 1000 g ha^{-1}] to terminate the grass stand in early April. The soil was plowed to a depth of 30 cm, followed by disking and harrowing to 10 cm, and marked with flags to delineate the experimental plots before seeding in early to mid-May. There were six rows by plot unit, and corn was sown at 81 × 10^3 plants ha^{-1}. Starter fertilizer N (30 kg N ha^{-1}) as urea (46% N) was band-applied (5 cm from the seeding row) using a disk opener (5 cm deep). Starter fertilizer P as TSP was band-applied by hand (5 cm from the seeding row) and buried (5 cm deep) with a rake the day following planting. Additions of manure P (0, 35; 30; 25; 20; and 15 kg available P ha^{-1}) to reach the target of 35 kg available P ha^{-1} (we assumed that 35% of manure P is available during the year of application) and a supplement of N fertilizer as ammonium nitrate to meet the local recommendation of 150 kg N ha^{-1} (including manure N) were side dressed at the 6-leaf stage to supply P and N for the rest of the growing season. The volume of manure for each combination treatment was measured in 11-L buckets and hand applied closed to the corn rows. Manure used in the corn experiments was collected in three dairy farms in the Frazer valley and their general chemical characteristics are presented in **Table 4**. At the 3-leaf stage, a mixture of post-emergence herbicides atrazine (1353 g ha^{-1}) and related triazines (87 g ha^{-1}) [2-chloro-4-ethylamino-6-isopropylamino-1,3,5-trithemazine; 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methyl-ethyl)-(S)] and glyphosate (1000 g ha^{-1}) was sprayed to control weeds according to provincial recommendations (BC MA, 2012).

TABLE 4 | Average chemical characteristics of manure from three sources used in the corn experiments.

	Source1	Source2	Source3[b]
Dry matter (%)	2.75 (0.24)[a]	11.85 (0.18)	3.2
Total nitrogen (%)	0.12 (0.01)	0.48 (0.01)	0.21
NH_4-N (ppm)	665 (80.73)	1957.75 (44.26)	955
Total phosphorus (%)	0.03 (0.00)	0.10 (0.01)	0.04
Phosphate (%P as P_2O_5)	0.06 (0.01)	0.24 (0.01)	0.10
Total potassium (%)	0.13 (0.02)	0.33 (0.02)	0.18
Potash (%K as K_2O)	0.16 (0.02)	0.40 (0.03)	0.22

[a] *Values in parenthesis are standard deviations of the mean (n = 4).* [b] *Manure from source3 was analyzed without replicates.*

Plant Sampling and Measurements

Corn plants were harvested at the 3-leaf stage (early-June), the 6-leaf stage (early-July), and maturity (mid-September) in a staggered pattern on the four innermost rows of each experimental plot. For the 3- and 6-leaf stages, 10 young corn plants were harvested, while for mature corn, 20 plants were harvested at 10 cm above the soil surface using a machete. Fresh weight was recorded in the field, and plants were chopped and a sub-sample of approximately 500 g from each plot was scooped and dried at 60°C in a forced-draft oven for 3–5 days and ground (2-mm screen). Dried and ground samples of 0.1 g were digested using a fluxer (M4 Fluxer, Claisse) and analyzed as described by Kowalenko and Babuin (2014). Phosphorus concentrations were assessed with an Inductively Coupled Plasma Optical Emission Spectrometer (ICAP 7000 series, Thermo Scientific). The P offtake at each harvest was calculated by multiplying corn P concentration by dry matter weight (DMY).

Soil Analyses

Soils were analyzed for general properties. Briefly, pH was measured in distilled water with a 1:2 soil: water ratio (Hendershot et al., 1993). Total C and N were determined by dry combustion with a LECO CNS-1000 (LECO, Corp., St. Joseph, MI, United States). Mehlich-3 extractable P (P_{M3}), aluminum (Al_{M3}) and iron (Fe_{M3}) were determined by shaking 2.5 g of soil with a 25 mL of Mehlich-3 solution (pH 2.3) for 5 min (Mehlich, 1984). Acid ammonium oxalate extractable P (POx), aluminum (AlOx) and iron (FeOx) were determined according to Ross and Wang (1993). The concentrations of P_{M3}, Al_{M3}, Fe_{M3}, POx, AlOx, and FeOx were assessed with an Inductively Coupled Plasma Optical Emission Spectrometer (ICAP 7000 series, Thermo Scientific).

The PSI was related to Mehlich-3 extracts as follows (Sims et al., 2002; Messiga et al., 2013):

$$\mathrm{PSI} = (\mathrm{P/Al})_{\mathrm{M3}} \quad (1)$$

where P_{M3} and Al_{M3} are quantified in Mehlich-3 extracts (mg kg^{-1}).

The DPS was related to acid ammonium oxalate extracts as follows (Breeuwsma and Silva, 1992; Messiga et al., 2013):

$$\mathrm{DPS} = \mathrm{POx}/(\mathrm{Al} + \mathrm{Fe})\mathrm{Ox} \quad (2)$$

where POx, AlOx, and FeOx are quantified in acid ammonium oxalate extracts (mmol kg^{-1}).

Statistical Analyses

Data were tested for normality using SAS univariate procedure. Analysis of variance (ANOVA) for DMY and P uptake for corn at the 3- and 6-leaf stages was performed separately for each site using the PROC MIXED of SAS version 9.3 (SAS Institute, 2010) with blocks as random effects and P rate as fixed effects. When P rate was significant, contrast analysis was used to assess if the effect was linear or quadratic. Model selection was based on graphical and numerical methods by comparing R^2, lack of fit F tests (Cerrato et al., 1990), and residual sum of squares.

The linear model was described as follows:

$$\mathrm{Y} = \mathrm{a} + \mathrm{bx} \quad (3)$$

Where Y is the corn DMY (Mg ha^{-1}) or P uptake (kg P ha^{-1}), a is the intercept, b is the linear coefficient, and x is the starter P rate (kg P ha^{-1}).

The linear-plus-plateau model is described as follows:

$$\mathrm{Y} = \mathrm{a} + \mathrm{bx} \text{ if } \mathrm{x} < \mathrm{x0} \quad (4)$$

$$\mathrm{Y} = \mathrm{P} \text{ if } \mathrm{x} > \mathrm{x0} \quad (5)$$

where Y is the corn DMY (Mg ha^{-1}) or P uptake (kg P ha^{-1}), a is the intercept, b is the linear coefficient, x is the starter P rate (kg P ha^{-1}), C is the critical starter rate of fertilization, which occurs at the intersection of the linear response and the plateau lines, and P is the plateau corn DMY (Mg ha^{-1}) or P uptake (kg P ha^{-1}), a constant obtained by fitting the model to the data.

ANOVA for DMY and P uptake for corn at maturity was performed separately for each site using the PROC MIXED with blocks as random effects and P combination treatment as fixed effects. When P combination treatment was significant, differences among least square means (LSMEANS) for all treatment pairs were tested at a significance level of $P = 0.05$. In addition, standard errors of mean (SEM) were calculated.

RESULTS

Weather Conditions

In 2018, the growing season, from May to September, received 111.3 mm less rainfall than the 30-year normal of 407.7 mm (**Table 2**). More specifically, May with 23.3 mm, July with 30.3 mm and August with 18.5 mm rainfall were respectively at least 4, 2 and 3 times drier than the 30-year normal. In 2019, May with 41.0 mm and June with 72.4 mm rainfall were respectively 2.5 and 1.3 times drier than the 30-year normal. The mean growing season air temperatures of 17.7°C for 2018 and 17.5°C for 2019 were higher than the 30-year normal of 16.6°C. In 2018, the mean air temperature in June was similar to the 30-year normal of 16.2°C, but June 2019 was warmer. The warm air temperatures of June 2019 were translated into warmer soil temperatures compare with June 2018. In particular, during the periods May 26 and June 26, there were 13 successive days with soil temperatures below 19°C in 2018 (**Figure 1A**), compared with only 5 days in 2019 (**Figure 1B**). In addition, there were 8 days with soil temperatures below 18°C in 2018, but only 1 day in 2019. In brief, mean soil temperature between May 26 and June 26 was 1°C cooler in 2018 with 19.6°C than 2019 with 20.6°C.

Corn Dry Matter Yield

Corn DMY at the 3-leaf stage was not affected by starter P fertilization in all 11 sites in 2018 and 2019 (**Figure 2**). Corn DMY at the 3-leaf stage was on average 0.070 Mg ha^{-1} in site1, site2, and site3 (**Figure 2A**); 0.17 Mg ha^{-1} in site4, site7, and site9 (**Figure 2B**); 0.28 Mg ha^{-1} in site10 and site11 (**Figure 2B**); 0.41 Mg ha^{-1} in site8 (**Figure 2B**); and 0.78 Mg ha^{-1} in site5 and site6 (**Figure 2B**).

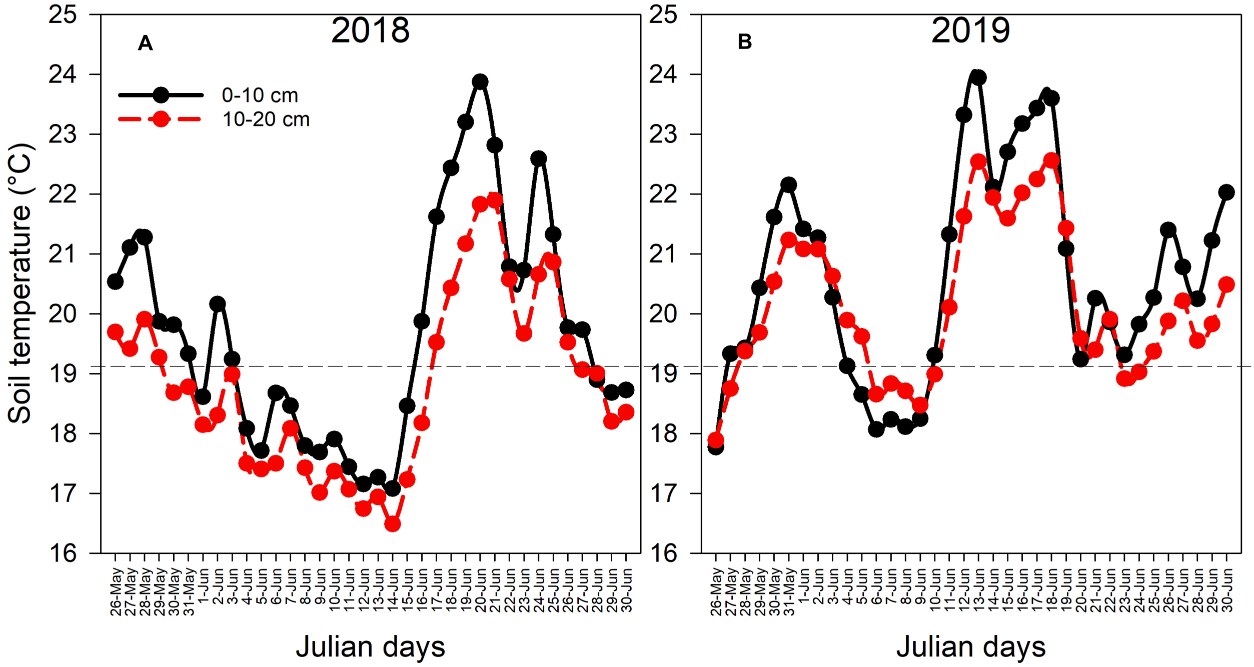

FIGURE 1 | Average soil temperature during the periods **(A)** May 26, 2018 and **(B)** May 26, 2019.

Corn DMY at the 6-leaf stage was affected by starter P fertilization in all three sites in 2018 (**Figure 3A**): site1 (*P* value = 0.014), site2 (*P* value = 0.033), and site3 (*P* value = 0.032). The trend of corn DMY response across the three sites was described by a linear-plus-plateau model. The critical starter P rates were 5 kg ha^{-1} for site1 and site3 and 7.5 kg ha^{-1} for site2. The plateau DMY were 0.39 and 0.37 Mg ha^{-1} for site1 and site3 and 0.66 Mg ha^{-1} for site2 (**Table 5**). In 2019, only six out of the eight sites were sampled at the 6-leaf stage due to workload and asynchrony with farmer's activities and planning. Corn DMY was not affected by starter P fertilization in the six sampled sites (**Figure 3B**). Corn DMY at the 6-leaf stage was on average 0.62 Mg ha^{-1} in site9; 0.91 Mg ha^{-1} in site10 and site11; and 1.48 Mg ha^{-1} in site5, site6, and site8 (**Figure 3B**).

Corn DMY at harvest varied with starter and manure P additions in the three sites (site1, site2, site3) in 2018 and only one site (site11) in 2019 for a total of 4 out of 11 sites. Among the four sites, the highest corn DMY were obtained with treatment combinations comprising low banded starter P rates at planting and high side-dressed manure P additions at the 6-leaf stage. In site1, corn DMY was 18.60 Mg ha^{-1} with T2 ($5P_{\text{starter}} + 30P_{\text{Manure}}$) and 15.15 Mg ha^{-1} with T5 ($20P_{\text{starter}} + 15P_{\text{Manure}}$), representing a change of 22.75% (**Figure 4A**); in site2, corn DMY was 27.20 Mg ha^{-1} with T1 ($0P_{\text{starter}} + 35P_{\text{Manure}}$) and 22.75 Mg ha^{-1} with T5 ($20P_{\text{starter}} + 15P_{\text{Manure}}$), representing a change of 19.48% (**Figure 4A**); in site3, corn DMY was 28.47 Mg ha^{-1} with T2 ($5P_{\text{starter}} + 30P_{\text{Manure}}$) and 24.78 Mg ha^{-1} with T4 ($15P_{\text{starter}} + 20P_{\text{Manure}}$) and T0 ($0P_{\text{starter}} + 0P_{\text{Manure}}$), representing a change of 14.95% (**Figure 4A**).

Corn P Uptake

Corn P uptake at the 3-leaf stage was affected by starter P fertilization in all three sites in 2018 (**Figure 5A**): site1 (*P* value = 0.001), site2 (*P* value = 0.003), and site3 (*P* value = 0.021). The trend of corn P uptake response across the three sites was described by a linear-plus-plateau model. The critical starter P rates were 15 kg ha^{-1} for site1 and site2 and 10 kg ha^{-1} for site3. The plateau P uptake was 0.33 kg ha^{-1} for site1 and site2 and 0.21 kg ha^{-1} for site3 (**Table 5**). Corn P uptake at the 3-leaf stage was affected by starter P fertilization in only one site (site6) in 2019 (**Figure 5B**). Corn P uptake at the 3-leaf stage was on average 0.57 kg ha^{-1} in site4, site7, and site10 (**Figure 1B**); 0.83 kg ha^{-1} in site9 and site11 (**Figure 1B**); 1.53 kg ha^{-1} in site8 (**Figure 1B**); and 3.44 kg ha^{-1} in site6 (**Figure 1B**).

Corn P uptake at the 6-leaf stage was affected by starter P fertilization in all three sites in 2018 (**Figure 6A**): site1 (*P* value = 0.001), site2 (*P* value = 0.022), and site3 (*P* value = 0.014). The trend of corn P uptake response across the three sites was described by a linear-plus-plateau model. The critical starter P rates were 7.5 kg ha^{-1} for site1 and 5.0 kg ha^{-1} for site2 and site3. The plateau P uptakes were 1.63 kg ha^{-1} for site1, 2.05 kg ha^{-1} for site2 and 1.06 kg ha^{-1} for site3 (**Table 5**). Corn P uptake at the 6-leaf stage was affected by starter P fertilization in only one site (site5) in 2019 (**Figure 6B**). Corn P uptake was on average 2.20 kg ha^{-1} in site9 (**Figure 6B**); 3.67 kg ha^{-1} in site10 (**Figure 6B**); 4.22 kg ha^{-1} in site11 (**Figure 6B**) and 5.12 kg ha^{-1} in site5, site6, and site8 (**Figure 6B**).

Corn P uptake at harvest varied with starter and manure P additions in two sites (site1 and site2) out of three in 2018 and

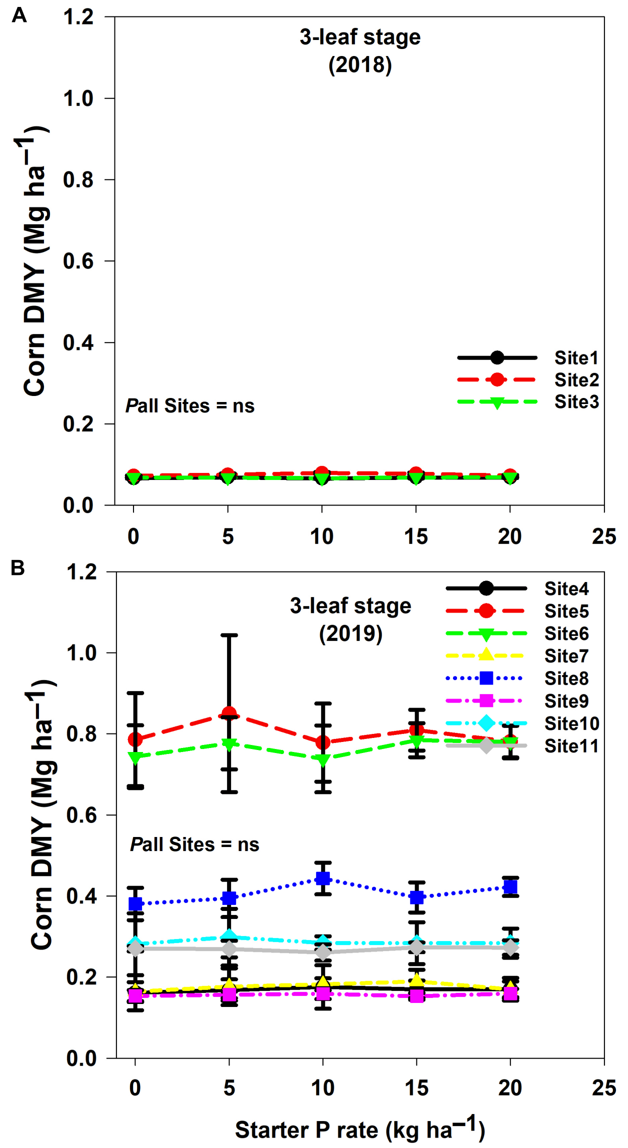

FIGURE 2 | Corn dry matter yield (DMY) at 3-leaf stage with application of starter P rates across 11 sites [2018 **(A)** and 2019 **(B)**] in the Fraser Valley, BC. Error bars represent standard deviations of the means.

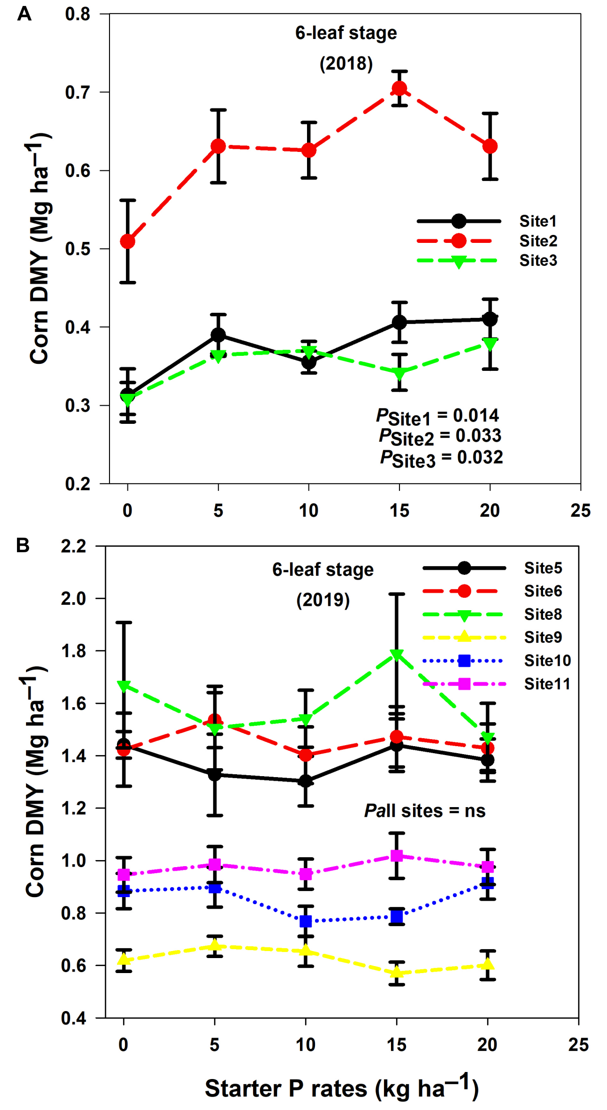

FIGURE 3 | Corn dry matter yield (DMY) at 6-leaf stage with application of starter P rates across 11 sites [2018 **(A)** and 2019 **(B)**] in the Fraser Valley, BC. Error bars represent standard deviations of the means.

seven sites out of eight in 2019 (**Figure 7**). Corn P uptake varied between 19.66 and 29.79 kg ha^{-1} in site1 (**Figure 7A**); 26.82 and 35.90 kg ha^{-1} in site9 (**Figure 7B**); 28.17 and 37.24 kg ha^{-1} in site7 (**Figure 7B**); 29.10 and 38.43 kg ha^{-1} in site4 (**Figure 7B**); 55.67 and 63.54 kg ha^{-1} in site3 (**Figure 7A**); 56.67 and 73.46 kg ha^{-1} in site2 (**Figure 7A**); 57.00 and 68.63 kg ha^{-1} in site11 (**Figure 7B**); 59.68 and 69.00 kg ha^{-1} in site5 (**Figure 7B**); 61.18 and 70.29 kg ha^{-1} in site8 (**Figure 7B**); 61.76 and 70.08 kg ha^{-1} in site10 (**Figure 7B**); and 67.14 and 74.72 kg ha^{-1} in site6 (**Figure 7B**). There was a trend of high corn P uptakes with treatment combinations comprising low banded starter P rates at planting and high side-dressed manure P additions at the 6-leaf stage, but low P uptake with treatment combinations comprising high banded starter P rates at planting and low side-dressed manure P additions at the 6-leaf stage (**Figure 7**).

Phosphorus Budget

Phosphorus budget was significantly lower under control compared with the other starter and manure P combinations in all 11 sites in 2018 and 2019 (**Figure 8**). Phosphorus budget was on average −31.26 kg ha^{-1} in site1, site4, site7, and site9 (**Figures 8A,B**) and −65.20 kg ha^{-1} in the other sites (**Figures 8A,B**) with 0 P addition. In contrast, phosphorus budget was on average 4.2 kg ha^{-1} in site1, site4, site7 and site9 (**Figures 8A,B**) and −29.73 kg ha^{-1} in the other sites (**Figures 8A,B**) with the different starter and manure P combinations.

TABLE 5 | Parameters of the linear-plus-plateau response curves (Y = bx + a) to P fertilization of corn dry weight at the 6-leaf stage, corn P uptake at the 3- and 6-leaf stages in 2018.

	b	a	C	P	*P*-value	MSE
Dry weight			(6-leaf stage)			
Site1	0.016 (0.006)	0.313 (0.026)	5.0	0.39	0.014	0.011
Site2	0.019 (0.008)	0.514 (0.052)	7.5	0.66	0.033	0.024
Site3	0.011 (0.005)	0.310 (0.021)	5.0	0.37	0.032	0.009
P uptake			3-leaf stage			
Site1	0.0067 (0.001)	0.239 (0.016)	15.0	0.339	0.001	0.008
Site2	0.0052 (0.002)	0.248 (0.016)	15.0	0.326	0.003	0.008
Site3	0.003 (0.001)	0.179 (0.011)	10.0	0.214	0.021	0.005
P uptake			6-leaf stage			
Site1	0.079 (0.019)	1.031 (0.119)	7.5	1.630	0.001	0.054
Site2	0.105 (0.042)	1.520 (0.187)	5.0	2.050	0.022	0.082
Site3	0.044 (0.016)	0.838 (0.072)	5.0	1.057	0.014	0.031

b is the linear coefficient, a is the intercept, C is the critical P rate of fertilization that occurs at the intersection of the linear response curve and the plateau lines, P represents plateau dry weight at the 6-leaf stage and plateau P uptake at the 3- and 6-leaf stages, R^2 is the coefficient of determination, and MSE represents the mean square error. Values in parenthesis represent standard error of the estimates.

Mehlich-3 P and P Saturation Indicators vs. Dry Matter Weight and P Uptake at Harvest

The 11 sites selected for the 2-year study represented a wide range of P_{M3}, Pox, Al_{M3}, AlOx, Fe_{M3}, and FeOx values (**Table 1**). The relationships between P_{M3}, DPS, PSI and corn DMY and P uptake were described by a quadratic relationship (**Figure 9**). The quadratic relationships obtained in this study are significant, with R^2 values varying between 0.65 and 0.94 (**Figures 9A,C**). These relationships encompassed a range of P_{M3} between 60 and 200 mg kg^{-1}, which is representative of high testing P soils of the coastal BC (**Figures 9A,B**), DPS between 14 and 38% (**Figures 9C,D**), and PSI between 3 and 14% (**Figures 9E,F**). However, site5 and site6 were not fitted by the functions. In addition, for the relationship between P_{M3} and P uptake, site4 and site7 were not fitted by the quadratic function (**Figure 9B**).

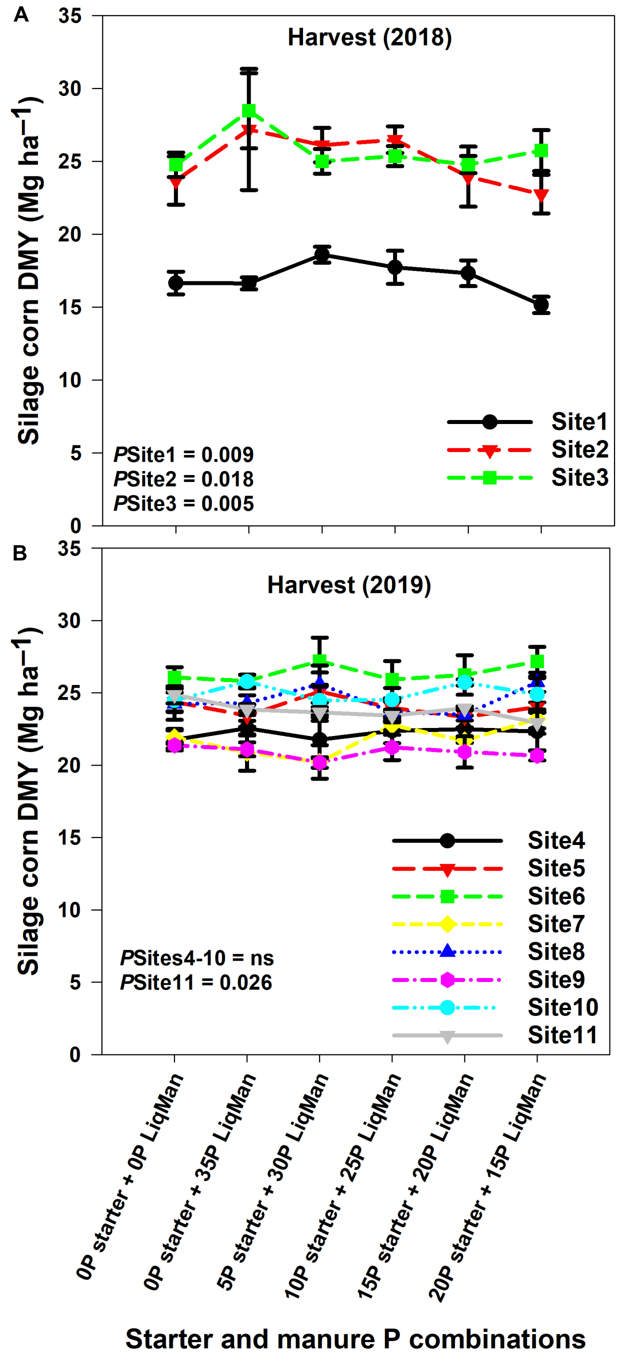

FIGURE 4 | Corn dry matter yield (DMY) at harvest with application of starter P rates and dairy manure across 11 sites [2018 **(A)** and 2019 **(B)**] in the Fraser Valley, BC. Dairy manure was applied at 6-leaf stage. Error bars represent standard deviations of the means.

DISCUSSION

The Effect of Starter P Fertilization on Growth of Young Silage Corn

The linear-plus-plateau response models of DMY and P uptake to starter P obtained in 2018 confirm the well-established negative effects of low soil temperatures on performances of corn plants at early growth stages (3- and 6-leaf stages). In 2018, starter P was beneficial for corn growth early in the growing season in the three study sites. This positive effect coincided with low soil temperatures that prevailed early in the growing season between emergence and the 6-leaf stage (May 26 and June 26, 2018). Daily soil temperatures were below 19°C for 13 successive days, and soil temperatures as low as 17°C were recorded (**Figure 1A**). In contrast, in 2019, starter P had no significant effect on the 3- and 6-leaf stage corn plants in all eight study sites, probably because soil temperatures during the same period dropped below 19°C for only 5 successive days with a minimum of 18°C (**Figure 1B**).

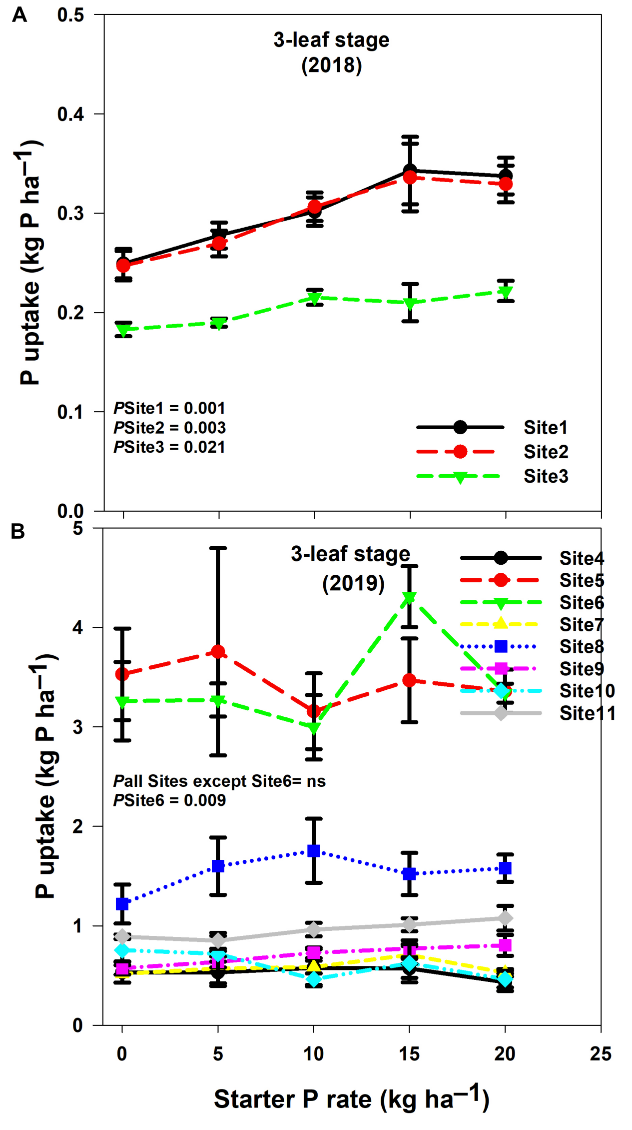

FIGURE 5 | Corn P uptake at 3-leaf stage with application of starter P rates and dairy manure across 11 sites [2018 **(A)** and 2019 **(B)**] in the Fraser Valley, BC. Dairy manure was applied at 6-leaf stage. Error bars represent standard deviations of the means.

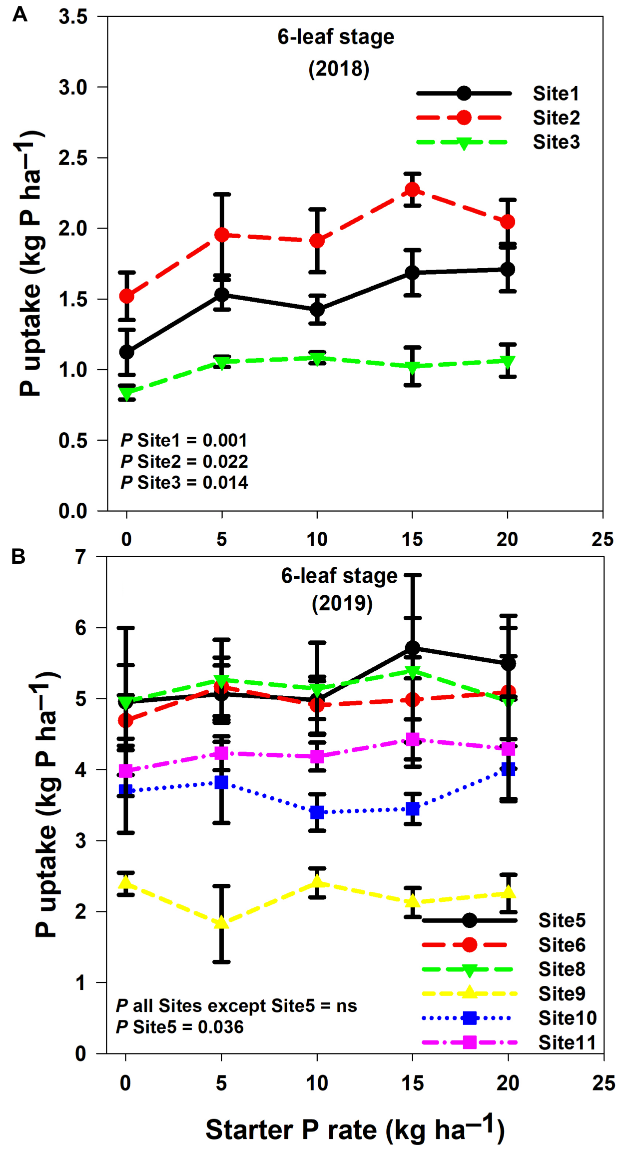

FIGURE 6 | Corn P uptake at 6-leaf stage with application of starter P rates and dairy manure across 11 sites [2018 **(A)** and 2019 **(B)**] in the Fraser Valley, BC. Dairy manure was applied at 6-leaf stage. Error bars represent standard deviations of the means.

Low soil temperatures early in the growing season reduce root growth and soil P availability even in high-P soils. One significant result of our study, however, is the critical starter P rates obtained from the linear-plus-plateau response models of DMY and P uptake to starter P at the 3- and 6-leaf stages. Overall, critical starter P rate was 5 kg P ha^{-1} at two sites and 7.5 kg P ha^{-1} at one site (**Table 5**). The *P* values and mean square error (MSE) indicated that linear-plus-plateau models accurately predicted the response of DMY and P uptake of young corn plants to starter P at the 3- and 6-leaf stages in 2018. The critical starter P rates obtained in our study are in the range of those recommended in other areas. Starter P fertilizer rates for silage corn are 5 to 15 kg P ha^{-1} in Ontario and Quebec (Preston, 2019) and 5 to 13 kg P ha^{-1} in the US (Jokela, 1992). In cool and moist coastal BC, almost all silage corn is side-banded at approximately 30–40 kg P ha^{-1}, and most of the corn receives manure (Zhang et al., 2018). The critical starter P rates obtained in our study are below local recommendations (British Columbia Ministry of Agriculture [BCMA], 2010). This indicates the need to refine and update recommendations for P fertilizer, particularly starter P fertilizer, for silage corn production in BC. Refining starter P fertilizer for silage corn will contribute to decreasing annual P inputs in this cropping system. A better match of P applications to silage corn needs will help to maintain or drawdown legacy P and reduce P losses to water sources.

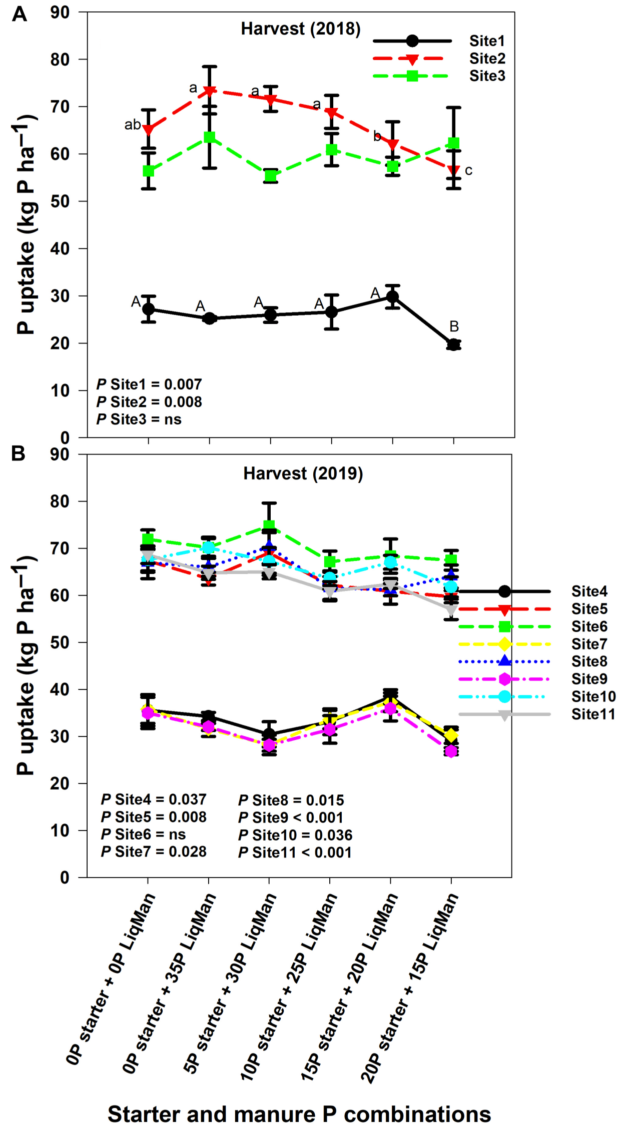

FIGURE 7 | Corn P uptake at harvest with application of starter P rates and dairy manure across 11 sites (2018 and 2019) in the Fraser Valley, BC. Dairy manure was applied at 6-leaf stage. Error bars represent standard deviations of the means.

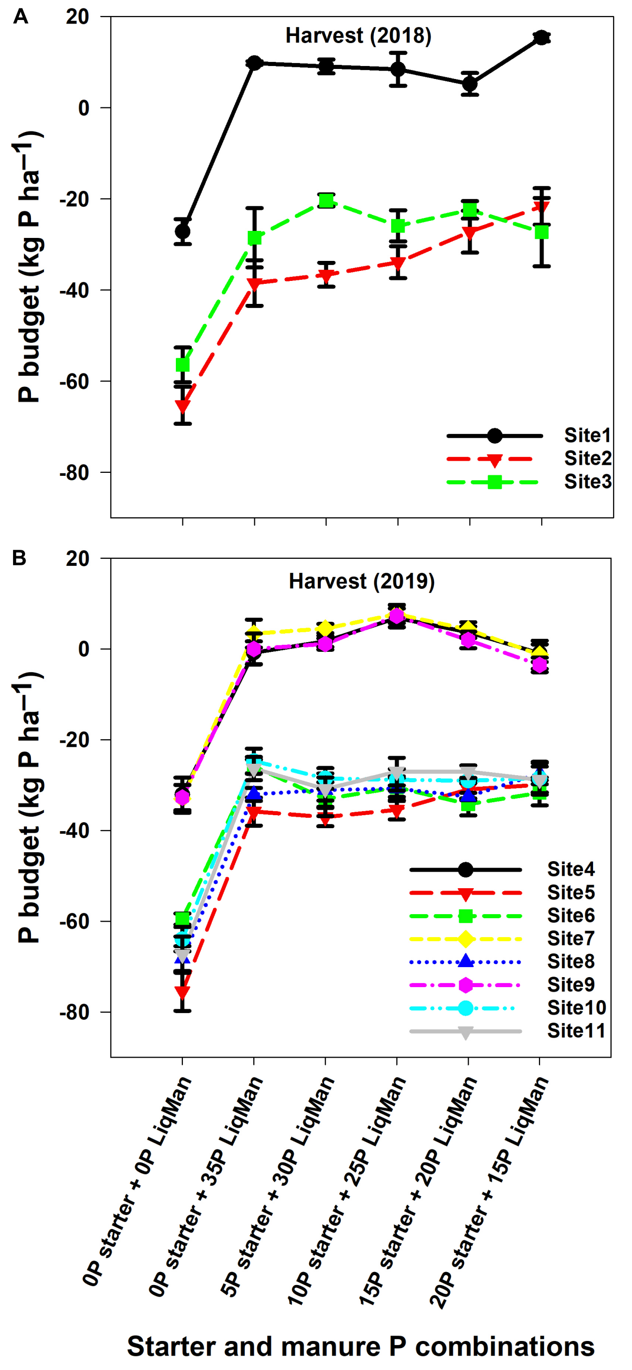

FIGURE 8 | P budget at harvest with application of starter P rates and dairy manure across 11 sites [2018 **(A)** and 2019 **(B)**] in the Fraser Valley, BC. Dairy manure was applied at 6-leaf stage. Error bars represent standard deviations of the means.

The Effects of Starter and Manure P Additions on Corn Dry Matter Yield and P Uptake at Harvest

Corn DMY values at harvest were in the range of those obtained by Zhang et al. (2018) in south coastal BC in a study investigating the effects of 11 year of different dairy manure fractions and mineral fertilizer on two consecutive silage corn productions with average DMY of 16.1 Mg ha^{-1} in 2015 and 22.7 Mg ha^{-1} in 2016. Our results are also in the range of those obtained by Ferreira and Teets (2017) in 2014 and 2015 at two commercial dairy farms located in Virginia. Our results are in the range of those obtained by Arriola et al. (2012) across several silage hybrids in Florida. In contrast, our results are higher than those of Jokela (1992), perhaps due to new and improved silage corn hybrids used in our study.

In our study, we were interested in understanding how a balanced P input including banded starter P fertilizer at planting

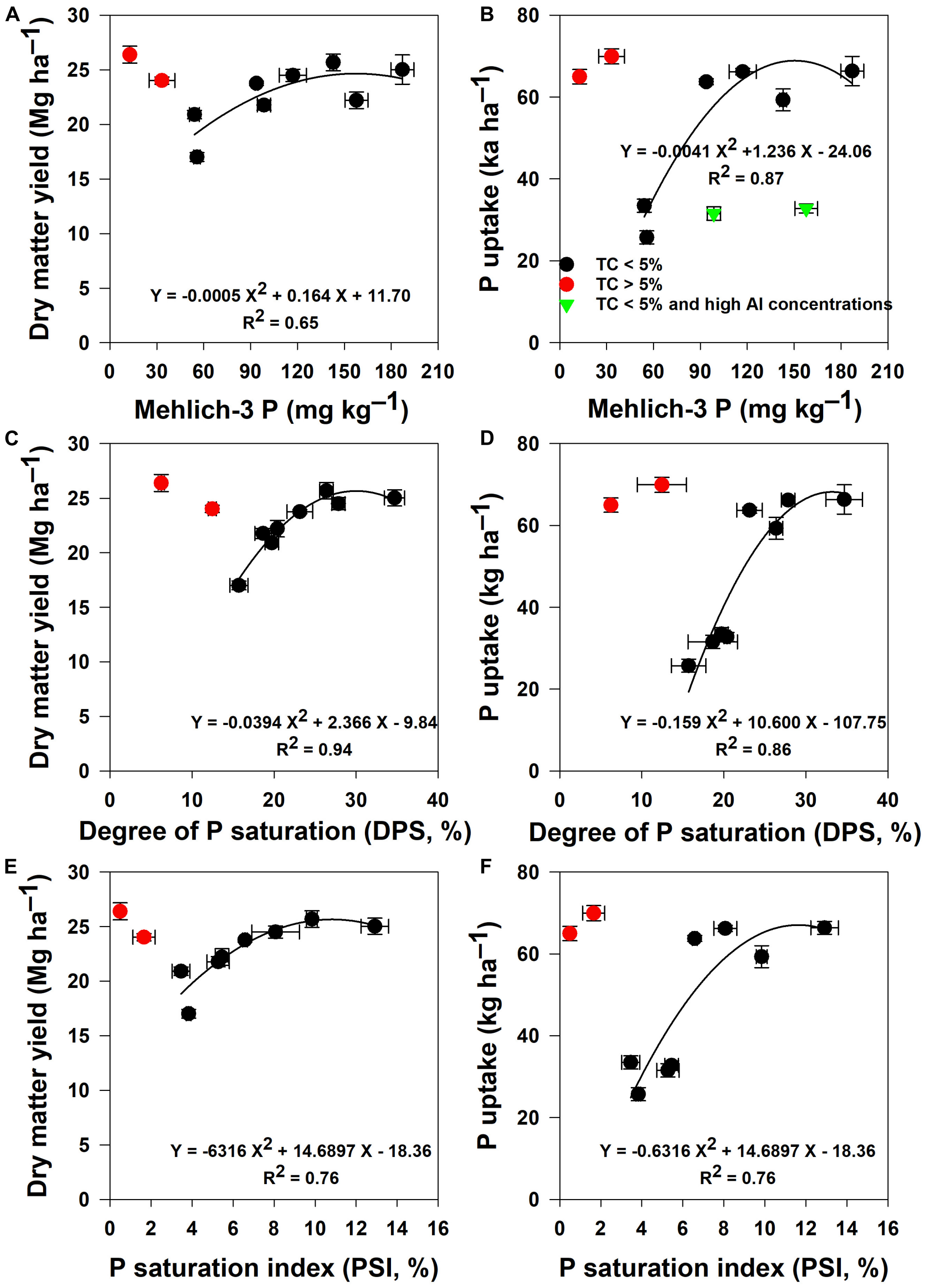

FIGURE 9 | Mehlich-3 P **(A,B)**; Degree of P saturation **(C,D)**; P saturation index **(E,F)** vs. Dry matter yield and P uptake at harvest with application of starter P rates and dairy manure across 11 sites in the Fraser Valley, BC. Dairy manure was applied at 6-leaf stage. Error bars represent standard deviations of the means.

and side-dressed manure P at the 6-leaf stage affects silage corn DMY and P uptake at harvest. Our results show that in 4 out of the 11 study sites, there was a trend of high DMY at harvest with treatment combinations comprising low starter P rates (<10 kg P ha^{-1}) and high side-dressed manure P (>25 kg P ha^{-1}) additions at the 6-leaf stage (**Figures 4A,B**). Corn P uptake showed similar trends in 9 out of the 11 study sites (**Figures 7A,B**). The final DMY and P uptake values indicate that the effect of starter P observed at the 6-leaf stage in 2018 was not carryover through harvest. It is well-known that most crops take up only 10 to 20% of P fertilizer during the year of application. The decreasing trend of corn P uptake at harvest with treatments including high starter P rates indicates that part of P fertilizer was fixed by the soil and therefore was not readily available for plant uptake later in the growing season. Kowalenko (2005) showed that soils of coastal BC exhibit high P retention or fixation capacity.

Zhang et al. (2018) also found that early plant response to starter P did not have a lasting effect on yield at final harvest even though starter P rate applied was 40 kg P ha^{-1}. Mallarino et al. (1999) in several corn experiments in Iowa also found that early growth and nutrient uptake to banded P did not translate into higher yield. Jokela (1992) in a study conducted on 12 soils with contrasting P levels also found that when soil test P is medium or high, the likelihood of the carryover effect of starter P on silage corn yield through harvest is low.

The 6-leaf stage coincides with the beginning of rapid nutrient uptake by corn plants. In our study, the synchrony between the timing of manure P application and the growth stage corresponding to high nutrient demand enhanced the potential for greater corn P uptake (**Figures 4A,B**). In coastal BC where dairy manure is an important source of P and other nutrients for dairy farmers, management practices that include combinations of low starter P rates and optimum rates of side-dressed manure P at the 6-leaf stage could be an alternative to current practices. The presence of other nutrients in dairy manure also contributed to a large extent to DM production and silage quality with optimum side-dressed manure P additions at the 6-leaf stage. In this study, nitrogen is the only element that was balanced among the treatment combinations (**Table 3**). Zhang et al. (2018) and other authors showed that interference in root P uptake with other nutrients such as zinc and calcium could occur in manure treated plots and result into lower P concentrations and P uptakes compared with control plots. We did not observe symptoms or other evidence of interference among nutrients on plant growth, probably because of the short-term nature of our study sites.

Relationship Between Silage Corn Yield and P Saturation Indicators

One important goal of this study is to improve our understanding of the mechanisms controlling P cycling in soils with high legacy P in coastal BC in order to reduce the risk of P losses to soils and water sources while building resilient cropping systems. In this study, we show that P_{M3}, DPS and PSI are highly correlated with silage corn DMY and P uptake with R^2 values varying between 0.65 and 0.94 (**Figure 9**). These relationships encompassed a range of P_{M3} between 60 and 200 mg kg^{-1}, which is representative of the high testing P soils of coastal BC (**Figure 9A**), DPS between 14 and 38% (**Figures 9A,B**), and PSI between 3 and 14% (**Figure 9B**). The significant relationship between PSI and DMY obtained in our study indicates that this proxy of DPS can be adapted for coastal BC soils as an agro-environmental indicator to assess the risk of P losses. In the Netherlands, DPS > 25% indicates a high risk of P transport with runoff water (Breeuwsma and Silva, 1992). In Wallonia, Belgium, a study using 57 agricultural topsoil samples subject to diverse P management showed that DPS values between 20 and 30% corresponded to the agronomic optimum of soil P content (Renneson et al., 2015). In North America, the PSI has provided much-needed information to support environmental assessment at watershed, local, and provincial scales (Khiari et al., 2000; Sims et al., 2002; Benjannet et al., 2018).

Among the sites that were used for this study, two were not fitted by the quadratic functions for DMY and P uptake (site5 and site6). These sites were located in the same area and were characterized by P_{M3} values lower than 30 mg kg^{-1} and TC content greater that 7%. Another contrasting behavior of these soils is that they yielded DMY in the high range at the 3-leaf stage (0.80 Mg ha^{-1}), the 6-leaf stage (1.40 Mg ha^{-1}) and harvest (25 Mg ha^{-1}), which could not be supported by the low P_{M3} values (**Figure 4B**). It is possible that Mehlich-3 and Oxalate ammonium extracting solutions are not adapted for these soils with high organic matter content. It is also possible that the high organic matter content contributes to P supply to plants through mineralization throughout the growing season. Two other sites were also not fitted by the quadratic function for P_{M3} vs. P uptake (site4 and site7). The sites were located in the same area and were characterized by P_{M3} values of 155 mg kg^{-1} for site4 and 90 mg kg^{-1} for site7. Even though the sites had high and very high soil test P, DMY at the 3-leaf stage (**Figure 2B**) as well as P uptake at the 3-leaf stage (**Figure 5B**) and harvest (**Figures 7A,B**) were in the low range. These two sites were located on poorly-drained soils, and their reactive Al were much higher than the other sites, indicating a higher P fixation capacity as shown by their DPS and PSI, which are aggregated among sites with P_{M3} in the low range (**Figures 9C–F**). Additional sites with similar characteristics will be needed to derive a recommendation for starter P that is different from what is suggested in this study.

Agronomic and Environmental Implications for Soils With High Legacy P

The site-year dependence of young corn plant growth and the linear-plus-plateau response model to starter P fertilization have implications for P fertilizer recommendations for silage corn production in BC. As noted in sections above, fertilization management for silage corn in coastal BC includes dairy manure (liquid or solid) spreading at rates 30 to 50 Mg ha^{-1}, usually in the spring just before moldboard plowing (British Columbia Ministry of Agriculture [BCMA], 2010). Phosphorus supplied by manure applications ranges from approximately 30 to 50 kg P ha^{-1}. In addition, starter fertilizer including P at rates 30 to 40 kg P ha^{-1} is banded 5 cm to the side and 5 cm below the corn row with the planter. Therefore, the total annual P input in most fields ranged between 60 and 90 kg P ha^{-1} for a crop that takes up approximately 35 kg P ha^{-1}. The excess P, on average 40 kg P ha^{-1} every year, accumulates in the soil and contributes to increasing the legacy soil P. Our results demonstrate that P fertilizer recommendations for silage corn can be refined by decreasing starter P rates to 5.0 to 7.5 kg P ha^{-1} without decreasing DMY at harvest. This result provides opportunities for the fertilizer industry in BC to prepare new starter fertilizer blends for silage corn production to address annual variations observed with soil temperatures early in the growing season (**Figures 1A,B**). These starter P rates will represent a cut of more than 75% of annual P input and could have a positive incidence on farmers' production costs and the dairy industry as a whole while protecting the environment. In southern BC, dairy farmers grow silage corn for five consecutive years followed by another five consecutive years of forage grass. It is crucial to understand whether annual applications of critical starter P rates are necessary and how they affect the growth of young silage corn plants in these types of rotation systems. Future studies are therefore needed to test the stability of annual applications of critical starter P rates during the silage corn phase. This knowledge will be useful to provide recommendations specifically tailored for dairy farmers and will contribute to better managing legacy soil P in silage corn and forage grass rotation systems in southern BC.

So far, most private laboratories in coastal BC use the Kelowna or modified Kelowna methods to assess the status of soil test P for P fertilizer recommendations. Our results show that the Mehlich-3 method, which is widely used in acidic to near-neutral soils in North America including Canada, can be used on the soils of coastal BC. Use of the Mehlich-3 method offers the opportunity to assess other cations such as Al and Fe and therefore make it possible to adapt a PSI for BC soils (Khiari et al., 2000; Sims et al., 2002; Benjannet et al., 2018). We showed that P_{M3}, DPS and PSI were all closely related to silage corn DMY and P uptake across 11 soils with a wide range of soil test P levels. Finally, future works including additional soils with contrasting properties will be needed to identify environmental P risk classes at the scale of coastal BC, Canada.

CONCLUSION

Silage corn DMY was affected by starter P fertilizer at the 6-leaf stage in one out of two seasons, and this response coincided with 13 consecutive days of low soil temperatures. Our results show that the response of DMY to starter P is described by a linear-plus-plateau model with critical starter P rate at 5.0 and 7.5 kg P ha^{-1}. Our results also show that in 4 out of the 11 study sites, there was a trend of high DMY at harvest with treatment combinations comprising low starter P rates and high side-dressed manure P additions at the 6-leaf stage. Thus, the effect of starter P fertilizer observed at early growth stages was not carryover through harvest. It appears that when high amounts of P fertilizers are banded as starter P (30 to 40 kg P ha^{-1}) to promote the growth of young corn plants early in the season when soil temperatures are still low, part of this P is fixed and therefore is not readily available for corn P uptake later in the growing season. This fixed P contributes to increased legacy soil P and the risk of P losses to water sources. Our results also highlighted the fact that P_{M3}, DPS and PSI are highly correlated with silage corn DMY and P uptake and therefore indicate that PSI as a proxy of DPS can be adapted for coastal BC soils as an indicator of the risk of P losses.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

AM conceived the idea and wrote the manuscript. CB and AM designed the methodology and planned all the field activities on the 11 sites, and analyzed the data and prepared the graphs and tables. CL, SK, YL, SY, CB, and AM established the experiments, applied the treatments of starter P and manure P, maintained all the sites, collected all the soil and plant samples and performed all the laboratory analyses, and contributed to the study and gave final approval for the manuscript.

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Soil Phosphorus Fractionation as Affected by Paper Mill Biosolids Applied to Soils of Contrasting Properties

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Industrial by-products such as paper mill biosolids (PBs) have been used as fertilizers and amendments in agriculture for many decades. However, their content of plant essential nutrients including phosphorus (P) varies largely with sources, and availability of these nutrients once applied to soil still remains unknown. Five PBs differing in their C/N ratio (12–47) were used in an incubation study to assess the P forms and availability from both PBs and PB-amended soils. Biosolids were added to three contrasting soils varying in texture and pH in 500-ml jars at the rate of 50 Mg wet ha^{-1}, supplying between 45 and 112 kg total P ha^{-1}. An unfertilized control and a mineral NP treatment (30 kg P ha^{-1}) were also included. Biosolids and soil P fractions were determined by a modified Hedley fractionation method following 2 and 16 weeks of incubation. Phosphorus forms in PBs varied across type of materials with labile P ranging from 20 to 72% and organic P (Po) ranging from 28 to 66% of total P. Soil incubated with PB showed changes in all P fractions due to amendment, soil type, and duration of incubation. Resin-P and labile P, the most available forms, were significantly higher with addition of high PB–P content. Soil labile P represented up to 50% of total P added by PB. Overall, most inorganic P (Pi) fractions and labile P further increased with incubation duration. Close relationships were established between resin-P and labile P content in PB and the increases of those fractions in incubated soils, notably after 2 weeks. The P recovery as labile P represented on average 34% of total P added or 68% in terms of mineral P. We concluded that PB could be used as an efficient source of P for fertilizing crops.

Keywords: phosphorus, paper mill biosolids, soil incubation, phosphorus fractions, phosphorus availability

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INTRODUCTION

The non-renewable status of extractable phosphorus (P) on Earth (Cordell et al., 2009) has resulted in an increasing interest of alternative sources such as the use of biosolids in agriculture (Shu et al., 2016). Biosolids are by-products of industrial or urban wastewater treatment which are commonly used as fertilizer and soil amendment to improve crop productivity (Su et al., 2007). Biosolids application can positively enhance crop dry matter production and yield through their N availability

(Cogger et al., 2006; Al-Dhumri et al., 2013). Henry et al. (1994) confirmed the potential of biosolids to increase the productivity of many forests.

Some concerns about biosolids application, such as potential environment contamination, make its management a very important part when used in agriculture soils. Biosolids are often applied based on crop N requirements, which may lead to soil P accumulation and increase the risk of P loss to surface or groundwater (Kelling et al., 1977; Shober and Sims, 2003; Pierzynski and Gehl, 2005). The N/P ratios for nutrient crop uptake in field crops varied from 4.5 to 8.7 (Sadras, 2006), which are considerably higher than those found in municipal biosolids (2.7) but much closer to PB (5.7) (Miller et al., 2000; Charbonneau et al., 2001; Maguire et al., 2001). Moreover, biosolids properties and their treatment plant process may have a significant impact on their fertilizer value, which can affect soil P availability (Frossard et al., 1996; Maguire et al., 2001; Penn and Sims, 2002; Torri et al., 2017).

Many studies documented soil P status once biosolids are applied. Maguire et al. (2001) recommended testing biosolids for P availability rather than total P, since it was a more appropriate indicator for predicting extractable P from biosolids-amended soils. Conversely, biosolids with similar total P may vary widely in the amount of P measured in various extracts (O'Connor et al., 2004). Different methods, including water-extractable P, Olsen P, or Mehlich-3 P (Wang et al., 2004; Su et al., 2007; Shu et al., 2016), can be used to investigate the effect of biosolids on soil P availability. Ippolito et al. (2007) reported that characterizing fractions into labile and non-labile pools could be a typical method to predict P availability in biosolids-amended soils. To address this, soil P fractionation can serve to predict the availability of P in soil as well as the likelihood of its transport (Sui et al., 1999).

The pulp and paper industry constitutes a major component of the Canadian economy, and their activities generate huge amounts of organic residues from the treatment of derived wastewater. Most recent statistics indicate that 1.5 million dry Mg paper mill biosolids (PBs) are produced annually (Rashid et al., 2006), but a low percentage (<25%) is applied on cropland whereas the rest of the residues are burnt or landfilled (MDDELCC, 2016). Large volumes of PBs are also available in the United States (5.5 million dry Mg year^{-1}; Thacker, 2007) and Europe (11 million dry Mg year^{-1} including rejects and ash; Monte et al., 2009). Land application of PB as municipal biosolids can benefit crop growth by supplying N and P while improving soil physical properties (Cabral et al., 1998; Camberato et al., 2006). Better knowledge of nutrient contribution from PB including P might help promote their appropriate use in the field.

Studies conducted to characterize municipal biosolids indicated that labile P fraction composes a small proportion of total P but this does depend on sludge type (Frossard et al., 1996; Sui et al., 1999; Ajiboye et al., 2004; Shober et al., 2006). For example, addition of lime or metal salts during the wastewater treatment process to stabilize biosolids can reduce solubility of P (Frossard et al., 1996; Pierzynski and Gehl, 2005; Su et al., 2007; Torri et al., 2017). With PB, the treatment of effluent generally consists of primary clarification to remove suspended solids followed by an activated secondary process in which microorganisms are used to reduce organic pollutants and biological oxygen demand (Nurmesniemi et al., 2007; Monte et al., 2009). To our knowledge, little is known about P forms in this particular material. When applied to soil, municipal biosolids were often reported to cause a net increase of relatively available Pi forms (H_2O, $NaHCO_3$, and NaOH) at the expense of more stable or recalcitrant forms (NaOH-Po, HCl-P, and residual P) (Sui et al., 1999; Kashem et al., 2004; Su et al., 2007; Alleoni et al., 2012). This transformation of NaOH-Po and residual P to more labile forms was also observed with PB after 6 years of application (Fan et al., 2010). All these studies, however, did not assess the P response of PB under different soil types and their possible interactions.

The objectives of this study were to (1) characterize the P forms in several PBs used in Canada and (2) determine the P forms after PB addition to soils with contrasting chemical properties after 2 (short-term) and 16 (typical duration of growing season) weeks under controlled conditions.

MATERIALS AND METHODS

Soils and Biosolids Characterization

The soils used for the incubation study were collected from the upper layer (0–15 cm) of three fields. Two soils came from near Quebec City, QC, Canada (47°N, 71°W): a Kamouraska silty clay (KAM; umbric Gleysol; FAO soil classification) from a cornfield and a St-Antoine sandy loam (StA; haplic Podzol) from a field under alfalfa/timothy pasture. The other soil came from near Lethbridge, AB, Canada (50°N, 113°W): a Chin fine sandy loam (CHIN; haplic Kastanozems) from a potato field. The soils were air-dried and sieved to 2 mm before incubation. Each soil was characterized in triplicate for particle size, pH, organic C, Mehlich-3 extractable elements, and P fractions (**Table 1**). The KAM and StA soils were selected to evaluate the response of PB in acidic soils, where P fixation occurs predominantly with Fe and Al oxides/hydroxides. The alkaline soil, CHIN, was selected to account for P fixation by Ca. According to critical P/Al or P/(Al + Fe) values, the KAM and StA soils had a greater capacity to retain P against losses into the environment (Pellerin et al., 2006; Wang et al., 2015).

Five different PBs were tested in this study: (1) mixed PB (primary and secondary treated sludge) from thermomechanical pulp (PB1); (2) mixed PB from bleached Kraft pulp with some municipal effluent (PB2); (3 and 4) mixed PB from bleached acid-treated Kraft pulp issued from different production times (PB3a and PB3b); and (5) de-inking sludge from recycled papers (PB4). These PBs have been previously used in field experiments (Gagnon et al., 2010, 2012b; Gagnon and Ziadi, 2012) and respected limits for trace metal contaminants (Camberato et al., 2006). Selected PBs differed largely in their properties, notably pH, total N and P, and C/N ratio (**Table 2**). PB2 and PB3b showed the highest concentrations in total N and P and the lowest C/N ratio.

TABLE 1 | Characteristics of selected soils used for the incubation study.

Attributes	Kamouraska (KAM)	Saint-Antoine (StA)	Chin (CHIN)
Sand (g kg^{-1})	302	683	554
Clay (g kg^{-1})	406	152	167
pH (1:2 soil to water)	5.32	5.89	7.51
Organic C (g kg^{-1})	30.2	16.3	14.3
Mehlich-3 P (mg kg^{-1})	38	36	119
Mehlich-3 Ca (mg kg^{-1})	2,551	1,023	2,511
Mehlich-3 Al (mg kg^{-1})	1,253	1,040	403
Mehlich-3 Fe (mg kg^{-1})	194	354	78
P fractions (mg kg^{-1})			
Resin-P	58	31	93
$NaHCO_3$-Pi	33	19	23
NaOH-Pi	118	59	20
HCl-P	415	116	227
ΣPi	624	225	363
$NaHCO_3$-Po	49	30	13
NaOH-Po	315	98	57
Residual-P	248	93	108
ΣPo	612	221	177
Σfraction P	1,236	446	540

TABLE 2 | Main properties of selected paper mill biosolids (PBs).

Attributes	PB1	PB2	PB3a	PB3b	PB4
pH_{water} (1:2)	7.80	6.77	4.98	4.51	7.23
Moisture (g kg^{-1})	707	782	639	693	553
Total C (g kg^{-1})	315	450	446	485	281
Total N (g kg^{-1})	12.8	36.3	19.1	39.7	6.0
C/N ratio	25	12	23	12	47
Total P (g kg^{-1})	4.2	5.7	2.9	7.3	2.0
PO_4-P (mg kg^{-1})	72	344	177	1,051	2
Total Ca (g kg^{-1})	8	51	6	2	149
Total Al (g kg^{-1})	36.5	3.7	9.1	9.1	13.0
Total Fe (g kg^{-1})	3.1	1.9	1.0	1.4	1.6
$(Fe + Al)_{total}$ (mmol kg^{-1})	1,463	204	374	387	539
Total P added with 50 Mg wet PB ha^{-1} (kg ha^{-1})	62	62	52	112	45

Values on dry matter basis except moisture. Total C was determined by loss to ignition, whereas total major nutrients by wet acid digestion with H_2SO_4-H_2SeO_3 (Isaac and Johnson, 1976). The water-soluble PO_4^{3-} content was extracted from fresh material to a solution ratio of 1:5 by weight.

Incubation Study

Biosolids were mixed with 100 g of air-dried soil at a rate equivalent to 50 Mg wet ha^{-1} (4.17 g fresh material per jar considering a depth incorporation of 10 cm and a soil bulk density of 1.2 g cm^{-3}) and incubated in 500-ml Mason™ glass jars. The PB supplied between 45 and 112 kg total P ha^{-1} (**Table 2**). Unamended controls containing soils only were prepared in a similar manner in order to estimate the contribution of soil-derived P. In addition, a mineral NP treatment [(30 kg KH_2PO_4-P ha^{-1} and 100 kg N ha^{-1} as NH_4NO_3 (35% N)] was added to evaluate the relative P contribution of each biosolids.

The experiment was laid out as a factorial of seven treatments (five PBs, NP, and the control) and three soils completely randomized in three replications. Distilled water was added to the soil–PB mixtures to adjust water-filled pore space to 60%. The jars were closed and incubated in the dark in a controlled environment chamber at 25°C for 16 weeks. Water loss was monitored twice a week and corrected if needed. At the same time, the soils were well aerated and carefully remixed before reclosing to maintain constant aerobic and mineralization conditions.

Soil Phosphorus Fractionation

Subsamples (5 g) of the soil–PB mixtures were collected after 2 and 16 weeks of incubation and analyzed for P fractions. The two sampling periods were chosen to simulate short-term (early root growth) and typical duration for a growing season in Canada. Soil samples were air-dried, sieved to pass a 2-mm screen, and then ground to 0.2 mm.

The Hedley sequential extraction procedure was performed as described by Tiessen and Moir (2008) with modifications for soil digestion as proposed by Zheng et al. (2001) and used in the Fan et al. (2010) study. Thus, a 0.5-g subsample of ground soil was weighed into 50- ml centrifuge tubes and sequentially extracted according to the following scheme:

1. Resin-P (Pi): 25 ml of water and two resin strips, shake for 16 h at 25°C, remove the strips, centrifuge, decant, and discard supernatant. P was recovered from strips in 25 ml of 0.5 M HCl.
2. $NaHCO_3$ (Pi and Po): 25 ml of 0.5 M $NaHCO_3$ at pH 8.5, shake for 16 h at 25°C, centrifuge, and collect supernatant.
3. NaOH (Pi and Po): 25 ml of 0.1 M NaOH, shake for 16 h at 25°C, centrifuge, and collect supernatant.
4. HCl (Pi): 25 ml of 1.0 M HCl, shake for 16 h at 25°C, centrifuge, and collect supernatant.
5. Residual P: 10 ml of 0.9 M H_2SO_4 and 0.5 g of $K_2S_2O_8$, digestion at 121°C in an autoclave for 90 min.

The concentration of Pi in extracts and digests was determined by the ammonium molybdate–ascorbic acid method (Murphy and Riley, 1962). The concentrations of Po in each of these two extractions ($NaHCO_3$ and NaOH) were calculated as the difference between total P determined after H_2SO_4–$K_2S_2O_8$ digestion and Pi. Total P in soils was also determined the same way as for residual P with 0.1 g of ground soil. This allowed evaluation of the P recovery from fractionation which varied from 83 to 103% (93 ± 5%). Additionally, the soil pH (1:2 soil:water) was determined on the air-dried 2-mm samples at the end of incubation.

Total Pi was the sum of resin-P, $NaHCO_3$-Pi, NaOH-Pi, and HCl-Pi, while total Po was the sum of $NaHCO_3$-Po, NaOH-Po, and residual P. Usually, but not exclusively, resin-P is considered as freely available Pi; $NaHCO_3$-P is assigned to Pi sorbed on crystalline Al and Fe oxides and to easily mineralized Po; NaOH-P is assigned to Pi sorbed to amorphous Al and Fe oxides and to stable Po; and HCl-P is considered to be Ca–P compounds (Negassa and Leinweber, 2009). According to the criteria of

Bowman and Cole (1978) and Tiessen et al. (1984), the sum of resin-P and $NaHCO_3$-P was considered as labile P, NaOH-P as moderately labile P, and HCl-P and residual P as stable P.

Biosolids Phosphorus Fractionation

Paper mill biosolids P was also fractionated before application using the same method as described for soil. Since the method was developed for mineral soils, some modifications were made considering the organic nature of the material. Thus, fresh PB (0.5 g wet basis for P fractionation and 0.1 g wet basis for total P) was used for the extraction, as suggested by Ajiboye et al. (2004). The supernatant of the resin step was kept to determine both Pi and Po (after digestion) in the water extract. No Pi was found in the water extract. Organic P in 1.0 M HCl was also determined as recommended by He et al. (2010) to provide a more accurate P characterization of the materials. Usually, this fraction is recovered in the residual P pool and has been simply assumed to be negligible. Finally, extracts of residual P and total P were digested twice to completely release Pi from materials.

Data Analysis

All data for soil incubation were checked for normality with Shapiro–Wilk. Only NaOH-Pi was transformed based on the Box–Cox model to improve the normality of distribution. Treatment effects were evaluated as a factorial of 3 × 7 replicated three times using the MIXED procedure (SAS Institute Inc., 2004) with replicates and replicates × treatments as random effects, soil types and treatments as fixed effects, and incubation time as repeated effect. Main treatment effects and their interactions were tested using differences of least squares means. The NP treatment in the CHIN soil was omitted due to the unexpected negative total Pi recovery (−10%) at the end of incubation as compared with KAM (86%) and StA (91%) soils. As a consequence, this treatment was excluded from the statistical analysis. Statistical significance was defined as $P < 0.05$.

RESULTS AND DISCUSSION

Biosolids Phosphorus Fractions

Total P content in PB ranged from 1,535 to 6,904 mg kg^{-1}, with the PB3b having the highest concentration and the PB4 showing the lowest (**Table 3**). The P recovery from fractionation was high, varying between 93% and 101%, in agreement with other studies performed on organic materials (79–113%; Ajiboye et al., 2004; Turner and Leytem, 2004; Gagnon et al., 2012a).

Total Po, with the exception of PB1, accounted for a large part of total P (46–66%, **Table 3**). This contrasts with municipal biosolids in which Pi was dominant (>71%; Sui et al., 1999; Ajiboye et al., 2004; O'Connor et al., 2004; Su et al., 2007). In this study, PBs have been mostly subjected to a secondary biological treatment for further purification and stabilization before discharge to the water bodies. This treatment increases the microbial activity and could explain the high Po proportion in such materials, a tendency also observed when sewage sludge was treated by the activated process (Frossard et al., 1996).

The relative contribution of P in each fraction varied with the material (**Table 3**). Resin-P (Pi + H_2O-Po), the most readily available form, ranged from 2% in PB4 to 40% and 59% in PB3a and PB3b, respectively. Moreover, a significant part (around 40%) of Po was found in resin + H_2O and $NaHCO_3$ extracts for PB3a and PB3b. These fractions are considered mobile in the soil and biologically available (Turner and Leytem, 2004). Either PB3a and PB3b was treated with phosphoric acid to stimulate P degradation by microorganisms and reduce odor (Gagnon and Ziadi, 2012). Both materials came from the same source and were subjected to the same treatment process. Despite differences in total P, they were more related to each other than to other materials.

The labile P (resin + $NaHCO_3$), which is considered plant available, was the highest with PB2, PB3a, and PB3b (44–72%). It constituted, however, only 20–26% of total P in the other two materials. In counterpart, PB1 and PB4 contained 36–40% of their P in the stable (HCl + residual) fractions. This could be attributed to their high pH, which may contribute to the formation of recalcitrant Ca phosphate minerals (Torri et al., 2017) and to the high total Al and Fe contents (**Table 2**), which were found to result in materials having a low-availability P concentration (Krogstad et al., 2005; Torri et al., 2017). Khiari et al. (2020) reported that biosolids with a $(Fe + Al)_{total}$ $>1{,}100$ mmol kg^{-1} were classified as medium to low for their P bioavailability and were similar to a slow-release source of P for crops. Overall, the relative distribution of P forms in PBs indicated that P in this material could constitute a good source to crops, sometimes more efficient than many municipal biosolids and composts (Sharpley and Moyer, 2000; Ajiboye et al., 2004; Xu et al., 2012). To this end, Ebeling et al. (2016) found that municipal biosolids with a biological P removal process had consistently the highest soil test P values in opposition to biosolids treated with alum or $FeCl_2$.

Effect of Incubation on Soil pH

Variations in soil pH during incubation induced by mineralization of PB may have a large impact on the distribution and form of soil P fractions. In acidic soils, Fe and Al phosphates are more dominant, whereas in alkaline soils, Ca phosphate dominates. In this study, addition of PB4 due to its high Ca content (**Table 2**) induced a large increase in soil pH, 0.1 unit in the alkaline CHIN but 1.6–1.8 in the acidic KAM and StA (**Table 4**). On the opposite, PB3a and NP decreased the soil pH by an average of 0.2–0.3 units, while PB3b caused the largest drop in pH with 0.6 units. PB3b was the most acidifying material of all those tested (**Table 2**).

Effect of Incubation Time, Soil Type, and Biosolids on Soil Phosphorus Fractions

There were significant changes in the different P fractions with time during the incubation (**Supplementary Table S1**). From the beginning to week 16, there was a general increase in all Pi fractions, notably the labile forms (resin-P and $NaHCO_3$-Pi; **Supplementary Table S2**). This was made at the expense of Po and residual P fractions for StA and CHIN and $NaHCO_3$-Po

TABLE 3 | Phosphorus fractionation of paper mill biosolids (PBs) used in the incubation study.

Fractions	**PB1**	**PB2**	**PB3a**	**PB3b**	**PB4**
			mg kg^{-1} dry weight		
Inorganic					
Resin	577 ± 45(13)	1,500 ± 86(26)	810 ± 78(29)	3,273 ± 102(47)	8 ± 4(1)
$NaHCO_3$	400 ± 32(9)	729 ± 30(13)	79 ± 17(3)	196 ± 5(3)	173 ± 5(12)
NaOH	1,293 ± 117(29)	153 ± 52(3)	1 ± 2(0)	0 ± 0(0)	246 ± 9(17)
HCl	894 ± 26(20)	695 ± 4(12)	37 ± 4(1)	84 ± 3(1)	228 ± 12(16)
Total Pi	3,164 ± 178(72)	3,077 ± 78(54)	927 ± 74(34)	3,554 ± 107(51)	656 ± 24(45)
Organic					
Resin + H_2O	66 ± 22(2)	126 ± 28(2)	303 ± 32(11)	818 ± 221(12)	28 ± 4(2)
$NaHCO_3$	94 ± 3(2)	172 ± 30(3)	358 ± 40(13)	708 ± 37(10)	76 ± 1(5)
NaOH	397 ± 33(9)	1,617 ± 73(28)	720 ± 52(26)	1,180 ± 115(17)	343 ± 4(24)
HCl	48 ± 6(1)	142 ± 10(2)	102 ± 48(4)	98 ± 18(1)	144 ± 33(10)
Residual	625 ± 14(14)	604 ± 66(11)	340 ± 41(12)	594 ± 48(9)	213 ± 2(15)
Total Po	1,230 ± 34(28)	2,661 ± 33(46)	1,823 ± 113(66)	3,397 ± 215(49)	804 ± 37(55)
Total					
Σfraction P	4,394 ± 173	5,738 ± 91	2,750 ± 181	6,951 ± 113	1,460 ± 59
Total P digestion	4,754 ± 99	6031 ± 69	2,838 ± 121	6,904 ± 156	1,535 ± 107
P recovery (%)	93	95	97	101	95

P recovery = (resin-Pi + $NaHCO_3$-Pi + NaOH-Pi + HCl-Pi + resin + H_2O-Po + $NaHCO_3$-Po + NaOH-Po + HCl-Po + residual P) × 100/total P in digestion. Values in parenthesis represent percentage of specific form P in the sum of fraction P.

TABLE 4 | Effect of paper mill biosolids (PBs) and mineral P fertilizer on the soil pH at the end of the 16-week incubation.

Treatment	**KAM**	**StA**	**CHIN**
PB1	5.17 ± 0.01	5.73 ± 0.07	7.54 ± 0.01
PB2	5.12 ± 0.02	5.79 ± 0.10	7.48 ± 0.03
PB3a	5.05 ± 0.01	5.42 ± 0.01	7.32 ± 0.04
PB3b	4.81 ± 0.00	5.01 ± 0.14	7.01 ± 0.03
PB4	6.82 ± 0.03	7.50 ± 0.01	7.71 ± 0.02
Mineral NP	5.07 ± 0.02	5.44 ± 0.10	7.43 ± 0.01
Control (0 NP)	5.23 ± 0.01	5.69 ± 0.13	7.61 ± 0.02
$LSD_{0.05}$	0.03	0.18	0.04

and residual P for KAM. Kashem et al. (2004) investigated the forms and distribution of P after 1, 4, and 16 weeks of incubation in a silty clay loam soil and reported that the amendment with municipal biosolids caused a net conversion of Po and residual forms toward H_2O-P, NaOH-Pi, and HCl-P fractions. Sui et al. (1999) and Su et al. (2007) also found a decrease in the most recalcitrant forms (HCl-P, NaOH-Po, and residual P) and a concomitant increase in labile forms (H_2O-P and $NaHCO_3$-Pi) after repeated applications of municipal biosolids. Fan et al. (2010) reported a large decrease in NaOH-Po and residual P after 6 years of continuous PB application, which was attributed to the mobility and/or mineralization of NaOH-Po and the transformation of recalcitrant P to more labile forms with time.

Soil type had a very significant effect ($P < 0.001$) on every soil P fraction (**Supplementary Table S1**). This was expected due to the significant variability in P distribution with the three soils selected in this study (**Table 1**). Globally, the greater P sorption capacity of KAM and StA soils made resin-P to be lower in these soils and NaOH-P associated with Al and Fe oxides to be higher as compared with CHIN (**Supplementary Table S2**). However, there was a soil × treatment interaction for resin-P and labile P, meaning that these P fractions behave differently depending on which soil the different PBs have been incorporated in.

Application of PB was particularly important for soil resin-P, a freely available fraction, and labile P (**Supplementary Table S1**). The increases reached 26 to 40 mg kg^{-1} in resin-P and 39 to 51 mg kg^{-1} in labile P (**Figures 1A,B**). The strongest differences were noted for resin-P in CHIN soil. Such wider range has been already observed in a preceding study evaluating different composts (Gagnon et al., 2012a) and was attributed to the relatively low potential of this soil to fix added P. The Fe and Al oxides are the most important phosphate adsorbents in soil (Parfitt, 1979; Borggaard et al., 1990), and phosphate sorption increases with decreasing pH (Lopez-Hernandez and Burnham, 1974), which can mask the differences between PBs. Conversely, application of P in soils of temperate climate was responsible for the largest variations over the years in labile P (Negassa and Leinweber, 2009). To this end, Fan et al. (2010) obtained a linear increase in resin-P and a tendency ($P = 0.07$) for increase in $NaHCO_3$-Pi after 3 years of continuous application of 90 Mg wet PB ha^{-1} to a loamy soil.

In this study, PB3b caused the largest increase in P concentrations in all soils (**Table 5** and **Figure 1A**). This is expected with the supply of a high total P amount by this material (112 kg P ha^{-1}; **Table 2**). In terms of total P added, PB3b (42–55%) followed by PB2 and PB3a (26–49%) was an interesting source to increase soil labile P. Unfortunately, PB1 and PB4 were relatively inefficient at releasing P in the alkaline CHIN soil (**Figure 1B**). Moreover, PB4 induced a shift from NaOH-Po to $NaHCO_3$-Pi (**Table 5**). Accordingly, it appears that its very high Ca content (**Table 2**) and good liming potential (40% $CaCO_3$ equivalent; Gagnon and Ziadi, unpublished data) promoted soil

TABLE 5 | Effects of paper mill biosolids addition on selected soil P fractions averaged over soils and time (mg kg^{-1} dry basis).

	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-P	Total Pi
PB1	28 ± 5c	23 ± 14c	69 ± 40bc	160 ± 124b	256 ± 127ab	418 ± 171b
PB2	30 ± 7b	25 ± 15b	69 ± 43b	168 ± 128ab	254 ± 125ab	426 ± 170b
PB3a	27 ± 5c	25 ± 14b	65 ± 42d	168 ± 127ab	247 ± 127c	408 ± 169c
PB3b	35 ± 6a	26 ± 14a	72 ± 45a	173 ± 124a	258 ± 127a	455 ± 172a
PB4	31 ± 8b	23 ± 13c	65 ± 40d	148 ± 118c	252 ± 123abc	405 ± 162cd
Untreated control (0 NP)	24 ± 5d	23 ± 14bc	66 ± 41cd	162 ± 121b	249 ± 124bc	399 ± 172d

Total Pi = resin-P + $NaHCO_3$-Pi + NaOH-Pi + HCl-P. For each factor, means followed by the same lowercase letter are not different at P = 0.05.

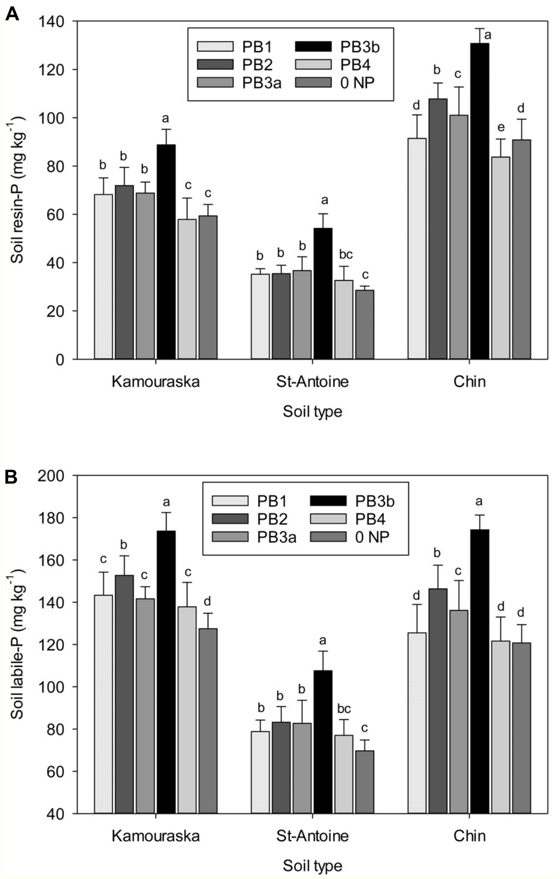

FIGURE 1 | Effects of paper mill biosolids (PBs) addition in function of soil type on the soil **(A)** resin-P and **(B)** labile P fractions. Values (mean ± standard deviation) are averaged over time.

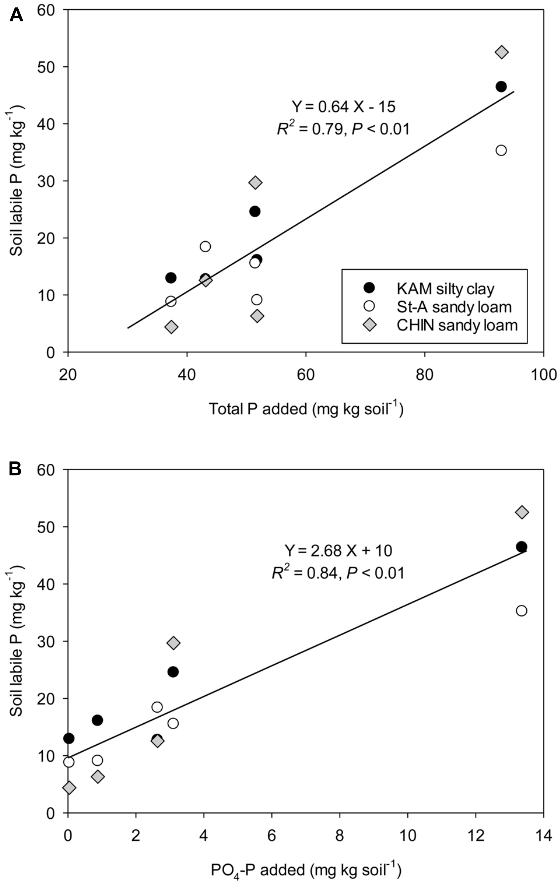

FIGURE 2 | Relationship between total P **(A)** and PO_4-P **(B)** added by the paper mill biosolids and the net increase (treated soil minus control) in soil labile P after 16 weeks of incubation.

pH increase (**Table 4**), which may have reduced the mobility of Al (Casséus et al., 2011).

Despite Po forming a large proportion of total P in some of these materials (**Table 3**), significant effects of PB were mostly detected on Pi fractions (**Supplementary Table S1**). This indicated that PB had a good potential for P mineralization in the studied soils. Increases in labile Pi were also observed with municipal biosolids even if these materials comprised Pi in less soluble forms (Sui et al., 1999). The net increase in labile P (soil labile P + PB minus soil labile P in 0 NP minus labile P applied with PB) was mainly negative, particularly for PB3a and PB3b (−11 and −21%, respectively). Negative values mean that PBs served more as a direct source of P fertilizer than soil P conditioner because they did not enhance the release of P from the soil.

Considering the application rate and the forms and distribution of P in the material, the PB in this study (excluding PB4 considered more as a soil conditioner) supplied 17 (6–38) kg resin-P ha^{-1} and 25 (9–55) kg labile P ha^{-1} after the early stage of application, based on the 2-week incubation data, and a little bit more for the entire incubation (18 and 28 kg P ha^{-1}, respectively). This covers a large part of the P requirements for corn and small cereals (26–28 kg P ha^{-1}; CRAAQ, 2010) produced in eastern Canada in the low-P KAM and StA soils. The P availability at week 2 is important for early plant growth, particularly in temperate areas where the soil is usually cool at springtime (Grant et al., 2001). Even if this study did not include a crop test, the accompanying NP treatment can be used to assess the potential direct P contribution of PB sources by assuming that labile P measured in the NP treatment is considered to represent plant available P (Tiessen and Moir, 2008). In terms of mineral P, the P recovery as labile P represented on average 68%, with the lowest recovery with PB1 (48%) and the highest with PB3b (87%). Hence, PB could constitute an excellent source of P, more than the conventional treated sewage sludge in which application to the field indicated a much lower P availability than the commercial P fertilizer based on soil $NaHCO_3$-P extraction and corn plant P concentration (Tian et al., 2016).

Relationships Between Phosphorus Fractions in Biosolids and Soils

Soil resin-P and labile P increases over the control were closely related to total P added at both week 2 (R^2 = 0.79–0.83) and week 16 (R^2 = 0.76–0.79) of the incubation (**Figure 2A**). This indicates a high potential for PB to be a valuable source of P for crops. Su et al. (2007) also reported an increase in these fractions with municipal biosolids addition to a sandy forest soil. Soil labile P increases were also related to the amount of PO_4-P added (**Figure 2B**). Total P concentration and water-extractable P have been reported as the best indicators for predicting P release from composts and manures (Sharpley and Moyer, 2000; Zvomuya et al., 2006). In this study, a relationship could be established between PO_4-P concentration and pH of materials, all determined by water extraction (R^2 = 0.53). This indicates that total P and pH might be selected as controlling variables to estimate P contribution from PB.

When expressed on a total P basis, close relationships were obtained between net increases in soil resin-P and labile P and a proportion of these fractions in biosolids at both 2 and 16 weeks (**Supplementary Table S3**). There was also a negative correlation between soil resin-P and labile P and materials with the most recalcitrant (NaOH-P, HCl-P, and residual) P fractions. Besides the magnitude of labile P, which can be used for evaluating potential environmental impact (Sharpley and Moyer, 2000; Ajiboye et al., 2004) and plant P bioavailability (Zvomuya et al., 2006), the fate of P of the organic amendments once applied to soil has always been hard to predict. Despite several attempts, the biogeochemistry of the soil plays a role in the fixation of phosphate ions by Al and Fe oxides in acidic soil and by Ca ions in alkaline soil and causes a redistribution of the different P forms with time.

CONCLUSION

This study revealed that PB could potentially be an efficient P source for fertilizing crops based on our soil *P* test. These materials generally contained a high percentage of freely available resin-P and labile P and low Al and Fe contents, which enhanced the P availability in soil. The increases in soil labile P, which is considered plant available, may represent up to 50% of total P added and can even reach amounts obtained with the mineral P fertilizer. All soils can benefit from application of PB, at least in the short term for the development of young plants. However, the total P content and P forms varied largely, even within the same production plant. These wide variations could therefore translate into differences in PB response when applied in field and various beneficial P effects on crops.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

XZ, a Ph.D. student under the supervision of NZ and ZH, did all the lab work, performed all calculations and statistical analyses, and wrote a first version of this manuscript. BG conducted the incubation study, validated statistical analyses, and contributed to the results and discussion. NZ initiated the study, supervised the Ph.D. student, and contributed to the whole manuscript. AC discussed and validated the statistical analyses and revised and commented on the manuscript. KA and ZH revised and commented on the manuscript.

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SUPPLEMENTARY MATERIAL

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An 11-Year Agronomic, Economic, and Phosphorus Loss Potential Evaluation of Legacy Phosphorus Utilization in a Clay Loam Soil of the Lake Erie Basin

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Legacy phosphorus (P) in agricultural soils has become a predominate source contributing to P loadings to Lake Erie since the mid-90s. The use of legacy P in soils can be an ultimate and effective way to mitigate the risk of agricultural P loss and to circumvent potential P rock reserve shortage, while sustaining crop production. A field experiment was conducted to assess the impacts of P draw-down (PDD) (i.e., use of legacy P in soils) on crop yields, P uptake and removal, and soil test P (Olsen P, an agronomic P calibration and environmental soil P risk indicator in the region) under a corn–soybean rotation in a clay loam soil of the Lake Erie Basin, southwestern Ontario, Canada, from 2008 to 2018. Corn and soybean grain yields with PDD were identical to those with continuous P addition (CPA), averaged at 7.7 Mg ha^{-1} for corn and 3.7 Mg ha^{-1} for soybean, over 11 years. Similarly, no significant differences in crop P uptake and removal were found between PDD and CPA. Compared to CPA, PDD increased net farming income by Canadian dollar (CAD) 104–125 ha^{-1} year^{-1} (i.e., USD 78.5–94.4 ha^{-1} year^{-1}), with savings on P fertilizer materials and associated application costs. Soil P loss risks with PDD reduced, as indicated by soil test P that, in the top layer (0–15 cm), decreased linearly with crop production year at 3.27 mg P kg^{-1} year^{-1} or 16.2 mg P kg^{-1} per 100 kg crop P removal per hectare, while in the lower soil layers, 15–90 cm, it remained unchanged. In comparison, CPA of 50 kg P ha^{-1} sustained soil test P in the entire soil profile, 0–90 cm, over the 11-year period. PDD can be a beneficial management practice utilizing legacy P in soils to achieve both agronomic and economic goals in an environmentally sustainable manner.

Keywords: soil test phosphorus, legacy phosphorus, crop yield, economic analysis, surface water quality, Lake Erie

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INTRODUCTION

Chemical fertilizer phosphorus (P) application often exceeds plant requirements, resulting in P accumulation in soils where it shows a strong affinity for the solid phase (Menezes-Blackburn et al., 2018). Manure application, commonly based on the N requirements for crop growth, also frequently results in the over-application of P due to the discrepancy in the N/P ratio between manure and field crop biomass. Across the world, long-term continuous applications of P have led to an agronomic surplus of 11.5 Tg P year^{-1}, most of which accumulates in agricultural soils as legacy P, leading to high soil test P (STP) and contributing to accelerated P losses and the eutrophication of surface water (MacDonald et al., 2011; Sharpley et al., 2013). Such a large amount of legacy P has become one of the major reasons causing the failure of conservation measures to deliver improvements in water quality in the last few decades (Sharpley et al., 2013). To address soil legacy P, one simple practice is to draw-down soil P concentration by halting P application until the potential for P loss decreases to an environmentally acceptable level (Dodd et al., 2012). However, two questions remain to be answered before adopting the P draw-down (PDD) approach to manage high-P soils. One is whether halting P applications impairs crop yield and farmers' profitability. The other is whether an environmentally acceptable soil test P level will be achieved in a reasonable or desired time frame (Dodd et al., 2012).

Applied P in soils, if not used by crops, is predominately in labile forms physically absorbing to soil particles and/or in moderately labile forms chemically absorbing to Fe and Al oxides, which are reversible through desorption and can be bioavailable to crops (Zhang and Mackenzie, 1997a,b; Zhang et al., 2004). For high-STP soils, legacy P can generally support adequate crop yields without further P application on certain soils for periods of 10 years or more (Rowe et al., 2016). For example, legacy P from a 28-year buildup was sufficient for 15 years of wheat cultivation without a significant decline in crop yield (Liu et al., 2015). Syers et al. (2008) further pointed out that up to 90% of P applied as fertilizer and manure can eventually be accessed by crops as stable forms of P break down into labile forms. These findings would confirm the agronomic feasibility of using legacy P to support crop growth in a way of reducing or ceasing P fertilization.

The risk of soil P loss is positively related to STP concentration (Wang et al., 2010). Under Ontario conditions, for example, soils with Olsen P < 30 mg kg^{-1} had an insignificant risk of soluble soil P loss in runoff water, while the P loss risk potential of those soils having Olsen P > 30 mg kg^{-1} increased quickly with increasing Olsen P concentrations (Wang et al., 2012, 2015). Halting P application often results in a decline in STP due to the removal of P by crops and through loss by runoff or erosion (Zhang et al., 2004; McDowell et al., 2016; Cade-Menun et al., 2017; Fiorellino et al., 2017). However, this decline is often slow, and it may be on the order of decades before environmentally acceptable STP levels are reached (Sharpley et al., 2013). McCollum (1991) found that without P application, it took 16–18 years to decrease STP (Mehlich-1 P) from 99 to 20 mg kg^{-1} under corn and soybean production. In North Central Iowa, Webb et al. (1992) reported that corn and soybean P removal decreased STP (Bray-1 P) from 75 to 10–15 mg P kg^{-1} after 15 years of zero P application. Zhang et al. (2004) noted that, for a field with an initial STP (Mehlich-3) of 55 mg P kg^{-1}, no significant reduction in STP occurred in the 4 years after halting P application; however, for a soil with an initial STP of up to 130 mg kg^{-1}, corn removal decreased the STP concentration at the rate of 3.9 mg P kg^{-1} year^{-1}. Generally, the rate of STP decline is related to farming P output, soil mineralogy, and the initial STP concentration (Dodd et al., 2012).

Elevated legacy P in surface soils contributes to the recently increased dissolved P loss and harmful algal blooms observed in Lake Eire (Daloğlu et al., 2012; King et al., 2017). In Ontario, calculated based on IPNI (2015), over 60% of agricultural soils have Olsen P > 30 mg kg^{-1}, where soil P loss may pose an environmental concern (Wang et al., 2012; Reid et al., 2019). Unfortunately, PDD is rarely studied in the Lake Erie drainage basin, and thus, its potential as a beneficial management practice (BMP) for managing high-P soils is unknown. The objectives of this study were to investigate the effects of long-term PDD on (1) crop yields and farmers' production profitability and (2) the risk potential of soil P loss in runoff water, indicated by the STP concentration, in comparison with continuous inorganic fertilizer P application (CPA).

MATERIALS AND METHODS

Experimental Site and Design

The experiment was conducted at the Honorable Eugene F. Whelan Experimental Farm, Woodslee, Ontario, Canada (42°13′ N, 82°44′ W), from 2008 to 2018. The soil at the site is a Brookston clay loam, classified as a fine-loamy, mixed, mesic, Typic Argiaquoll (Soil Survey Staff, 1999) or as Orthic Humic Gleysol (Soil Classification Working Group, 1998). The soil contained 280 g kg^{-1} sand, 350 g kg^{-1} silt, and 370 g kg^{-1} clay in the Ap horizon. The climate is humid and temperate, with a mean annual precipitation of 838 mm (Zhang et al., 2015). In the study, two tested treatments included a continuous P application (CPA) and a P draw-down (PDD), which were arranged in a randomized complete block design with two replicates. Each plot was 15 m wide by 67 m long, 0.1005 ha.

Treatment Implementation and Agronomic Management

The cropping system at the field site was a corn–soybean rotation, with fertilizer application occurring only in the corn years (i.e., 2008, 2010, 2012, 2014, 2016, and 2018). At the beginning of the study, soil Olsen P (i.e., an agronomic P calibration and environmental soil P risk indicator in the region) was greater in the soil of the PDD plots than that in the CPA plots (**Figure 4**). For the CPA treatment, triple super phosphate was applied at 50 kg P ha^{-1}, while fertilizer N as NH_4NO_3 and K as KCl were applied to both CPA and PDD treatments at 200 kg N ha^{-1} and 100 kg K ha^{-1}, respectively. Pesticides and herbicides were applied in consequence of the season's prevalent pests at rates which were determined based on local recommendations. Where applicable, the nutrients (i.e., N, K, and/or P) were pre-planting broadcast to the corn phase of the rotation in the spring and incorporated immediately or once weather conditions were permissible by disking, triple-K, and packing. Shortly after harvest in the fall, the plots were harrowed to about 15-cm depth with a chisel plow. The dates for performing fertilizer application, seed planting, and crop harvesting are presented in **Table 1**. Corn and soybean were seeded at

TABLE 1 | Agronomic practices of the study site, 2008–2018.

Cropping year	Crop	Surface broadcast of fertilizer	Fertilizer incorporation	Crop seeding	Crop harvesting
2008	Corn	June 2–3	June 17	June 18	November 12
2009	Soybean	–	–	May 12	October 20
2010	Corn	June 25–26	June 26	June 26	November 8
2011	Soybean	–	–	June 15	December 13
2012	Corn	May 24	May 24–25	May 25	November 5
2013	Soybean	–	–	May 16	October 9
2014	Corn	June 30	June 30	June 30	November 28
2015	Soybean	–	–	May 25	October 7
2016	Corn	May 20	May 24	May 25	November 8
2017	Soybean	–	–	May 30	September 28
2018	Corn	May 9	May 9	May 28	October 25

the local recommended rates of 76,800 and 486,700 seeds ha^{-1}, respectively.

Sample Collection and Analysis

The grain yields of corn and soybean were determined by harvesting the central 12 and 36 rows, respectively, from a total of 20 and 40 rows in each plot. The grain moisture content was determined using a Grain Analyzer (GAC2100, Dickey-John, Crop., Cornwall, ON, Canada). Stover biomass yields of corn were determined by randomly harvesting the aboveground portion of 16 plants in each plot, while soybean biomass yields were determined by randomly harvesting the aboveground portion of soybean plants of six 35-cm-long sections of row in each plot. The grain and stover samples were dried at 55°C, and then ground to pass through a 1-mm sieve. The ground plant samples were digested using the H_2SO_4–H_2O_2 procedure (Thomas et al., 1967), with the P concentrations determined using a flow injection autoanalyzer (QuikChem FIA + 8000 series, Lachat Instruments, Loveland, CO, United States) with the ammonium molybdate ascorbic acid reduction method of Murphy and Riley (1962). Grain P removal was calculated by multiplying the grain yield with the P concentration. Crop total P uptake was the P contained in all aboveground biomass and grains.

Composite soil samples were collected at 0- to 15-cm soil depth shortly after harvest each year from 2008 to 2018 to determine the temporal patterns of soil test P. In order to evaluate the treatment effects on the downward movement of P in the soil profile over the study period, soils were also sampled at depths of 0–15, 15–30, 3–50, 50–70, and 70–90 cm in 2009 and 2016, respectively. All soil samples were air-dried and passed through a 2-mm sieve for chemical analysis. Soil test P was determined using the Olsen method, which has been the test regionally recommended for both agronomic calibration and environmental soil P loss risk indication (OMAFRA, 2017). Soil Olsen P concentration was measured by shaking 2.5 g soil in 50 ml of 0.5M $NaHCO_3$ (pH 8.5) solution for 30 min (Schoenau and O’Halloran, 2008). The determinations for P were conducted using a Flow Injection Auto-Analyzer (QuikChemFIA + 8000 series, Lachat Instruments, Loveland, CO, United States) with the ammonium molybdate ascorbic acid reduction method of Murphy and Riley (1962).

Economic Analysis

In the study, corn and soybean grain yields were largely identical between PDD and CPA, as discussed in Section “Results.” Compared to CPA, therefore, the economic benefit of PDD was gained by saving on fertilizer P and the associated application costs. The annual economic benefit (Y) of PDD was calculated as follows:

$$\mathrm{Y} = \frac{\sum_{i=1}^{6} (\text{Application rate} \times \text{fertilizer price} + \text{application cost})_i / 2}{6} \tag{1}$$

where i represents corn crop year (i.e., 2008, 2010, 2012, 2014, 2016, and 2018), when fertilizer applications occurred in the current study. Because no fertilizer was applied in the soybean year of the rotation, the annual economic benefit of PDD was calculated by dividing the summed P fertilizer and associated application costs saved for the PDD treatment in each corn year by 2. In the CPA plots, fertilizer P was applied at the rate of 50 kg ha^{-1} in the corn year. The prices for inorganic P fertilizer (i.e., triple superphosphate in the current study) were CAD 1,072, 670, 849, 758, 811, and 833 in 2008, 2010, 2012, 2014, 2016, and 2018, respectively, which were collected by Ridgetown Campus (2008–2018), University of Guelph, in the survey conducted for “Ontario Farm Input Monitoring Project.” Based on the Survey for the Ontario Custom Farmwork Rates charged in 2015 and 2018 (OMAFRA, 2016, 2019b) and 2019 Field Crop Budgets (OMAFRA, 2019a), the associated fertilizer application costs were CAD 8, 9, 10, and 11 per acre in 2012, 2015, 2018, and 2019, respectively. According to the temporal trend of fertilizer application costs, we assumed that the fertilizer application costs were CAD 7.0, 7.5, 8.0, 9.0, 9.5, and 10.0 in 2008, 2010, 2012, 2014, 2016, and 2018, respectively. In Ontario, some farmers may apply fertilizer in a band beside and below the seed in the case of row crops or with the seed of cereals. Banding requires fertilizer boxes and metering systems on the planter or drill and an extra opener on planters to place the fertilizer into the soil. In this case, we assume that there is no extra cost for fertilizer application in addition to the planting cost. In the study, therefore, we calculated the economic benefit of PDD under two scenarios. One is that the economic benefit is gained by saving on both the P fertilizer and associated fertilizer application costs when fertilizer application and planting are performed separately. The other is that the economic benefit is gained by saving on P fertilizer only when the fertilizer is applied together with planting.

Data Analyses

Under PDD conditions, P uptake by crops not only comes from the plant-available P pool, which is Olsen solution extractable, in the soil but also may come from the biogeochemical conversion of soil P originally not plant available to plant-available P (Fiorellino et al., 2017). In the current study, the percentage (Y) of crop P removal deriving from the conversion of soil P originally not plant available extracted by the Olsen P extractant to the total crop P removal will be calculated as follows:

$$Y(\%) = \frac{(\text{Total P removal } - \text{Olsen P decline over time} \times 0 - 15\,\text{cm soil weight})}{\text{Total P removal}} \times 100 \qquad (2)$$

where total P removal is the amount of P removed out of the field by the harvested crop grain between 2008 and 2018 under PDD; Olsen P decline is the difference in the soil Olsen P concentration between 2008 (i.e., the start of PDD) and 2018 (i.e., the end of PDD) under PDD; 0–15 cm soil weight is the total soil mass in the depth in a 1-ha area, where the soil bulk density was 1.2 g cm^{-3}.

All statistical analyses were performed using SAS (SAS Institute, 2009). Significances of the differences in crop yield and P removal and uptake between PDD and CPA were tested with analysis of variance using the general linear model (GLM) procedure. Linear regression analysis between the soil Olsen P concentration and the cropping year was performed using the REG procedure.

RESULTS

Crop Yield and P Uptake and Removal

Corn grain yields with PDD were largely identical to those with CPA over the 11-year period from 2008 to 2018, with an average yield of 7.7 Mg ha^{-1} (**Figure 1**). Similarly, soybean grain yields were also similar between PDD and CPA, with the exception of 2009 and 2011, in which PDD treatment had a slightly lower (i.e., 6% in 2009 and 8% in 2011) grain yield compared to CPA. From 2008 through 2018, the average soybean grain yield was 3.7 Mg ha^{-1} across both CPA and PDD treatments (**Figure 1**). Regarding crop stover yields, they were similar between PDD and CPA treatments from 2008 through 2018 (**Table 2**).

Crop P uptake and removal were also similar between the CPA and PDD treatments in the study (**Table 2**). From 2008 through 2018, the average P uptakes of corn and soybean were 21.7 and 23.9 kg ha^{-1}, respectively, with average P removals of 17.6 and 18.3 kg ha^{-1}, respectively.

Economical Benefit of PDD

Because corn and soybean grain yields were largely similar between PDD and CPA, PDD practice obviously generated more economic benefits than did CPA by saving on fertilizer P and the associated application costs. Compared to CPA, PDD increased the net farming income by CAD 104 (i.e., USD 78.5) per hectare per year by saving on fertilizer P only, or up to CAD 125 (i.e., USD 94.4) per hectare per year by saving on both fertilizer P and the associated application costs from 2008 through 2018 (**Figure 2**).

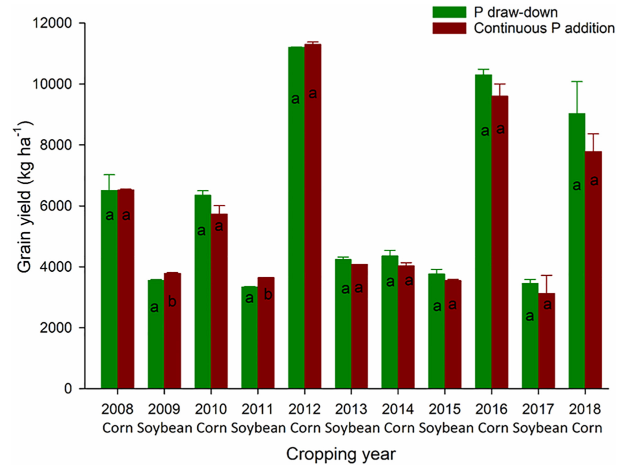

FIGURE 1 | Corn (2008, 2010, 2012, 2014, 2016, and 2018) and soybean (2009, 2011, 2013, 2015, and 2017) grain yields with continuous fertilizer P addition and soil P draw-down. *Bars* (means) labeled with *the same lowercase letter above each year* are not significantly different at $P \le 0.05$ levels. The *vertical T-bars* are standard errors (n = 2).

TABLE 2 | Corn (2008, 2010, 2012, 2014, 2016, and 2018) and soybean (2009, 2011, 2013, 2015, and 2017) stover yields, P removal (i.e., P contained in grain), and uptake (i.e., P contained in all aboveground biomass and grains) with continuous fertilizer P addition (CPA) and soil P draw-down (PDD).

	Stover yield (kg ha^{-1})		P removal (kg ha^{-1})		P uptake (kg ha^{-1})	
	PDD	CPA	PDD	CPA	PDD	CPA
2008	3,714 a	4,138 a	5.4 a	6.4 a	6.78 a	7.92 a
2009			18.5 a	18.3 a		
2010	3,521 a	3,632 a	25.3 a	16.8 a		
2011	4,091 a	4,674 a	20.5 a	21.2 a		
2012	8,173 a	8,364 a	21.9 a	20.0 a	28.5 a	26.2 a
2013	6,939 a	6,698 a	20.7 a	18.6 a	27.0 a	25.5 a
2014	5,218 a	5,440 a	12.4 a	10.3 a	17.0 a	14.9 a
2015	7,285 a	7,746 a	16.5 a	14.3 a	21.7 a	22.2 a
2016	7,342 a	7,261 a	26.9 a	23.9 a	33.8 a	29.0 a
2017	5,514 a	5,233 a	17.8 a	16.1 a	24.3 a	23.0 a
2018	6,343 a	5,018 a	24.0 a	18.6 a	29.2 a	23.3 a

Means followed by the same letter are not significantly different at $P \le 0.05$ between PDD and CPA.

Soil Profile Distribution of Soil Test P

For both treatments of PDD and CPA, the soil Olsen P concentrations decreased with depth up to 50 cm below the ground and remained very low below 50 cm (**Figure 3**). The differences between PDD and CPA were obvious: at 0- to 15-cm soil, Olsen P concentrations were significantly lower in 2016 than those in 2009 under PDD, while under CPA, no significant

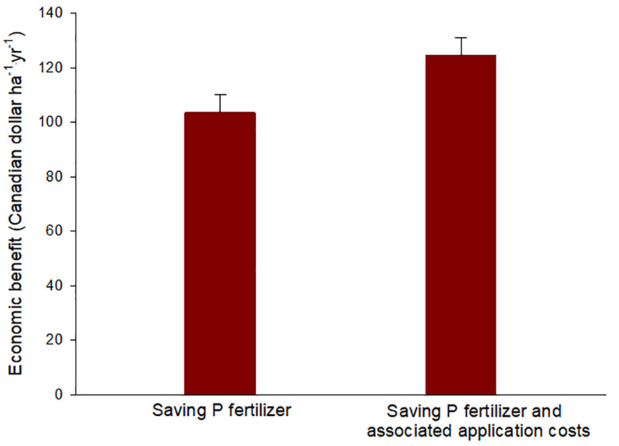

FIGURE 2 | Estimated annual economic benefit with continuous P draw-down under two scenarios: (1) economic benefit was gained by saving on P fertilizer only when the fertilizer was applied together with planting, and (2) economic benefit was gained by saving on both P fertilizer and associated fertilizer application costs when the fertilizer application and planting were separately performed. The *vertical T-bars* are standard errors (*n* = 6).

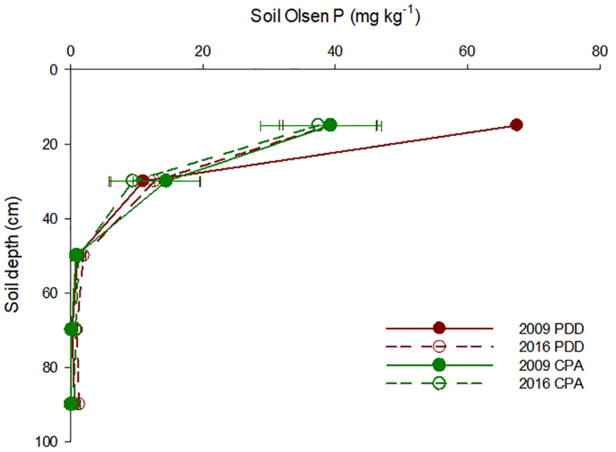

FIGURE 3 | Soil Olsen P concentrations in the profile of the soil managed under continuous fertilizer P additions (*CPA*) and soil P draw-down (*PDD*) between 2009 and 2016. The *horizontal T-bars* are standard errors (*n* = 2).

differences in Olsen P concentrations were observed between 2009 and 2016. For the soil depths below 15 cm, Olsen P concentrations were similar between 2009 and 2016 for both tested treatments.

Temporal Change of Soil Test P

In order to quantify temporal changes in soil test P under PDD and CPA, regression analyses were conducted for Olsen P in the surface 0- to 15-cm soil layer against the cropping years (**Figure 4A**). Between 2008 and 2018, the soil Olsen P concentration with PDD decreased linearly with crop production year at a rate of 3.27 mg kg^{-1} year^{-1}. Overall, soil PDD caused a soil Olsen P decline of 32.7 mg kg^{-1} between 2008 and 2018 in the current study. Meanwhile, the soil Olsen P concentration of the 0- to 15-cm soil was also linearly related to cumulative crop P removal, with 6.2 kg P removal per hectare required to decrease soil Olsen P by 1 mg kg^{-1} (**Figure 4B**). In the current study, 50 kg P ha^{-1} application maintained soil Olsen P over the 11-year period (**Figure 4A**).

DISCUSSION

Legacy P on Agronomic and Economic Benefits

In Ontario, fertilizer P is not recommended for corn and soybean if the Olsen P is above 30 and 15 mg kg^{-1}, respectively (OMAFRA, 2017). In the current study, therefore, P application at 50 kg ha^{-1} was able to provide sufficient available P to ensure optimum crop production in the CPA plots, where the soil Olsen P remained consistently around 36 mg kg^{-1}. Obviously, similar yield values between the PDD and CPA treatments are overall consistent with previous reports that soil legacy P were potentially plant available and could support adequate crop yields without further P application for up to 10 years or more (Liu et al., 2015; Rowe et al., 2016). The soil Olsen P concentrations were around 60 mg kg^{-1} in 2009 and 2011 in the PDD-treated plots. Thus, the decreased soybean yields of PDD in 2009 and 2011 may indicate that, under some conditions, soybeans with small and shallow rooting systems may need a booster of P to compromise the temporal shortage of soil-available P, especially in early spring. This needs to be further tested under field conditions. In Ontario, there is approximately 2.0 million hectares of crop land having soil Olsen P > 30 mg kg^{-1}, above which fertilizer P is not recommended for corn and soybean grain, and meanwhile runoff-soluble P losses may become an environmental concern (Wang et al., 2012, 2015; OMAFRA, 2017; Reid et al., 2019). Assuming that our PDD results apply to all these high-P soils and PDD can be properly adopted, Ontario farmers would receive a total net income of CAD 208–250 million (i.e., USD 157–189 million) each year based on our economic benefit analysis (**Figure 2**).

Legacy P on Soil Test P and P Availability to Crop Uptake and Runoff Loss

Our results indicated that the impact of PDD on soil Olsen P was restricted to 0- to 15-cm soil. Liu et al. (2015) had a similar finding that P fertilization and cessation did not impact the soil P of subsurface soils. Regarding the soil Olsen P declining rate of PDD, our result (i.e., 3.27 mg kg^{-1} year^{-1}) agreed with previous reports that soil test P decreased at rates of 3.3–3.9 mg kg^{-1} year^{-1} after halting P application (Randall et al., 1997; Zhang et al., 2004). Kamprath (1999) and Zhang et al. (2004) reported that 4.2–7.9 kg P removal per hectare in harvested corn and soybean was required to decrease Mehlich-3 P by 1 mg kg^{-1}, which was comparable to our finding that 6.2 kg P removal per hectare was required to decrease the soil Olsen P by 1 mg kg^{-1}.

In the current study, PDD reduced the soil Olsen P concentration by 32.7 mg kg^{-1} between 2008 and 2018, which

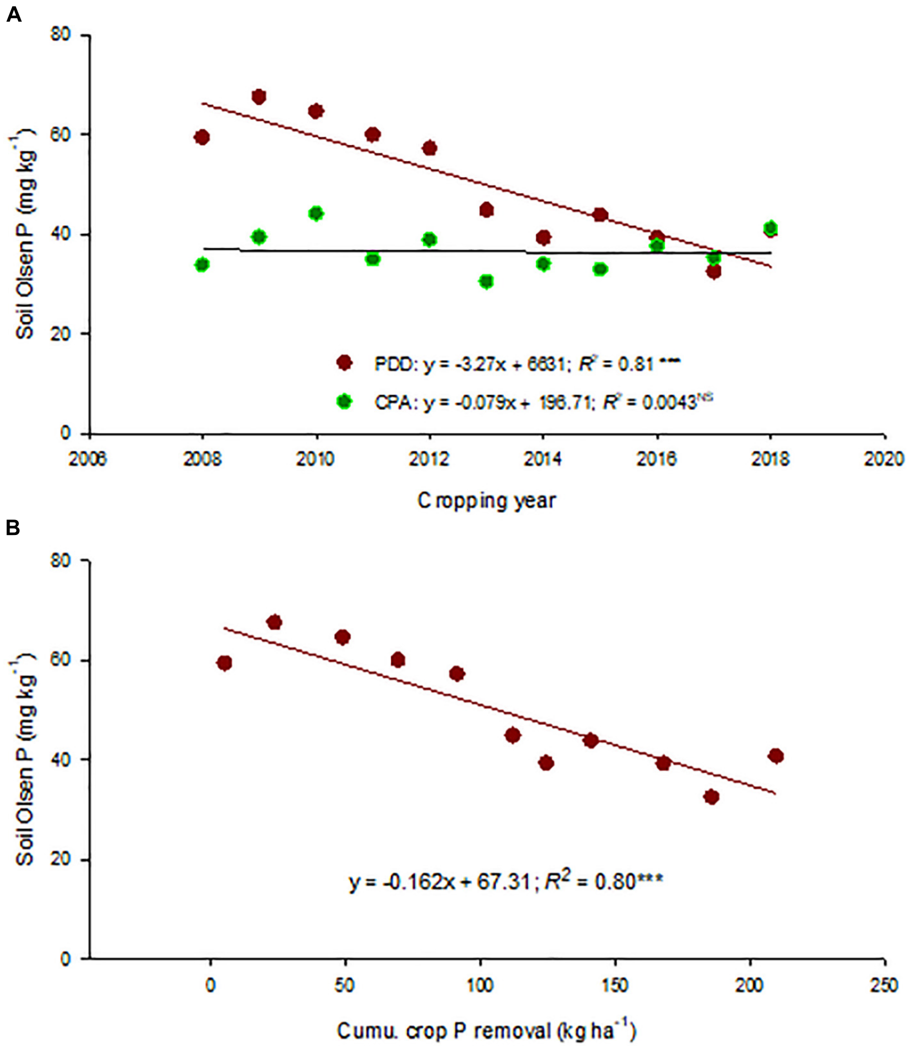

FIGURE 4 | (A) Temporal changes of the soil Olsen P concentration in the 0- to 15-cm soil layer with continuous fertilizer P addition (*CPA*) and soil P draw-down (*PDD*). **(B)** Relationship between cumulative crop P removal and soil Olsen P concentration (0–15 cm) under PDD. ***Significant at the $P \leq 0.01$ level. *NS*, not significant at the $P \leq 0.05$ level.

was equivalent to a reduction of 58.9 kg P ha^{-1} in soil Olsen P stored in the 0- to 15-cm soil depth (soil bulk density = 1.2 g cm^{-3}). In comparison, the harvested corn and soybean removed a total of 210 kg P ha^{-1} from the PDD-treated soil between 2008 and 2018 (**Table 2**). As mentioned above, soil PDD operations only impacted the P concentrations of 0- to 15-cm soil, indicating that the P removed by the crops did not come from the soil depths below 15 cm. Therefore, soil plant-available P measured by the Olsen P extractant only contributed 58.9 kg P ha^{-1} to the amount of P removed by the crops, leaving the rest of 151 kg P ha^{-1} unaccounted for. Regarding the sources of such a large amount of unaccounted P removal, Kamprath (1999) and Rowe et al. (2016) suggested that soil moderately labile P (e.g., NaOH-extractable P) and/or non-labile stable P could become plant available through a biogeochemical conversion when the available P was depleted due to crop uptake. Meanwhile, Zhang et al. (2004) pointed out that soil residual P and 0.1M NaOH-extractable organic P, both of which are not directly available to plants, could provide available P for crop uptake by mineralization when the soil-available P levels were low. Liu et al. (2015) reported that when P fertilization was discontinued, Ca–P species [i.e., hydroxyapatite and $Ca_3(PO_4)_2$] can be chemically converted to plant-available P for crop uptake. Based on Equation 2, we calculated that up to 72% of the P removed by the harvested grain was derived from the conversion of soil P originally not plant available to plant-available P in the PDD-treated plots.

In order to understand the non-changes of soil Olsen P over time in the CPA plots, a P budget was calculated by subtracting crop P removal from the amount of P applied. A total of 300 kg P ha^{-1} was added in the study period from 2008 to 2018, during which an amount of 185 kg P ha^{-1} was removed by the crops (**Table 2**). This resulted in an amount of 115 kg P ha^{-1}

appearing as legacy P in soil over the 11-year period. In reality, the actual amount of legacy P accumulated in the soil would have been less than the 115 kg P ha^{-1} due to the occurrence of soil P losses in surface runoff and tile drainage water. Zhang et al. (2015) reported that P losses were up to 1.1 kg ha^{-1} $year^{-1}$ in tile drainage water from the field adjacent to the current experimental site. Based on long-term field experiments ranging from 3 to 32 years, it was indicated that a positive P budget of 100 kg ha^{-1} increased the Olsen P concentration by 2.0–5.8 mg kg^{-1} (Messiga et al., 2010). However, the cumulative P budget values cited by Messiga et al. (2010) fell within a wider range of values than those in the current study. For example, the cumulative P budget ranged from −369 to 1,058 kg ha^{-1} in the study of Gallet et al. (2003). Consequently, it would be reasonable to believe that the insignificant changes in soil Olsen P with CPA might have been due to the increase in soil Olsen P that was too small to be detectable in the current study. In addition, soil-extractable P is often convertible to non-extractable forms over time after P additions, particularly when the soil P levels are high (Kamprath, 1999; Dodd and Mallarino, 2005). Phosphorus application greater than crop removal is often required to maintain the initial values of the soil test P in soils with higher levels of P. Zhang et al. (2004) observed that increasing the P application rates favored the soil P transformation from $NaHCO_3$–Pi (i.e., Olsen P) to NaOH–Pi, which is not extractable with the Olsen P procedure.

Implications on Legacy P Management

Biogeochemical conversion of soil P less solubility to plant-available P makes it scientifically sound to adopt the soil PDD approach in order to manage high-P soils for agricultural production. It not only increases the farmers' net income by saving on fertilizer and the associated application and transport costs but also helps to circumvent potential P rock reserve shortage by recycling the legacy P stock in soils (Gilbert, 2009). Meanwhile, biogeochemical transformation of soil P less solubility to available P would encourage our efforts to reduce agricultural P loss and improve surface water quality. Growing evidence indicates that the chronic release of soil legacy P is one of the major factors causing the failure of many agricultural conservation and nutrient management programs to reduce soil P loss and achieve improved water quality within timescales predicted originally (Sharpley et al., 2013). Soil P loss risk potentials are positively related to the soil P levels (Wang et al., 2010). Vadas et al. (2018) reported that reducing high soil P levels in all agricultural soils across the state of Maryland, United States, to optimum agronomic levels could reduce P loss to Chesapeake Bay by 40%. In our current study, it took 11 years to reduce the soil Olsen P concentration to 32 mg kg^{-1} from 65 mg kg^{-1}. For high-P soils, it can be on the order of decades before reaching environmentally acceptable P loss levels (Sharpley et al., 2013).

In Ontario, fertilizer P is not recommended for corn and soybean if the Olsen P is above 30 and 15 mg kg^{-1}, respectively (OMAFRA, 2017). Wang et al. (2012, 2015) suggested that soil test P greater than 30 mg Olsen P kg^{-1} may cause a significantly increased runoff-soluble P loss. In the current study, the overall Olsen P concentration was reduced to 32 mg kg^{-1} after 11 years of PDD operation. It is expected to see some reductions in crop yields and soil P losses without further P additions in the next few years. Subsequently an excellent opportunity would likely appear to determine a critical soil Olsen P concentration for P re-application that ensures maximum crop economic yields, with minimized P loss.

CONCLUSION

Legacy P in soils was at least partially plant available and could support adequate crop yields without further P application for up to 11 years. This led to an increased net farming income of CAD 104–125 (i.e., USD 78.5–94.4) per hectare per year and a decreased soil test P in the top layer of 0–15 cm at a declining rate of 3.27 mg kg^{-1} $year^{-1}$. The latter would indicate a reduced soil P loss from high-P agricultural lands to water resources, such as the Lake Erie, as it is closely related to soil P losses in both surface runoff and tile drainage water. Considering its significant agronomic and economic benefits, as well as the potentials for alleviating the adverse effects of agricultural production on water quality and for circumventing P rock reserve shortage, the utilization of legacy P in soils appears a promising BMP, although its efficacy on soil P loss needs to be confirmed with quantification in future studies.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

AUTHOR CONTRIBUTIONS

TZ proposed, designed, and facilitated the studies. All authors contributed to the conduction of field trials. YW and TZ contributed to the preparation of the manuscript.

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Legacy Phosphorus After 45 Years With Consistent Cropping Systems and Fertilization Compared to Native Soils

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Agricultural practices affect the status of legacy phosphorus (P) in soils and consequently the P bioavailability and susceptibility of losses to water resources. Previous studies have primarily assessed P status within agroecosystems, and rarely have these results been compared to native conditions. We evaluated the effects of long-term (45 years) consistent cropping [continuous corn (CC), corn-oats-alfalfa-alfalfa rotation (CR), and continuous bluegrass sod (CB)] with and without P fertilization on changes in P fractions of different bioavailability in a Brookston clay loam, as compared to an adjacent forest native soil. Soil P was separated into various inorganic P (P_i) and organic P (P_o) fractions using a modified sequential fractionation method. Phosphorus in native soil was predominated by moderately labile P_o (NaOH-P_o), 44%, followed by moderately stable P_i (HCl-P), 26%. Compared to the native soil, consistent cropping without P fertilization significantly decreased all P fractions except for water-extractable P_o, with the largest decrease in labile P_i (water-P_i + NaHCO$_3$-P_i) and moderately labile P_o of 65 and 73 mg kg^{-1}, respectively, over 45 years. Consistent cropping with fertilization retained comparable amount of total P in CC and RC, but increased total P in CB, relative to the native soil. Averaged over cropping systems, labile P_i, NaOH-P_i, and HCl-P increased by 129, 74, and 20 mg kg^{-1}, respectively, whereas labile P_o and moderately labile P_o decreased by 8 and 60 mg kg^{-1}, respectively, compared to the native soil. This study indicates that long-term cropping significantly enhanced the rate of moderately labile P_o mineralization, irrespective of fertilization. The increases of total P and P_o in the fertilized CB plots suggest that P accumulation in long-term grass fields is a concern as far as potential P contamination in surface waters.

Keywords: long-term fertilization, cropping system, grass land, phosphorus fraction, inorganic phosphorus, organic phosphorus, forest ecosystem, native soil

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Abbreviations: CC, continuous corn; CB, continuous bluegrass sod; CR, corn-oats-alfalfa-alfalfa rotation; P, phosphorus; P_i, inorganic P; P_o, organic P.

INTRODUCTION

Soil P status plays important roles in P bioavailability and mobility from soil to water (Simard et al., 1995; Zheng and Zhang, 2012). Phosphorus accumulates in soil when applied in excess of crop removal (Zhang et al., 2004; Hao et al., 2018). However, P decline in soils under agricultural production has also been observed after long-term cessation of fertilization relative to native soils due to P removal in the harvested crops. Hedley et al. (1982) found that total P in three grassland soil associations of the Canadian prairies after 60 to 70 years of cultivation was 12 to 29% lower than that of the adjacent permanent pastures. For two of the three soil associations, essentially all P losses were accounted for by P_o. Bowman et al. (1990) reported that half of total P decline was stemmed from the decrease in P_o pool. Tiessen et al. (1982) considered that soil P was primarily lost from P_o fraction until this fraction depleted sufficiently to allow dissolution of apatite to occur. A significant relationship between P reduction and soil organic matter loss was observed by Tiessen et al. (1983). The supply of plant availability P was regulated by the rate of mineralization of P_o (Cole et al., 1977). Those studies, as well as the ones reported recently (e.g., Tiecher et al., 2018), indicated that the mineralization of P_o in cultivated soil plays important roles in both plant available P and P transformation in soil. Hedley et al. (1982) considered that P loss from cultivated soil resulted mainly from crop removal, which would account for the 75% of the decrease in total P loss from soil.

Cropping systems have large impacts on soil P status. Bowman and Halvorson (1997) reported P availability increased significantly in the 0- to 5-cm depth with continuous wheat compared with wheat fallow in central great plain of the United States, and they attributed this increase to the high return of crop residues. Zheng et al. (2003) reported larger pools of labile inorganic P (Pi) and organic P (Po) extractable with $NaHCO_3$ (pH 8.5) and moderately labile Po extractable with NaOH (0.1 *M*) in 30- to 60-cm soil in a barley-alfalfa-alfalfa rotation compared to monoculture barley. Great root biomass from perennial crops contributed to P accumulation in the soil layer. Although there have been research efforts dedicated to assess P transformations in soils under diverse cropping systems (Zhang and MacKenzie, 1997a,b; Saltali et al., 2007; Takeda et al., 2009; Ahmed et al., 2019), little information is available on comparison of soil P dynamics among cropping systems, especially using native soils as a reference.

The addition of fertilizer P not only supplies an essential nutrient to plants, but it also influences P distribution in pools of various bioavailability and loss susceptibility to water. Increases in labile P_i and moderately labile P_i in fertilized soils have been observed (Wagar et al., 1986; Zhang and MacKenzie, 1997a,b; Zheng et al., 2002; Zhang et al., 2004; von Sperber et al., 2017). It was reported that moderately labile P_i is the primary sink of added fertilizer P and source of labile P_i under long-term cropping practice in a Labarre silty clay (Zheng et al., 2002) and a Chicot sandy clay loam (Zhang et al., 2004). However, changes in moderately stable P_i extractable with 0.1*M* HCl were not consistent in P fertilized soils. Zhang and MacKenzie (1997a,b) reported moderately stable P remained constant in a Chicot sandy clay loam over the short term, whereas Mckenzie et al. (1992), Richards et al. (1995), and Crews and Brookes (2014) found moderately stable P increased along with addition of fertilizer P in the long-term up to 100 years. Total P_o in the fertilized soil remained unchanged (Zhang and MacKenzie, 1997b) for short-term cultivation. On the other hand, Agbenin and Goladi (1998) found the fractions of P_o and residual P, predominated by Po, decreased in the P fertilized soil. The change in P_o pools depends on the rate of mineralization (Tiecher et al., 2018), which is governed by complex mineralogical, chemical, and biological processes. A path analysis indicated that the role of P_o pools was more important than P_i pools for soil P transformation, and labile P_o acted as a transitory pool rather than as a sink or source of P in a Labarre silty clay (Zheng et al., 2002) and in a very clayey Oxisol under non-tillage (Tiecher et al., 2018). Soil P dynamics in fertilized soils can be form-interactive and temporally cumulative. There is information shortage on the long-term effect of fertilizer P addition on soil P, which impairs management practices being developed in a crop production that is profitable and at the same time in an environmental-friendly manner. The objective of this study was to assess the P status and P transformation in a Brookston clay loam after 45 years of contrasting (monoculture corn vs. crop rotations) cropping systems with and without P fertilization, relative to the native forest soil.

MATERIALS AND METHODS

Experiment Design and Plot Management

An experiment was initiated in 1959 at Eugene Whelan Research farm, Woodslee, Ontario, Canada (42°13′ N latitude, 82°44′ W longitude). The mean annual air temperature is 8.7°C, and the mean annual precipitation is 876 mm. The soil was classified as Brookston clay loam (Typic Argiaquoll), consisting of 28% sand, 35% silt, 37% clay, 619 mg kg^{-1} Al (Mehlich-3 extractable), 310 mg kg^{-1} Fe (Mehlich-3 extractable) (Wang et al., 2012), and 2.3% organic carbon in the Ap horizon.

Treatments consisted of combinations of three cropping systems and two regimes of fertilization. Cropping systems included conventionally tilled continuous corn (*Zea mays* L., CC), corn-oats (*Avena sativa* L.)–1st alfalfa (*Medicago sativa* L.)–2nd alfalfa rotation (CR), and continuous Kentucky bluegrass sod (*Poa pratensis* L., CB). Each phase of the corn-oats-alfalfa-alfalfa rotation was presented in each year. However, only the corn phase of the rotation, CC and CB, was selected for this study. The plot size was 76.2 × 12.2 m each. Conventional tillage consisted of a moldboard plowing to 0.18-m depth in the fall and a disking and harrowing in the spring just prior to planting. Conventional tillage was conducted every year for CC plots and in 2 of 4 years for the rotation treatments (i.e., following the second year alfalfa and following the rotation corn harvest). The CB plots were not tilled. Corn was planted at 55,000 seeds ha^{-1} with 1.0-m row spacing. All fertilized plots received 8-32-16, which provided 16.8 kg N ha^{-1} as ammonium nitrate (NH_4NO_3), 67.2 kg P_2O_5 ha^{-1} as triple superphosphate, and

36.2 kg K_2O ha^{-1} as potassium chloride (KCl) prior to planting each year. In addition, corn also received 112 kg N ha^{-1} of side-dressed NH_4NO_3, applied in bands 15 cm on either side of the row (2- to 5-cm depth) when corn was at six-leaf stage (usually in early–mid June). Herbicides were applied at regionally recommended rates for weed control in corn and oat production.

Corn yields were measured annually by harvesting 33-m lengths of 10 individual rows. Grain moisture content was determined on corn grain subsamples. Alfalfa was cut and baled two to three times a year and removed from the field. Cereal and forage biomasses were estimated, and plant samples collected using the sampling square technique. The plant P removal was the product of dry matter and P concentration in all harvested plant tissues. The harvest index of 0.5 for corn was used to estimate shoot biomass (Bolinder et al., 2007). The shoot–root ratios of 5, 3, 2, and 2 were used to calculate the root and stubble biomass of corn, oats, alfalfa, and bluegrass sod, respectively (Bolinder et al., 1997, 2002). The national average P concentrations were used for the harvested tissues of corn, oats, alfalfa, and sod, and P concentrations in root and stubble biomass were estimated as 0.5% of their concentrations in harvested plant tissues (National Research Council, 1982). The P budget after 45-year cropping practices is shown in **Table 1**. The soil at adjacent woodlot (native forest) was considered to be native soil. The vegetation species for the woodlot are native grasses and deciduous trees. Not only was the woodlot not fertilized, but it was also not tile drained, whereas all of the field plots were systematically tile drained.

Soil Sampling and P Fractionation

Soil samples were taken from 0- to 20-cm top layer in May 2003, using a 3.2-cm-diameter auger. Soil samples were air-dried, ground, and passed through a 2-mm sieve. Soil subsamples were further ground and passed through a 100-mesh sieve.

Soil P was fractioned using a modified Hedley (Hedley et al., 1982) sequential extraction procedure (Zhang et al., 2004). In brief, 0.5-g soil was extractable sequentially with 30 mL of deionized water, 0.5 *M* $NaHCO_3$, the first 0.1 *M* NaOH, 1.0 *M* HCl, and the second 0.1 *M* NaOH by shaking the suspension for 16 h, centrifuging for 10 min at 16,000*g*, and passing through a 0.45-μm Millipore membrane filter (mixed cellulose ester). An aliquot (10 mL) of the $NaHCO_3$ and NaOH extractants was acidified to precipitate extractable organic matter, and the supernatant was analyzed for P_i. The 2nd NaOH extraction used in this study enabled the extraction of P (either P_i or P_o), which is held more strongly at internal surface of soil aggregates (Hedley et al., 1982; Zhang and MacKenzie, 1997b). Another aliquot (10 mL) of the deionized water, $NaHCO_3$, and NaOH extracts was digested in an autoclave (103.4 kPa, 121°C for 1 h) with 10 mL of 9 *M* H_2SO_4 and ammonium persulfate $[(NH_4)_2S_2O_8]$ (0.5 g) and analyzed for total P (P_t). The difference between P_t and P_i was considered as P_o (Tiessen and Moir, 1993). Residual P in soil after sequential extraction was determined using the digestion method with concentrated H_2SO_4 and H_2O_2. Phosphorus concentrations were determined colorimetrically using a flow injection autoanalyzer (Quikchem FLA 8000 series; Lachat Instruments, Loveland, CO, United States) with the molybdate–ascorbic acid procedure (Murphy and Riley, 1962). Consequently, soil P was separated into water-extractable Pi and Po (water-P_i and P_o), $NaHCO_3$ extractable P_i and P_o ($NaHCO_3$-P_i and -P_o), NaOH extractable P_i and P_o (sum of the first and the second NaOH extractions), acid P (HCl-P), and residue P (Res-P). Both water- and $NaHCO_3$-P were considered as labile P. The NaOH-P was considered as moderately labile P. The HCl-P was referred as moderately stable P. Res-P was considered to be stable P. Soil P was expressed in mg P kg^{-1} soil. Total extractable P_i or P_o was the sum of P_i or P_o extractable by water, $NaHCO_3$, NaOH, and HCl reagents. Total P was measured directly by digesting soil subsamples with H_2SO_4 and H_2O_2, with P concentrations determined using the same approach as for other samples described above. The differences between the total P and the sum of P factions were within ± 3%.

Statistical Analysis

The statistical analysis of the cropping treatments and the native soil was performed separately for the fertilized and non-fertilized plots using the least significant difference (LSD) *t* test at a significance level of $P \leq 0.05$ (SAS Institute, 1999 Cary, NC, United States, 1999).

RESULTS AND DISCUSSION

Phosphorus Budget

The P budget was calculated as the difference between the cumulative fertilizer P inputs and the cumulative P removal by plants using the available data from the establishment of these plots. The P deficits were expectedly observed after 45-year consistent cropping without P fertilization in both CC and CR plots, whereas no changes were anticipated in the CB plots as the grasses were not removed from the plots (**Table 1**). However, there was a great P surplus in the fertilized CB plots, which was attributed to no P removal, as the grasses were only cut and not removed in addition to the annual inputs of fertilizer P. This treatment simulated a grass buffer strip. A slight P deficit was found in the fertilized CC and CR plots, suggesting an apparent P balance in the two corn plots after 45 years of long-term cropping with annual fertilization.

Total Soil P

Total P content in native soil was 667 mg kg^{-1} (**Figure 1**). The P_o extractable with 0.1 *M* NaOH (NaOH-1-P_o + NaOH-2-P_o) was the dominant P fraction, accounting for 44% of total P, followed by HCl-P 26%. Labile P (water-P_i and -P_o + $NaHCO_3$-P_i and -P_o) and Res-P accounted for 20% and 16%, respectively. The high proportion of NaOH-P_o may have been due to two extractions by 0.1 *M* NaOH in our study. The small proportion of labile P in our Brookston clay loam was comparable to the native forest Brown Chernozemic at 10% labile P (Schoenau et al., 1989) and permanent Black Chernozemic pasture soil at 12% labile P (Hedley et al., 1982). The content of HCl-P in our native Brookston clay loam was higher than those in native Gleysolic and Gray Luvisolic soils (Schoenau et al., 1989) and lower than those in Chernozemic soils (Hedley et al., 1982;

TABLE 1 | Phosphorus budget after 45-year contrasting cropping practices in a Brookston clay loam at Woodslee, ON, Canada.

Cropping system	Cumulative P input	Cumulative P uptake[†]	Cumulative P input in crop residuals[‡]	Cumulative P removal	Soil P budget[£]
	kg P ka^{-1}				
			Fertilized		
CC	1,291	1,486a[£]	71c	1,415a	–124b
CR	1,291	1,497a	120b	1,377a	–86c
CB	1,291	928b	928a	0b	1291a
			Non-fertilized		
CC	–	368a	18b	350a	–350a
CR	–	345a	21b	324a	–324a
CB	–	297b	297a	0b	0b

[†] *Cumulative P uptake included P contained in both above ground and below ground (i.e., roots) of crop tissues.* [‡] *Cumulative P input in crop residuals is referred to the crop P remained in soil after harvests, including crop stubbles and roots.* [£] *Soil P budget = cumulative P input - cumulative P removal.* [£] *LSD test significance level at $P \leq 0.05$. CC, continuous corn; CB, continuous bluegrass sod; CR, corn-oats-alfalfa-alfalfa rotation.*

Schoenau et al., 1989). The HCl solution extracts the relatively insoluble apatite-type materials, and HCl-P may show the magnitude of soil weathering. Guo et al. (2000) found higher HCl-P in slightly weathered soil than highly weathered soil. The content of HCl-P in our native Brookston clay loam was much lower than the low weathered Humic Cryaquept in Quebec in which the phyllosilicate mineralogy of clay fraction is dominated by vermiculite, hydrous mica, and feldspars (Zheng et al., 2003).

Continuous cropping without P fertilization significantly decreased total soil P content relative to the native soil after 45-2 cropping practices, ranging from an 18% to 30% decrease or 122 to 202 mg P kg^{-1} soil (**Figure 1**). The result was coincident with studies reported by Crews and Brookes (2014) that significant soil P depletion occurred over time from 1893 to 2009 with Park Grass perennial hay meadow, by Hedley et al. (1982) that total P in soils after 60 to 70 years cultivation was lower than that of adjacent permanent pasture, and by Bowman et al. (1990) that large declines of P (41%) occurred after 60 years of cultivation. The sequence of total P reduction among our cropping systems followed the following order: CC > CR > CB. The reduction of total P by cultivation was mainly due to P removal by plant (Hedley et al., 1982; Schoenau et al., 1989). Leaving the grass in place (i.e., the grass was consistently cut but not removed) in CB compared to crop removal in CC or CR plots would have reduced the total P loss from soils (**Table 1**). These results are consistent to the previous study that showed the larger P accumulation in grass field was mainly due to more roots of perennial species compared to annual crops (Zheng et al., 2003). In addition, total P reduction in the cultivated plots relative to the native soil would have also been attributed to soil P losses in tile drainage. An annual P loss of 1.5 kg P ha^{-1} was observed in tile drainage water (Zhang et al., 2015), which contributes to greater than 70% of soil P loss in the study region (Tan and Zhang, 2011; Zhang et al., 2013).

Application of fertilizer P resulted in a comparable total P in CC and CR plots, but significantly increased total P by 31% in CB relative to native soil (**Figure 1**). This was in agreement with the calculated P budget (**Table 1**). The P accumulation in soil with long-term application of fertilizer P has been reported by Mckenzie et al. (1992), Zhang et al. (1995), and Hao et al. (2018). The continuous application of fertilizer P, coupled with no crop removal in the CB plots, contributed to the increase of total P (**Table 1** and **Figure 1**). The large increase in total P of grassland soils may increase the risk of P loss in runoff, leaching, and preferential flow (Zhang et al., 2015, 2017).

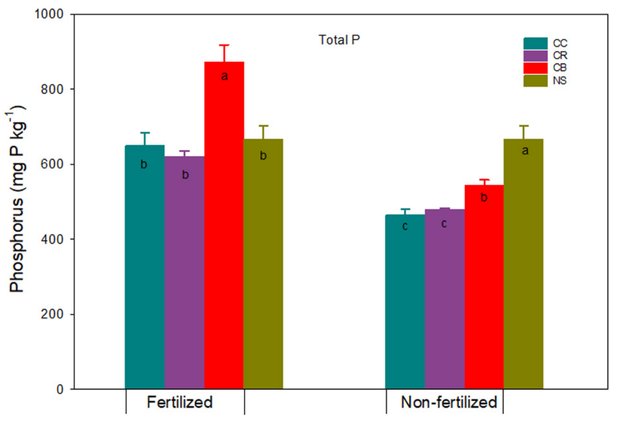

FIGURE 1 | Total P and standard errors ($n = 3$) (mg kg^{-1}) in a Brookston clay loam at Woodslee, ON, Canada, after 45-year consistent cropping with and without P fertilization relative to its native forest soil (NS). CC, continuous corn; CB, continuous bluegrass sod; CR, corn-oats-alfalfa-alfalfa rotation. Letters in the bars are significant levels (at $P \leq 0.05$) with LSD test conducted separately for the fertilized and non-fertilized treatments.

Inorganic P

Consistent cropping without P fertilization significantly decreased all fractions of P_i, compared with native soil, by an average of 6, 24, 31, and 21 mg P kg^{-1} for water-P_i, NaHCO$_3$-P_i, NaOH-P_i, and HCl-P, respectively (**Figure 2**). This resulted in a decrease in total extractable P_i by an average of 82 mg P kg^{-1} relative to native soil (**Figure 3**). Labile P_i (water-P_i + NaHCO$_3$-P_i) significantly decreased by 57 to 67% relative to native soil, which was greater than the 45% decrease reported by Hedley et al. (1982) for a soil after 65 years of cultivation. This decline was attributed to plant uptake of readily available P in the top layers of soil, as evidenced by the larger P removal relative to P input in crop residues including root and stubble biomass

(**Table 1**). Although NaOH-P_i and HCl-P in the non-fertilizer plots remained unchanged over a 5-year period (Zhang and MacKenzie, 1997a), this study showed a significant reduction in the two P pools in soil after 45-year cropping (**Figure 2**), suggesting moderately labile Pi was desorbed and the moderately stable P_i dissolved to replenish readily labile P_i pool in long-term cultivated soils. This transformation was previously reported by Crews and Brookes (2014) and (Sheklabadi et al., 2015). Few recent studies further concluded that the decline in HCl-P is caused by the conversion from HCl-P to readily available P_i (von Sperber et al., 2017; Tiecher et al., 2018).

The decrease in labile $NaHCO_3$-P_i and moderately labile NaOH-P_i in CR and CB plots without P fertilization was significantly larger than in CC plots (**Figure 2**). The larger decrease of both P pools in CR plots could be explained by more P export of crop removal and resultant P desorption. The mean yield over 45 years in non-fertilized CR plots (3.84 ± 0.21 t ha^{-1} $year^{-1}$) was significantly greater than in CC plots (1.24 ± 0.09 t ha^{-1} $year^{-1}$). This suggests that there was more available-P removal by crops in the CR treatment (**Table 1**), resulting in transformation of moderately labile P_i to replenish the lower labile P_i. The readily available P appeared to be in equilibrium with moderately labile NaOH-P_i (Guo et al., 2000; Zhang et al., 2004). The larger decrease in both P fractions in CB plots than in CC plots could be attributed to the stronger immobilization of P_i to P_o, as was evidenced by the CB treatment having the highest content of P_o among the three cropping systems (**Figure 5**). The immobilization of P_i to P_o in grassland soil was reported previously by Herlihy and McGrath (2007) and Saltali et al. (2007). The HCl-P pool among the three cropping systems was not significantly different (**Figure 2**).

In P fertilized treatments, the concentrations of all P_i fractions increased except for water-P_i in the CC and CR plots and HCl-P in the CR plots, compared to the native soil (**Figure 2**). The increases of P_i were, on average, 13, 50, 65, and 34 mg P kg^{-1} for water-P_i, $NaHCO_3$-P_i, NaOH-P_i, and HCl-P, respectively, and the resultant total extractable P_i significantly increased with added fertilizer P, ranging from 63 to 237 mg P kg^{-1}, as compared to the adjacent native forest soil (**Figure 3**). The addition of fertilizer P significantly elevated, as expected, the concentration of labile P_i fractions (Wagar et al., 1986; Mckenzie et al., 1992; Zhang and MacKenzie, 1997a). The observations from this study indicated that the addition of fertilizer P encouraged the formation of NaOH-P_i, reinforcing the conjecture that the NaOH-P_i acted as a sink of added P_i under long-term fertilization (Zhang and MacKenzie, 1997b; Zheng et al., 2002; Zhang et al., 2004). Conversely, NaOH-P_i acted as a buffer for the available P in the non-fertilized soils (Zhang and MacKenzie, 1997a; Guo et al., 2000) or in soils that are running low in bioavailable P supply (Zhang et al., 2004). Clearly, the long-term response of P pools was controlled mainly by the dynamics of NaOH-P_i (von Sperber et al., 2017).

The increase in total extractable P_i among cropping systems followed the sequence CB > CC > CR with increases of 237, 173, and 63 mg P kg^{-1}, respectively (**Figure 3**). The lower total extractable P_i in the fertilized CC and CR plots than in the fertilized CB plots was attributed to the higher biomass yields and the greater removal of available P from soil (**Table 1**), whereas there was no P removal in the CB plots as they were not harvested. A small increase in total labile P_i (**Figure 3**) and moderately labile P_i (**Figure 2**) in CR did not yet result in the concurrent increase of HCl-P (**Figure 2**), suggesting the transformation from labile and moderately labile P_i pools to relative stable P pool was restricted in the rotation system. However, the concurrent increases of labile P_i, NaOH-P_i, and HCl-P in CB and CC plots relative to native forest soil indicated the greater transformation from labile and moderately labile P_i to HCl-P in these two treatments. This observation confirms the conclusion that P is transformed from readily available P forms to more resistant forms with the consecutive P fertilizer addition in monoculture systems (Zheng et al., 2002). Water-P_i and the resultant total water-P in CB plots were 44 and 46 mg P kg^{-1}, which were significantly greater than in the other plots. It was speculated that the increase in water-P, especially water-P_i, might have resulted from the increase in the degree of soil P saturation, as evidenced by the increase in $NaHCO_3$-P_i, a form of P that is predominately sorbed physically or physicochemically on the soil surface. Water-extractable P has been deemed a soil test indicative of environmental risk of soil P loss (Wang et al., 2010, 2012). Recent studies have found that concentrations of dissolved, bioavailable, and particulate P in runoff increased linearly as water-extractable P increases (Schroeder et al., 2004; Wang et al., 2010, 2012). The high water-P_i in CB plots could result in great potential P losses by runoff and leaching (Zhang et al., 2015).

Organic P

Consistent cropping without fertilization significantly decreased extractable P_o fractions except for water-P_o, which was significantly increased compared to native soil (**Figure 4**). The NaOH-P_o and $NaHCO_3$-P_o decreased, on average, across the three cropping systems, by 58 and 15 mg P kg^{-1}, respectively. Although the proportion of increase was large, the increment of water-P_o averaged approximately only 1.1 mg kg^{-1}. Averaged across cropping systems, the decrease of NaOH-P_o accounted for 43% and 84% of total P and total extractable P_o reductions, respectively (**Figures 1**, **5**). This proportion of decrease was consistent with those reported by Hedley et al. (1982), 68% of total P_o loss after 65-years cultivation was attributed to the NaOH-P_o decrease. This was confirmed by the result from Zhang and MacKenzie (1997a), who found NaOH-P_o decreased by 46 kg P ha^{-1} in the non-fertilized plots. These studies suggest that NaOH-P_o plays an important role as sink or source in P transformation in soils. For cropping systems, the sequence of total extractable P_o decrease was CC > CR > CB (**Figure 5**). The CC and CR cropping practices decreased not only easily mineralized P_o ($NaHCO_3$-P_o), but also moderately labile NaOH-P_o, especially for the portion of extractable P after HCl extraction (NaOH-2-P_o) (Zhang and MacKenzie, 1997a). The greater decreases in NaOH-P_o in CC and CR plots than in CB plots, compared with native soil (**Figure 5**), indicate that the rate of P_o mineralization was enhanced by agronomic practices such as tillage. Tiecher et al. (2018) assessed linkage between soil P forms in contrasting tillage systems using path analysis and concluded that the organic P pool has a greater direct

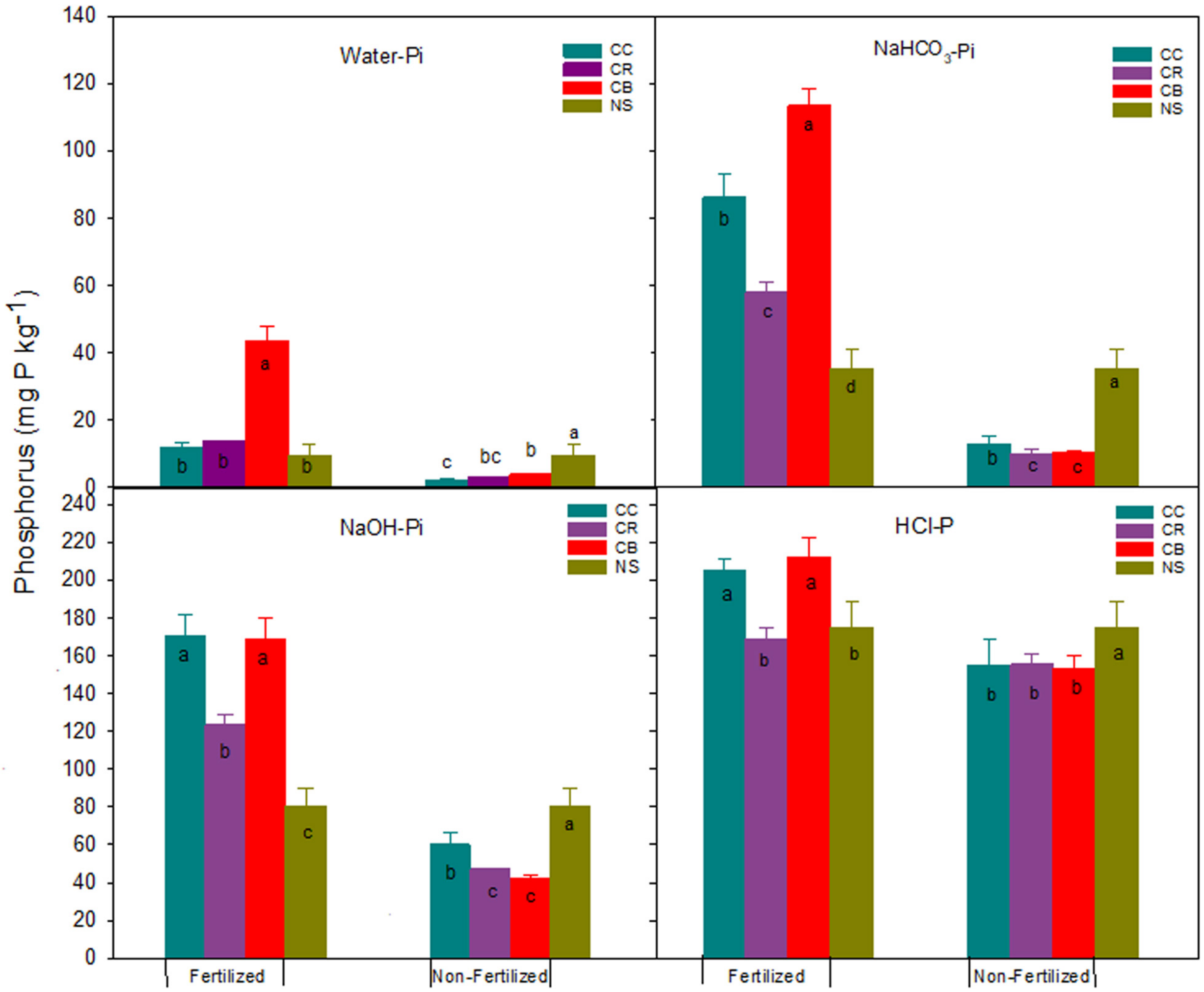

FIGURE 2 | Water-P_i, $NaHCO_3$-P_i, NaOH-P_i, HCl-P, and standard errors ($n = 3$) (mg kg^{-1}) in a Brookston clay loam at Woodslee, ON, Canada, after 45-year consistent cropping with and without P fertilization relative to its native forest soil (NS). CC, continuous corn; CB, continuous bluegrass sod; CR, corn-oats-alfalfa-alfalfa rotation. Letters in or above the bars are significant levels (at $P \leq 0.05$) with LSD test conducted separately for the fertilized and non-fertilized treatments.

contribution to buffer resin-P under conventional tillage (94%) than under non-tillage (35%), due to higher mineralization of organic P forms with moderate lability caused by soil disturbance. A small decrease in total extractable P_o in CB plots might have partially been due to the tradeoff of more P_o being returned to soil in grass residues including shoots, as well as the massive roots (**Table 1** and **Figure 5**).

In P fertilized plots, a significant decrease in all P_o fractions was found in CC and CR plots, except for water-P_o in CC and $NaHCO_3$-P_o in CR plots where they remained unchanged, relative to the native forest soil (**Figure 4**). This resulted in a significant decrease in the total extractable P_o by 129 and 85 mg P kg^{-1} in CC and CR plots, respectively, as compared to the native soil (**Figure 5**). Res-P also decreased by 32 and 18 mg kg^{-1} in CC and CR plots (data not shown), respectively. Data obtained by Haas et al. (1961) for the soils from 15 dry-land experiment stations in the US Great Plains showed that total P was reduced by an average of 8% by cropping over 30 to 48 years, with most of the loss being P_o. In contrast, with increases in NaOH-Po (**Figure 4**), total extractable P_o in CB plots increased by 10 mg P kg^{-1} relative to native soil, although labile P_o, sum of water-P_o and $NaHCO_3$-P_o, decreased slightly by 8 mg P kg^{-1} (**Figure 5**), due to the strong immobilization process in grassland (Herlihy and McGrath, 2007; Saltali et al., 2007).

In general, either soil labile P_o or total extractable P_o in the fertilized CC and CR treatments decreased, compared to the native soil (**Figure 5**). While the total labile P_o decreased slightly, averaged at 8 mg P kg^{-1} across three cropping systems, the predominant P_o loss was observed in the form of NaOH-P_o, with an average of 60 mg kg^{-1} across CC and CR. This confirms the previous observations reported by Zhang and MacKenzie (1997a). Tran and N'dayegamiye (1995) suggested the decrease of NaOH-P_o in P fertilized treatments was probably due to the

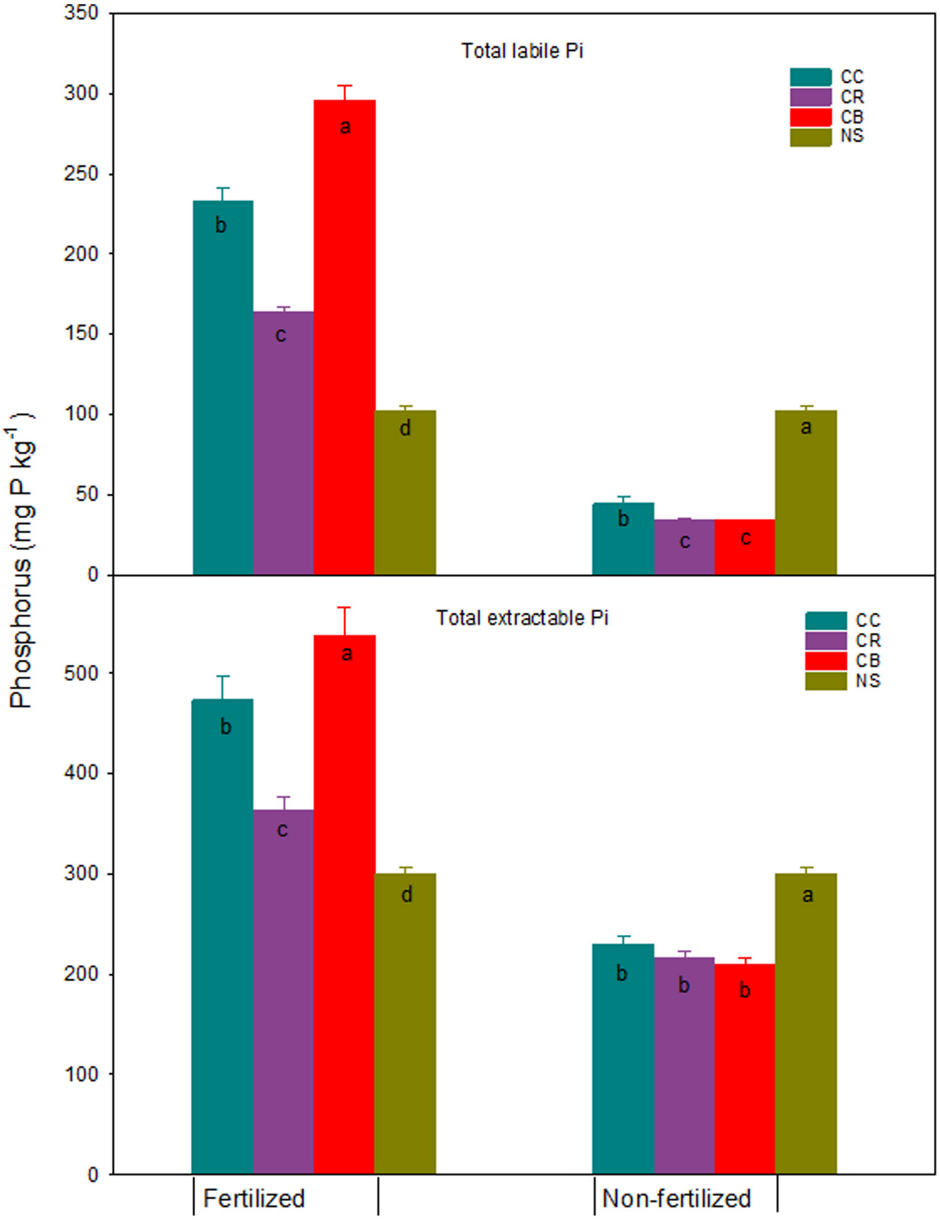

FIGURE 3 | Total extractable P_i and labile P_i and standard errors (n = 3) (mg kg^{-1}) in a Brookston clay loam at Woodslee, ON, Canada, after 45-year consistent cropping with and without P fertilization relative to its native forest soil (NS). CC, continuous corn; CB, continuous bluegrass sod; CR, corn-oats-alfalfa-alfalfa rotation. Letters in the bars are significant levels (at $P \leq 0.05$) with LSD test conducted separately for the fertilized and non-fertilized treatments.

synergetic effect of N and P on soil P_o mineralization. Significant decreases in total extractable P_o in the fertilized CC and CR plots imply that fertilizer application alone cannot remedy P_o loss by cropping practices, apart from returning more plant residues.

CONCLUSION

The significant decrease of total P and all P fractions by cropping practices was found in the non-fertilized soil relative to the native forest soil except for water-extractable P_o. The labile P_i decrease accounted for the most proportion of total extractable P_i decrease. The moderately labile NaOH-P_o was the dominant pool accounting for the loss of P_o and total P in the non-fertilized soils. The addition of fertilizer P significantly increased all P_i pools under the three cropping systems, except for water-P_i with CC and CR and HCl-P with CR where no changes were observed. The NaOH-P_i largely increased after 45 years in the fertilized soils, whereas it decreased in the non-fertilized soils. This study revealed that long-term cropping practices significantly enhanced the rate of P_o mineralization regardless of whether they were fertilized. Further, NaOH-P_i and -P_o played important roles by

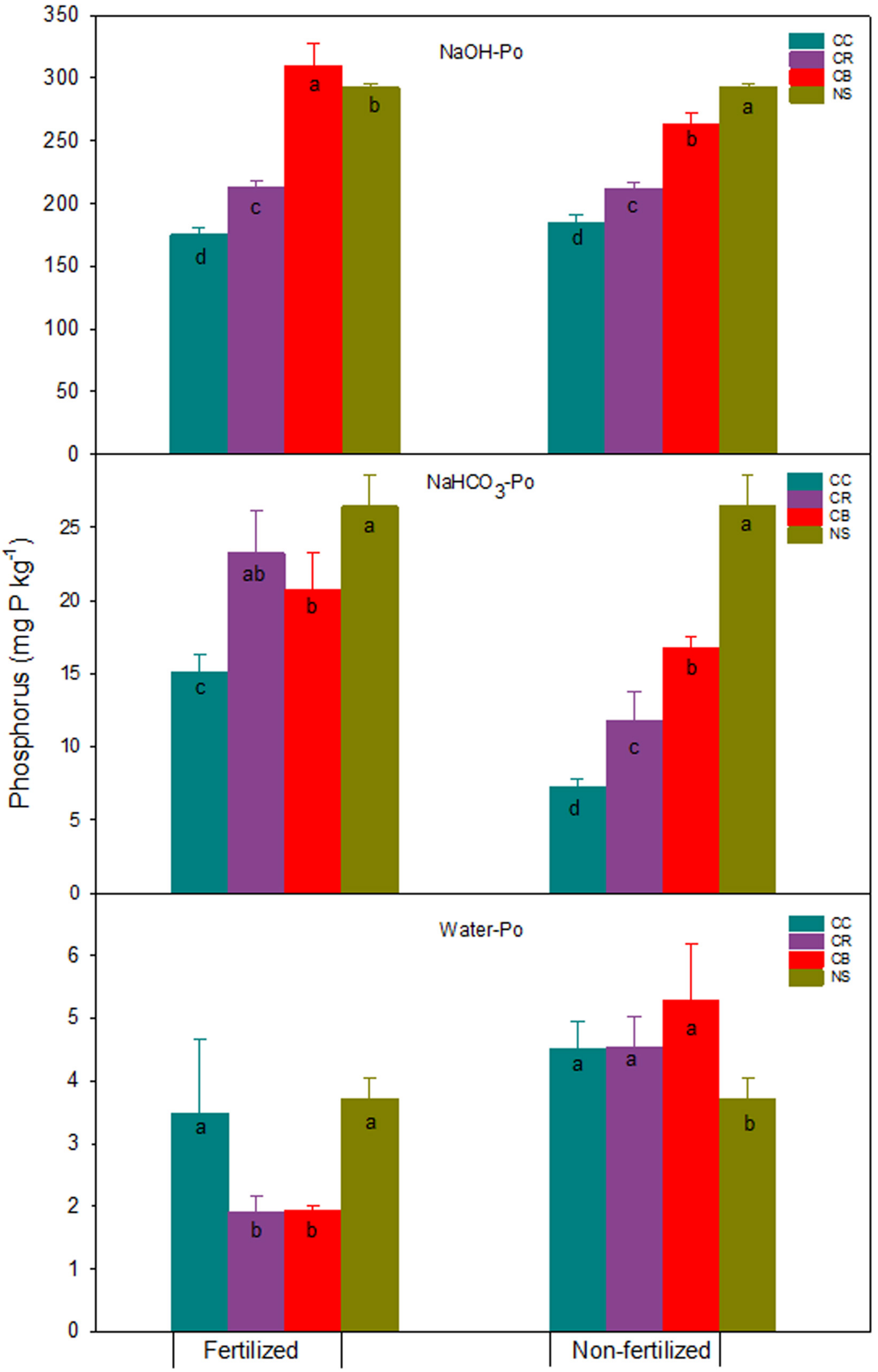

FIGURE 4 | Water-P$_o$, NaHCO$_3$-P$_o$, NaOH-P$_o$, and standard errors (n = 3) (mg kg^{-1}) in a Brookston clay loam at Woodslee, ON, Canada, after 45-year consistent cropping with and without P fertilization relative to its native forest soil (NS). CC, continuous corn; CB, continuous bluegrass sod; CR, corn-oats-alfalfa-alfalfa rotation. Letters in the bars are significant levels (at $P \leq 0.05$) with LSD test conducted separately for the fertilized and non-fertilized treatments.

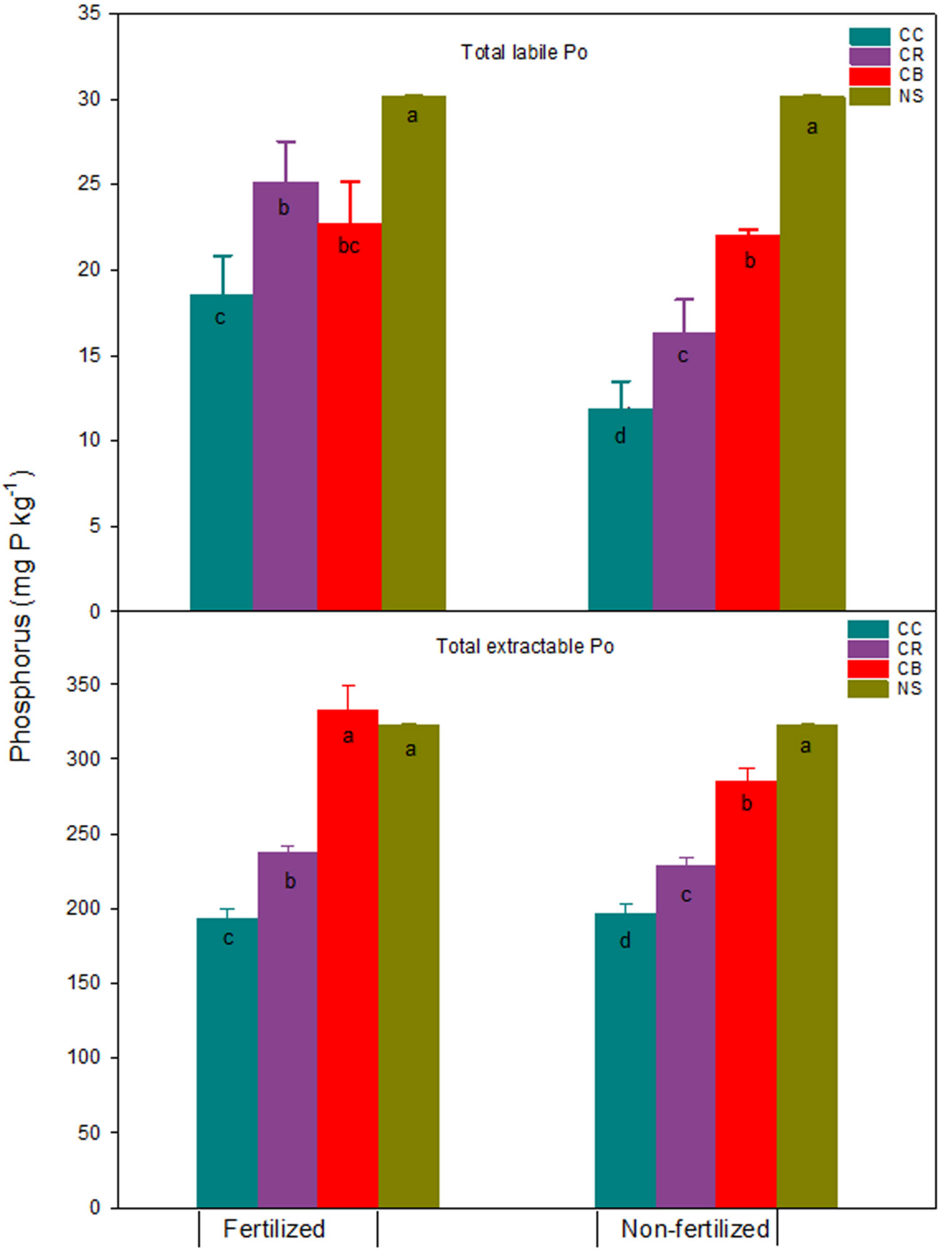

FIGURE 5 | Total extractable P_o and labile P_o and standard errors (*n* = 3) (mg kg^{-1}) in a Brookston clay loam at Woodslee, ON, Canada, after 45-year consistent cropping with and without P fertilization relative to its native forest soil (NS). CC, continuous corn; CB, continuous bluegrass sod; CR, corn-oats-alfalfa-alfalfa rotation. Letters in the bars are significant levels (at $P \leq 0.05$) with LSD test conducted separately for the fertilized and non-fertilized treatments.

acting as a sink or source of P in this soil. The return of grass residues in the CB treatment resulted in the increases in total P and total P_o in the fertilized plots relative to the native soil, suggesting P accumulation in grass fields is a concern as a risk of P pollution to adjacent fresh waters.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are available upon request.

AUTHOR CONTRIBUTIONS

TZ proposed and facilitated the study. CD and few previous scientists in Harrow Research and Development Center (formerly Harrow Research Center or Greenhouse and Processing Crops Research Center), AAFC, maintained the long-term plots and provided the yield data. TZ, ZZ, QH, CD, and CT contributed to the preparation of the manuscript. All authors contributed to the manuscript and approved the submitted version.

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Modelling Changes in Soil Phosphorus When Phosphorus Fertiliser Is Reduced or Ceases

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In order for land managers and policy makers to manage excessive soil phosphorus (P) concentrations and reduce the risk of this particular source of P from impacting water bodies, models of soil P decline under various scenarios are needed. We modelled the decrease in calcium chloride-extractable P ($CaCl_2$-P), and sodium bicarbonate-extractable P (Olsen-P and Colwell-P) using data from six Australian grazed pasture soils with contrasting P sorption properties, over a period of 4.5 years. Each soil had four initial soil P concentrations (P_{init}), each of which received four on-going rates of P fertiliser (P_{fert}). The model predicts the final P concentration (P_{final}) by taking into account the P concentration previously measured ($CaCl_2$-P, Olsen-P or Colwell-P), P_{fert} applied since measurement, and time since previous measurement: Final P concentration = (previously measured P concentration + e_p x P fertiliser applied) exp (-d_p x years since previous P concentration measurement). Where e_p is the increase in soil P for each unit of applied P and d_p is the decay constant representing how quickly the soil P decreased. The greatest decreases in proportion to P_{init} occurred for $CaCl_2$-P, followed by Olsen-P, and then Colwell-P. The model tended to fit the dataset well for Olsen-P and Colwell-P, with mean overestimation (modelled P_{final} concentration greater than actual P_{final}) of the P_{final} concentrations of 6.1 (32%) and 4.3 mg/kg (10%), respectively. Although there was less $CaCl_2$-P data, the model successfully described it, with a mean overestimation of P_{final} $CaCl_2$ of 3.1 mg/kg (26%). The overestimation of P_{final} $CaCl_2$ was possibly due to the high $CaCl_2$-P concentrations of the low P buffering index soils. The model predicted an average of 32 years (ranging from 26 to 49 years) for Olsen-P concentrations of between 55 and 96 mg/kg to decrease to an agronomic optimum of 17 mg/kg. Agronomic optimum was not a reliable indicator of environmental risk as some soils did not exceed the $CaCl_2$-P environmental threshold until Olsen-P concentrations were twice the agronomic optimum, whereas low P sorbing soils tended to exceed the threshold before reaching agronomic optimum. Further work with more soils is required to examine the influence of soil properties – such as P sorption – on decreases in soil P.

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INTRODUCTION

Excessive soil phosphorus (P) concentrations are a concern in parts of Europe, the United States of America and Australia (Paulter and Sims, 2000; Sharpley et al., 2001; Gourley et al., 2015). Soils with more P than is needed for agronomic purposes can result in unnecessarily high concentrations of P in runoff (Sharpley and Rekolainen, 1997; Burkitt et al., 2010) which can, in addition to other landscape and land management factors, contribute to eutrophication of waterways (Carpenter et al., 1998). These excessive soil P concentrations can be the result of P inputs (fertiliser, livestock feeds and manures) exceeding P removal in produce. Some land managers still apply P fertiliser when soil P concentrations are above agronomic optimum, despite being aware of the environmental risks, especially in intensive pasture industries (Nicon Rural Services, 2010). This practice is used as a form of risk management to ensure that soil P does not suddenly decrease below the agronomic optimum and result in a subsequent decrease in pasture production and consequent economic loss. However, this approach results in inefficient use of P fertiliser, a finite resource which is in increasing world demand (Smil, 2000; Condron et al., 2005).

In response to increasing P concentrations in some water resources, policies have been implemented in some countries to prevent further increases in soil P concentrations that are already above agronomic optimum (European Parliament, 2000). Models predicting increases in plant available P concentrations are well established, however, few models predict declines. In order for land managers and policy makers to better understand how to manage soil P so as to decrease excessive concentrations without risking production losses, models of the changes in soil P – particularly decreases – under various scenarios are needed.

A number of researchers have described an exponential decrease in extractable soil P after P fertiliser application has ceased (McCollum, 1991; Paris et al., 2004; van der Salm et al., 2009). A number of mechanistic (Russell, 1977) and descriptive (Cox et al., 1981; Schulte et al., 2010; Dodd et al., 2012) models have been devised to model soil P decline. Schulte et al. (2010) and Dodd et al. (2012) developed models across a range of initial P (P_{init}) concentrations and soils, with Schulte et al. (2010) also presenting a number of negative P-balance scenarios. However, these models did not include the influence of different on-going P fertiliser (P_{fert}) rates on decreases in extractable soil P, a practice which commonly occurs for intensive pasture soils and agricultural enterprises more generally. Coad et al. (2014) described changes in extractable soil P in a range of field soils which each had a wide range of initial soil P concentrations and on-going fertiliser P rates.

In this current paper, we use a Bayesian statistical framework to describe the changes in environmental P ($CaCl_2$-P) (Houba et al., 2000) and agronomic P (Olsen-P and Colwell-P) (Olsen et al., 1954; Colwell, 1963) using datasets from the field study of Coad et al. (2014). We use a Bayesian approach to allow for uncertainty in measurement and parameters to be incorporated in a natural way through the appropriate prior specification. Bayesian methods also allow complex models to be more easily constructed (Brooks, 2003). Our aim was to develop a model to predict the final extractable soil P concentration (P_{final}) after a specified time period for various soil and P fertiliser scenarios. This model could be used by policy makers and land managers to assist their decision making and to set realistic timeframes to return soils that have excessive soil P to agronomic optimum concentrations and/or concentrations that pose low environmental risk.

MATERIALS AND METHODS

Site Management and Soil Characterisation

The materials and methods employed to obtain the dataset used to develop the Bayesian model are published in Coad et al. (2014). Briefly, six field sites were selected that had a wide range of P sorption capacities (**Table 1**). Subsequently a wide range of P rates were applied as a single initial application to generate varying initial soil P concentrations. Then P was applied every 6 months at rates of zero, 0.5, 1, and 2 times maintenance rates for up to 4.5 years. The experimental design was fully factorial with 3 replicates at each site. The sites were sampled regularly for up to 4.5 years. Agronomic (Colwell and Olsen) measures of P and an environmental indicator (0.01 M $CaCl_{2-}P$) of P status were measured on soil samples. We also measured both unadjusted P buffering index (PBI) (Burkitt et al., 2008) – commonly used in Australia – and the amount of P sorbed after addition of 1000 mg P/kg (Ps_{1000}) for the initial and final soil samples for all treatments. Both PBI and Ps_{1000} were undertaken using the PBI method (Burkitt et al., 2008; Rayment and Lyons, 2011). Briefly, 1 g of soil was shaken for 17 h in 0.01 *M* $CaCl_2$ with an addition of 1000 mg P/kg soil (Burkitt et al., 2008). The difference between the added P and the final P concentration in the equilibrating solution was the amount of P sorbed (Ps_{1000}), with PBI calculated as P sorbed (mg/kg)/[final P solution $(mg/L)^{0.41}$]. Phosphorus sorption capacity (PSC) was calculated for each soil according to Borling et al. (2001; **Table 1**).

Bayesian Statistics

Bayesian inference is the process of fitting a probability model to a set of data and summarising the results via probability distributions on the parameters of the model. In a Bayesian model, the parameters are considered to be uncertain and are therefore represented by probability distributions, known as prior distributions, or priors. These are combined with the model likelihood to obtain posterior distributions that provide their most probable values given the data and modelling assumptions (Berger, 1993). The posterior distributions are summarised using summary statistics. Using a Bayesian framework allows the inclusion of uncertainty in parameters and models, the probabilistic representation of the model outcomes, as well as conveniently constructing very complex models.

Model Construction

We had previously observed that the greater the P concentration, the greater the rate of decline in soil P (Coad et al., 2014).

TABLE 1 | Selected chemical and physical properties of the surface 10 cm of pasture soils sampled from the six field sites prior to P fertiliser treatment.

	Soil					
Characteristic	**1**	**2**	**3**	**4**	**5**	**6**
Soil Texture^A	**Silty loam**	**Loam**	**Sand**	**Sandy loam**	**Silty clay loam**	**Sandy loam**
Clay^B (%)	23	20	6	11	18	14
Sand^C (%)	47	58	90	70	51	77
Silt^D (%)	30	22	4	19	31	9
pH ($CaCl_2$)	5.4	4.8	3.9	4.9	4.8	4.6
Olsen-P (mg/kg)	12	12	16	18	18	37
Colwell-P (mg/kg)	40	39	30	43	69	72
Organic C (g/kg)	95	28	114	26	60	87
CEC (meq/100 g)	21	9	19	5	24	13
Oxalate-extr. Al (mg/kg)	5900	2600	300	700	5200	800
Oxalate-extr. Fe (mg/kg)	4700	4000	800	2300	12300	700
PBI^E	480	220	<10	60	400	60
PSC^F(mmol/kg)	151	84	13	34	206	21
Soil Classification^G	Red Ferrosol	Brown Kurosol	Podosol	Grey Dermosol	Brown Dermosol	Hydrosol

^{A}*International Soil Texture Classification System (Atterberg, 1905), ^{B}<0.002 mm, ^{C} 0.02 – 2 mm, ^{D}0.002 – 0.02 mm, ^{E}Phosphorus buffering index unadjusted (Burkitt et al., 2008), ^{F}Phosphorus Sorption Capacity (Borling et al., 2001), ^{G}Australian Soil Classification (Isbell, 2002).*

Consequently we developed a model with an exponential decay function. The model was based on (1) P concentration previously measured (P_{init} concentration), and (2) the amount of P added as on-going P_{fert}, with P concentration for each plot being repeatedly measured over time. Although P_{init} and P_{fert} were used in constructing data summaries, they were not independent of each other within the model due to the overlapping treatment structure i.e., the 16 treatments comprised the combination of the four P_{ferts} with the four P_{init} concentrations. Time was measured in 12 month intervals for $CaCl_2$-P and 6 month intervals for Olsen-P and Colwell-P. Thus, the $CaCl_2$-P parameter estimates were based on less than half the data of Olsen-P and Colwell-P. All treatments were measured for Olsen-P and Colwell-P, while $CaCl_2$-P was measured from nine selected treatments; the two lowest and the highest P_{init} concentrations receiving either zero or one of the two highest P_{fert} rates. The model was fitted using Markov Chain Monte Carlo techniques by means of JAGS software, version 3.1, using 500 000 iterations, a burn-in of 250 000, and thinning every 250 iterations.

The model used Eq. 1 to reflect the changes in soil P that were offset by P_{fert} application. In Eq. 1 $Y_{p,t}$ was the soil P concentration at time (t) for each treatment plot (p); $Y_{p,t-1}$ represented the previous soil P concentration (P_{init}); $A_{p,t-1}$ was the amount of P applied since the previous soil P concentration measurement (divided by 1000 for numerical convenience); S represented the time in years since previous measurement; and e_p and d_p were parameters which were estimated and were required to be positive. e_p represented the increase in extractable soil P for each unit of applied P, and d_p represented a decay constant that reflects how quickly the extractable soil P decreases i.e., moves from the labile to unavailable pools, is removed in product, or otherwise lost from the soils.

$$Y_{p,t} = \left(Y_p, t_{-1} + e_p A_p, t_{-1}\right) \exp\left(-d_p S\right) \tag{1}$$

$$\text{Final Pconcentration} = (\text{previously measured P concentration} + e_p\, x \text{ P fertiliser applied}) \exp\left(-d_p\, x \text{ years since previous P concentration measurement}\right) \tag{2}$$

The model is universal for all three P extractions (mg/kg), P fertiliser applied is expressed as kg P/ha, and estimated soil specific e and d values are provided (see **Table 4**). The observed P_{final} concentrations for each of the measures were denoted by $P_{p,t}$, which was assumed to be normally distributed, with means given by the modelled value $Y_{p,t}$, and with reciprocal variance τ (Eq. 3).

$$P_{p,t} \sim N\left(Y_{p,t}, \tau\right) \tag{3}$$

Prior distributions were assigned to the parameters of the Bayesian model (**Table 2**). Vague priors were used since they make minimal assumptions, resulting in the inferences being

TABLE 2 | Assignment of priors to parameters e_p and d_p for a Bayesian model of soil P concentration change across six soils, each with a range of P_{init} concentrations and P_{ferts}.

P_{fert} constant	**Decay constant**
$e_p = N\left(e_s, \tau_{e_p}\right)$	$d_p = N\left(d_s, \tau_{d_p}\right)$
$e_s = N\left(e_0, \tau_{e_s}\right)$	$d_s = N\left(d_0, \tau_{d_s}\right)$
$e_0 = N\left(0.1, \tau_{e_0}\right)$	$d_0 = N\left(0.1, \tau_{d_0}\right)$
$Y_{p,1} \sim U\left(0, 1000\right)$	

The observation precision, τ, in Eq. 3 and all precisions in this table (τ_{e_s}, τ_{e_p}, τ_{e_0}, τ_{d_p}, τ_{d_s}, τ_{d_0}) were assigned vague gamma priors with shape and rate parameters set to 0.01; e.g., $\tau_{e_s} \sim Ga\,(0.01, 0.01)$. The initial predicted value; $Y_{p,1}$ was unlike later predictions in that it was assumed to not be subject to earlier P applications, but informed only by the first observed P value, and was assigned a uniform distribution so that any value between the upper and lower limits was equally probable.

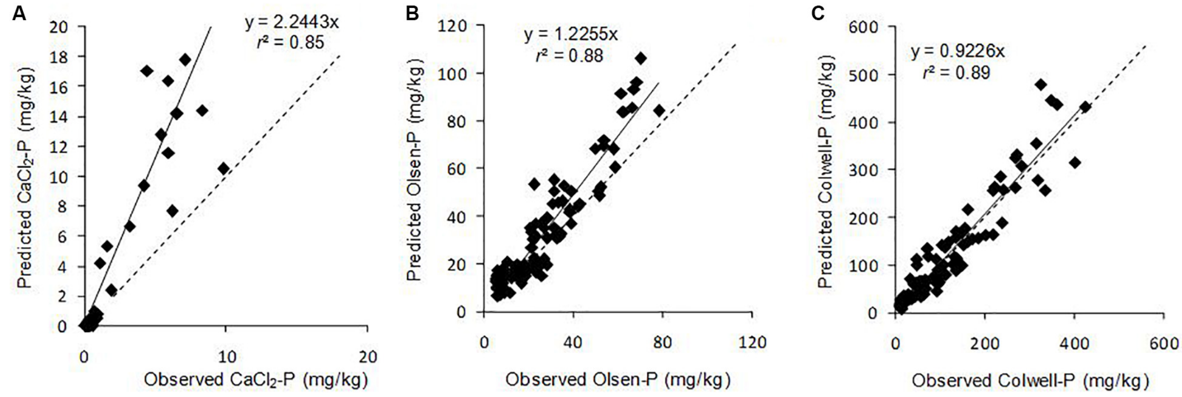

FIGURE 1 | Relationship between observed and predicted final **(A)** $CaCl_2$-P, **(B)** Olsen-P, and **(C)** Colwell-P concentrations of the surface 10 cm for six Australian grazed pasture soils and all P_{init} concentrations and P_{ferts}. Solid line is the fitted regression and dashed line represents $x = y$. Points above the dashed line represent overestimation of P_{final} concentration (modelled P_{final} concentration greater than actual P_{final}), while points below represent underestimation of P_{final} concentration.

informed by the data rather than the prior parameters. The parameters e_p and d_p were assumed to be normally distributed, each with soil specific means, e_s and d_s, where the subscripts *p* and *s* are indices for plot and soil. The e_s and d_s were assumed to be normally distributed with common global means, e_0 and d_0, respectively. Thus, plot parameters could vary around their soil means, which themselves were distributed around a global mean. The normal distributions were truncated on the left at zero so that the parameters remained positive (**Table 2**).

Using the model, the time required for P_{init} to decrease to agronomic optimum – defined as 17 mg/kg for Olsen-P (Gourley et al., 2007) and as 55, 40, 23, 29, 55, and 29 mg/kg for Soils 1 to 6, respectively, for Colwell-P (Gourley et al., 2007) and an environmental threshold (defined as $\leq$0.25 mg/kg for $CaCl_2$-P), was calculated. We chose the $CaCl_2$-P threshold of 0.25 mg/kg (equivalent to solution concentration of 0.05 mg/L based on a 5:1 solution:soil extraction ratio) because the phosphorus trigger value for slightly disturbed lowland streams in Tasmania and Victoria at the time of the study was 0.05 mg/L (ANZECC, 2000) and $CaCl_2$ extractable P (mg/L) has been shown to approximate runoff P concentrations in a number of studies (Dougherty et al., 2008; Burkitt et al., 2010).

RESULTS AND DISCUSSION

Predicted Versus Observed Final $CaCl_2$-, Olsen- and Colwell-P Concentrations

Posterior predictions and observed soil $CaCl_2$-P, Olsen-P and Colwell-P concentrations (**Figure 1**) were highly correlated ($r^2 > 0.85$), showing the model successfully fitted the dataset well for all three P extractions based on 4.5 years of data for 6 soil which ranged from extremely low to high P sorption capacity. Final Olsen-P and Colwell-P concentrations, which were initially approximately twice agronomic optimum or lower, were the most accurately predicted. The precision of the model for P concentrations which were initially near agronomic optimum should be reassuring to land managers and policy makers as it limits the chances of soil P concentrations decreasing below agronomic optimum faster than predicted. However, the large range in extractable soil P concentrations of the dataset resulted in modelled values diverging from observed values as soil P concentrations increased (**Figure 1**). This divergence meant that the model overestimated the P_{final} concentration i.e., the greater the P_{init} concentration, the greater the predicted P_{final} concentration was compared to the actual P_{final} concentration (**Figure 1**). This may have been the result of the smaller population of data at high P_{init} concentrations, for example, Soil 3 did not contain high Olsen and Colwell P_{init} concentrations.

Since the dataset used to develop this model was sourced from grazed pasture field soils, some decreases in extractable soil P would be the result of P being exported in grazing animal products (estimated to be between 2.9 and 12.3 kg P/ha.yr depending on site), and environmental losses. These estimated exports changed according to soil and P_{init} concentration, as would any environmental losses which may occur. The current model includes these exports as contributing to the decreases in extractable soil P concentrations. Further population of the model with data which compares decreases in extractable soil P and various rates of P export from a soil is required, and may lead to incorporation of a P export term. Nevertheless, the majority of the decrease in extractable soil P may be driven by readily extractable P being sorbed by the soil (Barrow, 1980) and conversion to forms not extractable by $CaCl_2$ or bicarbonate, for example organic P (Po) or recalcitrant inorganic-P (Pi).

It could be postulated that our model was predicting decreases in the effectiveness of the capital fertiliser applied at trial site establishment. As a result, the P_{final} concentrations we observed may be expected to be lower than those in soils where similarly high P_{init} concentrations were established over a longer period. However, if that were the case, our model would more closely represent the higher P_{final} concentrations since the model overestimated P_{final}, primarily for high P_{init} soils. Irrespective of this, greater decreases in soil extractable P have been reported for high P_{init} concentrations, whether built-up over a short or long period (McCollum, 1991). McCollum (1991) found that the

TABLE 3 | Absolute and percentage error (in brackets) model prediction, compared to the observed P_{final} concentrations for each P measure and soil, and across all soils.

Soil	$CaCl_2$-P	Olsen-P	Colwell-P
1	−0.15 (−66)	7.7 (20)	25.6 (7)
2	−0.11 (−39)	9.6 (25)	17.1 (9)
3	10.89 (175)	7.7 (110)	4.1 (29)
4	0.68 (36)	2.4 (< 1)	−6.3 (−10)
5	−0.13 (−54)	−0.7 (−4)	−37.4 (−20)
6	7.24 (104)	9.3 (39)	22.8 (44)
All	3.07 (26)	6.1 (32)	4.3 (10)

loss constants for Mehlich-1 P_{init}, when the P_{init} was achieved either through a single application of P fertiliser or progressively over 8 years of P fertiliser applications, had similar loss constants of −0.097 (r^2 = 0.89) and −0.073 (r^2 = 0.81), respectively, for initial Mehlich-1 P of 100 g m^{-3}; −0.068 (r^2 = 0.73) and −0.080 (r^2 = 0.72) for initial values of 40–60 g m^{-3}; and −0.047 (r^2 = 0.52) and −0.048 (r^2 = 0.41) for initial values of 20–30 g m^{-3}. When averaged across all soils P_{init} concentrations and P_{fert}, our model overestimated final Olsen-P and Colwell-P concentrations by 6.1 mg/kg (32%) and 4.3 mg/kg (10%), respectively (**Table 3**). The overestimation for final Colwell-P was within analytical error, suggesting that the model was most successful at predicting final Colwell-P concentrations. Still, the overestimation for Olsen-P was relatively small considering the range in P_{init} concentrations and soils. The Olsen-P method involves a short extraction (0.5 h) and generally extracts soluble orthophosphate P, including P that can be rapidly desorbed from soil particles (Coventry et al., 2001). The longer extraction time (16 h) for the Colwell-P method measures orthophosphate as well as some molybdate-reactive non-orthophosphate P compounds (Coventry et al., 2001). Thus, in addition to the background P exports, the model reflects the conversion of P from readily available to less available forms, and to lesser degree, the rapid and reversible sorption reactions which would have influenced the decreases in $CaCl_2$-P and Olsen-P concentrations.

Similar to Olsen-P and Colwell-P, the divergence of the predicted $CaCl_2$-P concentrations from the observed concentrations was greater as P_{init} concentrations increased (**Figure 1**). The greater a soil's $CaCl_2$-P concentration, the greater the amount of P available to be converted to less available P through processes such as sorption and precipitation, to be removed in animal products, and to be lost to the surrounding environment. The model overestimated average final $CaCl_2$-P concentrations by 3.07 mg/kg (26%) (**Table 3**). The P_{final} concentrations for Soils 3 and 6, with the highest $CaCl_2$-P concentrations and lowest PBIs, were overestimated by a mean of 9.1 mg/kg (140%) (**Table 3**). Whilst these may be large overestimations when compared to the environmental $CaCl_2$-P threshold i.e., ≤0.25 mg/kg (0.05 mg/L), they were not when compared to the initial $CaCl_2$-P concentration range of the dataset (**Table 5**). We hypothesise that the extremely high $CaCl_2$-P concentrations measured from Soils 3 and 6, and the fewer data available for $CaCl_2$-P, contributed to the overestimation. The extreme $CaCl_2$-P concentrations of Soils 3 and 6 appear to be due to these soil's low PBI. Soils 3 and 6 contained the lowest oxalate-extractable iron and aluminium contents, lowest clay and highest sand contents, and some of the highest organic carbon contents, of all the soils examined in the study (**Table 1**).

Phosphorus movement from the surface 10 cm, and conversion to forms not $CaCl_2$-P extractable, may have had a greater contribution to P decreases compared to sorption processes for Soils 3 and 6. Indeed, the greater a $CaCl_2$-P concentration, the greater the risk of environmental loss (Burkitt et al., 2010). This is supported by the modelled P_{final} concentrations being much closer to the observed P_{final} concentrations for the very low PBI Soil 4 which had lower initial $CaCl_2$-P concentrations than the extremely low and very low PBI Soils 3 and 6, respectively (**Table 3**). Final $CaCl_2$-P concentrations for Soils 1, 2, and 5, which had much lower initial $CaCl_2$-P concentrations and higher PBIs than Soils 3, 4, and 6, were underestimated by an average of −0.13 mg/kg (−53%).

Influence of P_{fert} on the Modelled P_{final} Concentration

It is important to examine the influence of P_{fert} on the modelled P_{final} concentration because it is a variable that the land manager can control. Whilst some land managers would rather not omit P_{fert} inputs altogether, soil P concentrations may still decrease if the P_{fert} applied is less than that required to maintain soil P concentrations. There was a range in e_s values (increase in extractable soil P concentration for each unit of applied P) across the soils, where smaller e_s values indicated soils which were less responsive to P_{fert} when presented in isolation from the soil effect (**Table 4**). Changes in P concentrations of Soils 3 and 6 were the least sensitive to P_{fert} when extracted with $CaCl_2$ (**Table 4**); contrasting with current assumptions that relatively low rates of P fertiliser are required to increase fertility of low PBI soils (Burkitt et al., 2001). This is likely to largely be due to the on-going P_{fert} moving beyond the surface 10 cm of these very low PBI, coarse textured soils which receive high annual rainfall (∼1100 mm). Indeed, difficulty was encountered in increasing agronomic extractable soil P concentrations for the extremely low PBI Soil 3. Despite application of 500 kg P/ha, split over 12 months, the Olsen-P of Soil 3 only increased from 13 to 19 mg/kg. Alternatively, the high initial $CaCl_2$-P concentrations of these soils, and conversion of the applied P_{fert} to forms unextractable by $CaCl_2$, such as Po through incorporation into the plant biomass and microbial population, may have also contributed to this low sensitivity to the applied P_{fert}. Olsen-P and Colwell-P concentrations of Soils 3 and 6 were more sensitive to P_{fert} than Soils 1 and 2 which had higher PBI (**Table 4**). This suggests that Soils 3 and 6 had some capacity to weakly sorb P which was not $CaCl_2$-P extractable, and/or desorb molybdate-reactive non-orthophosphate P compounds.

Influence of Soil on the Modelled P_{final} Concentration

Despite soil PBI being an important factor in determining the appropriate P fertiliser rate to increase soil P concentrations, few studies have modelled P decreases across a range of soils.

TABLE 4 | The $CaCl_2$-P, Olsen-P and Colwell-P posterior statistics of *d* and *e* in isolation from each other, on a global (d_0 and e_0) and soil (d_s and e_s) level.

Soil	Mean *d*	SD *d*	Mean *e*	SD *e*
$CaCl_2$-P				
Global	0.3769	0.3725	0.6005	0.7713
1	2.1332	1.1271	1.0555	1.2675
2	1.6377	0.9493	1.1279	1.3648
3	0.1146	0.0604	0.9510	1.0540
4	0.7471	0.1890	1.1756	1.4114
5	1.9853	1.2190	1.1683	1.5647
6	0.0611	0.0455	0.8212	0.8336
Olsen-P				
Global	0.0271	0.0241	0.2533	0.3029
1	0.0260	0.0140	0.3237	0.3038
2	0.0515	0.0142	0.3405	0.3013
3	0.1215	0.0175	0.4849	0.5905
4	0.0058	0.0052	0.5035	0.6312
5	0.0043	0.0040	0.5975	0.7944
6	0.0432	0.0130	0.4059	0.4167
Colwell-P				
Global	0.0355	0.0323	5.4581	10.1002
1	0.0200	0.0100	3.0582	2.6847
2	0.0094	0.0074	4.7165	4.2606
3	0.2239	0.0574	33.5996	22.1170
4	0.0050	0.0043	38.1874	14.6719
5	0.0022	0.0021	127.6211	18.0604
6	0.0569	0.0121	4.7348	4.1758

e represents the increase in extractable soil P for each unit of applied P, and d represents a decay constant that reflects how quickly the extractable soil P decreases. The larger the number, the more sensitive the extraction is to changes in soil P.

Kerridge et al. (1990) reported that the negligible P sorption capacity of the soils they studied meant that a soil term did not need to be included in the model they used. However, the dataset that was used to construct the current model, showed that extractable P concentration decreases did vary according to soil.

Olsen-P and Colwell-P concentrations decreased fastest (highest d_s values) for Soils 3 and 6 (**Table 4** and **Figure 2**), despite having some of the lowest P_{init} concentrations. It is thought that vertical movement of P beyond the soil sampling depth, and conversion to forms not directly extractable, were the two most likely pathways responsible for the decreases in extractable P for Soils 3 and 6. However, despite absolute decreases in $CaCl_2$-P concentrations of Soils 3 and 6 being the largest, they had the lowest *ds,* suggesting the $CaCl_2$-P concentrations of the two lowest PBI soils decreased more gradually than those of higher PBI soils (**Table 4**, and **Figure 2**). Since the $CaCl_2$-P concentrations of Soils 3 and 6 were similar to the Olsen-P concentrations, it is possible that their high organic carbon content may have led to greater hydrolysis of Po to readily available P. The acidic molybdate-solution may cause hydrolysis of Po so that the molybdate-blue colour reaction of Murphy and Riley (1962) does not exclusively represent Pi (Haygarth and Sharpley, 2000). Thus, it is possible that the gradual decreases in $CaCl_2$-P concentrations were partly due to decreases in Po.

We have successfully modelled decreases across a range of soil types, and identified varying decreases according to P extraction, soil type, and PBI. However, we acknowledge that these findings are based on a limited data set of 6 soils sampled over a period of 4.5 years and we suggest more data on P decreases across a wider range of soil types are needed before a term based on PBI can be incorporated into our model. If such a soil term could be incorporated, we suggest the accuracy of the predictions would increase further.

Influence of P_{fert} and Soil on Modelled P Decrease

The magnitude of the effect of P_{fert} was less than soil since the decreases in P were more variable between soils than between rates of P_{fert} (**Table 4**). This may in part be due to different animal product exports (2.9 to 12.3 kg P/ha.yr according to soil and soil P concentration), and potential for surface runoff and leaching of P according to soil, from the trial sites which populated our model. Schulte et al. (2010) reported that their large P deficit due to P export in fodder removed from their trial sites was the driver of P decreases (contributing 63%) for eight moderate to low PBI soils. Despite this, and the small range in PBI of the soils studied by Schulte et al. (2010) they reported a relationship between Morgan-P decreases and parent material of the individual soils that was marginally insignificant ($P = 0.052$). The greater influence of soil than P_{fert} in the current study was thought to be due to the large range of soils examined and supports the need to investigate P decreases across a more diverse set of soils which cover a wider range of P sorption and their properties.

Since there was one experimental site per soil, each of the site-level parameters corresponds to a soil type with associated properties such as PBI. However, a highly significant ($P < 0.001$; $r^2 = 0.95$) linear relationship existed between Ps_{1000} and the d_p parameter for $CaCl_2$-P. Due to the PBI scale not being linear; i.e., a PBI of 400 does not have double the sorption capacity of a PBI of 200, there was a highly significant ($P < 0.001$) logarithmic relationship between PBI and the d_p parameter for $CaCl_2$-P ($r^2 = 0.97$). There was also a significant ($P < 0.05$) linear relationship between Ps_{1000} and d_p for Olsen-P and Colwell-P ($r^2 = 0.24$ and 0.38, respectively). However, the polynomial relationship for d_p of Olsen-P was stronger ($r^2 = 0.40$) than the linear relationship, as was the exponential relationship for Colwell-P d_p ($r^2 = 0.57$). Although the parameters for this model are soil specific, information on their magnitude and interrelationship will assist in estimating decreases for other soils. For example, changes in P concentrations of low PBI soils which are coarse textured, contain high organic matter contents, and have low clay and oxalate-aluminium and oxalate-iron contents, would be expected to be different to medium and high PBI soils.

Time Until Environmental and Agronomic Optimum P Concentrations Are Reached

Based on the current data set, the model estimated that for the majority of the soils, it would take up to 2.7 years for $CaCl_2$-P concentrations of $\leq$1.71 mg/kg ($CaCl_2$-P concentrations of Soils

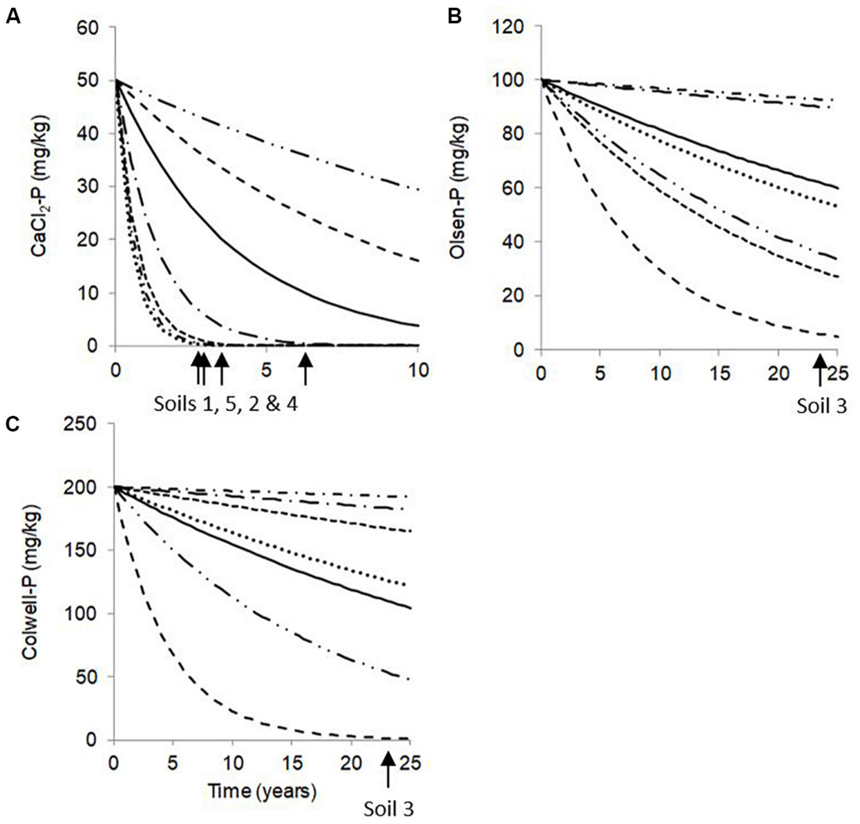

FIGURE 2 | Predicted **(A)** $CaCl_2$-P, **(B)** Olsen-P, and **(C)** Colwell-P generic curves with zero P_{fert} (solid lines) which used the global parameters across all soils (d_0); and for Soil 1 (. . .), Soil 2 (- - -), Soil 3 (– –), Soil 4 (–.–), Soil 5 (-.-), and Soil 6 (–..–), using soil parameters (d_s). Labelled arrows indicate the estimated time required for soils to reach environmental and agronomic thresholds. Note x- and y-axes are differently scaled.

1, 2, 4 and 5) to decrease to an environmentally acceptable level of ≤0.25 mg/kg (0.05 mg/L) (**Table 5**). This is a shorter period of time than that reported by Dodd et al. (2012) of 22–44 years for surface 7.5 cm water extractable-P (WEP) concentrations of 0.039–0.141 mg/L to decrease to an environmentally acceptable level of 0.02 mg/L (0.10 mg/kg). When Dodd et al. (2012) reported WEP and $CaCl_2$-P concentrations were plotted, there was no relationship between the two measures. This explains why Dodd et al. (2012) did not report exponential decreases in $CaCl_2$-P for three of their four sites, while they did report exponential decreases in WEP for three sites.

Dodd et al. (2012) estimated that WEP concentrations of the two soils with the greatest P retentions (PR) would take twice as long to reach the target concentration than a soil with a lower PR. We converted the PR (%) reported by Dodd et al. (2012) to approximate PBI values using data from Burkitt et al. (2002) to conclude that the PBI range was approximately 90–600 for their lowest P fertiliser treated soils. Based on this conversion, the PBI's of two of Dodd et al. (2012) soils were less than 150, as were three of our soils. When we modelled our two lowest PBI soils the model estimated it would take on average 20 years, ranging from 3 to 35 years, for $CaCl_2$-P concentrations to decrease from between 19 and 28 mg/kg for Soil 3, and on average 34 years, ranging from 17 to 41 years, to decrease from between 10 and 29 mg/kg for Soil 6; to ≤0.25 mg/kg (**Table 5**). For our $CaCl_2$-P concentrations to decrease to Dodd et al. (2012) WEP threshold of 0.10 mg/kg, our model predicted it would take 37 years, ranging from 4 to 53 years for Soil 3, and 47 years, ranging from 21 to 51 years, for Soil 6. While the latter mean estimates were similar to those reported by Dodd et al. (2012) for WEP, in contrast to Dodd et al. (2012) our lowest PBI soils took longer to decrease than higher PBI soils. This may be due to our initial $CaCl_2$-P concentrations, which were greatest for the lowest PBI soils, being much greater than the respective $CaCl_2$-P and WEP concentrations of Dodd et al. (2012). This suggests that the extreme $CaCl_2$-P concentrations of our two lowest PBI soils, and high $CaCl_2$-P concentrations of our high PBI soils, decreased more rapidly than those reported by Dodd et al. (2012) supporting the exponential decrease we modelled for $CaCl_2$-P.

Our findings are in line with simple model calculations by Schoumans and Groenendijk (2000) which showed that it would take many decades before high soil P concentrations in non-calcareous low P sorbing sandy soils would be reduced to environmentally acceptable concentrations. It is assumed that Po at least partly buffers the decreasing extractable P concentrations. Considering Pi is the dominant P form in highly P fertilised soils (Dougherty et al., 2006) that are able to hold P, it is assumed that in low PBI soils which only hold small amounts of Pi, Po supply via microbial processes can buffer loss of Pi that occur via desorption.

An important finding of the model is that even if some soils are at or close to agronomic optimum, environmental losses can still occur. For the extremely low PBI Soil 3 and very low PBI Soil 6, $CaCl_2$-P concentrations were above the environmental threshold when Olsen-P and Colwell-P concentrations were at or just above agronomic optima. These findings suggest the use of such soils for agricultural purposes could pose a long-term elevated risk to the environment as optimum agronomic production is unlikely to be achieved without a high risk of P loss. Similarly, for the low PBI Soil 4, Olsen-P concentrations below agronomic optima resulted in $CaCl_2$-P concentrations above the environmental threshold. Since the $CaCl_2$-P concentrations of Soil 4 were not as great as those of Soils 3 and 6, the time taken to reach the environmental threshold was less than 6 years. However, not all soils will pose an environmental risk when soil P concentrations are at agronomic optimum. From **Table 5**, $CaCl_2$-P concentrations of Soils 1 and 2 were not above the environmental threshold until Olsen-P concentrations were twice, and Colwell-P concentrations three to four times the agronomic optima. For Soil 5, Olsen-P and Colwell-P concentrations at or near the agronomic optima were associated with $CaCl_2$-P concentrations close to the environmental threshold.

The current model predicts it would take, on average, 14 years, ranging from 11 to 20 years for Olsen-P concentrations of between 34 and 44 mg/kg to decrease to agronomic optimum of 17 mg/kg. An initial Olsen-P concentration of between 55 and 96 mg/kg would take on average 32 years, ranging from 26 to 49 years, depending on the soil (**Table 5**). In comparison, Dodd et al. (2012) reported a shorter period of up to 7 years for initial Olsen-P concentrations (0–7.5 cm) to decrease from 51 to 25 mg/kg. This is equivalent to an initial Olsen-P of ∼43 mg/kg decreasing to 22 mg/kg if sampled to 10 cm (Coad et al., 2010) which our model suggests would take 25 years when using global d and e values. The time reported by Dodd et al. (2012) for

TABLE 5 | Modelled mean time (years) and range in time (in brackets) until various initial $CaCl_2$-P, Olsen-P and Colwell-P concentrations (mg/kg) reach environmental threshold or agronomic optima, averaged across all on-going P_{ferts} for each soil.

$CaCl_2$-P		Olsen-P		Colwell-P	
Initial concentration (mg/kg)	**Time (years)**	**Initial concentration (mg/kg)**	**Time (years)**	**Initial concentration (mg/kg)**	**Time (years)**
Soil 1					
0.13	0	8	0	33	0
0.15	0	16	0	65	5 (4–8)
n/a	n/a	39	17 (13–24)	181	35 (27–49)
0.85	0.6 (0.4–1.2)	96	50 (33–118)	461	65 (47–135)
0.18	0	11	0	34	0
Soil 2					
0.21	0	19	2 (1–3)	67	19 (15–27)
n/a	n/a	44	15 (12–19)	159	47 (38–64)
1.71	1.1 (0.7–2.7)	93	19 (17–23)	335	67 (54–87)
Soil 3					
23.2	37 (3–156)	17	0	24	0.2 (0.2–0.3)
19.24	44 (3–181)	16	0	22	0
n/a	n/a	16	0	20	0
28.02	13 (4–16)	20	1.0 (1.0–1.3)	30	1.1 (0.9–1.5)
Soil 4					
0.45	0.7 (0.6–1.0)	16	0	49	22 (18–26)
0.53	0.9 (0.7–1.2)	21	8 (7–11)	65	35 (29–43)
n/a	n/a	42	32 (27–40)	139	75 (62–95)
6.96	4.4 (3.5–5.9)	73	34 (30–39)	274	96 (80–120)
Soil 5					
0.22	0	15	0	76	19 (16–22)
0.33	0.1 (0.06–0.3)	20	6 (5–7)	102	34 (30–39)
n/a	n/a	34	25 (22–30)	168	70 (61–81)
0.74	0.5 (0.3–1.4)	55	40 (35–47)	280	108 (94–126)
Soil 6					
11.22	27 (20–41)	23	11 (7–27)	47	10 (8–15)
10.33	22 (17–32)	33	18 (13–32)	73	17 (14–22)
n/a	n/a	40	15 (12–21)	90	18 (15–23)
29.51	22 (18–29)	57	15 (13–18)	134	21 (18–26)

Environmental threshold for $CaCl_2$-P was defined as <0.25 mg/kg, while agronomic optima for Olsen-P was defined as 17 mg/kg, and Colwell-P as 55, 40, 23, 29, 55, and 29 mg/kg for Soils 1 to 6, respectively. n/a means not applicable since P_{init} was already below agronomic optimum, or was not analysed for $CaCl_2$-P.

Olsen-P concentrations to decrease to agronomic optimum was shorter than the time they reported for WEP concentrations to decrease to the environmental threshold, despite WEP decreasing at a greater rate. This suggests that the majority of the soils they studied may have soil properties similar to Soils 3, 4 and 6 of the current study, in that they cannot be maintained at agronomic optimum without environmental loss. Colwell-P agronomic optimum varies according to soil and as such, it would take on average 9 (mean range of 7 to 11 years), 47 (mean range of 39 to 59 years), and 84 years (mean range of 69 to 117 years) for initial Colwell-P concentrations of 30–90, 102–181, and 274–461 mg/kg to decrease to their respective agronomic optima for Soils 1 to 6 (**Table 5**).

From **Table 5**, and as mentioned above, there is a large range in predicted decreases for our model. Schulte et al. (2010) reported an uncertainty in their model predictions of 3 to >20 years for Morgan-P to decrease from Index 4 to 3. Dodd et al. (2012) reported an estimate of 23 years could be 21 or 31 years for WEP. Our greater range in predicted decrease period may be due to the greater range of soils used. In addition, our model extrapolated our dataset more than Dodd et al. (2012) who had field data for 7, 16, 21, and 26 years, depending on site. However, the advantage of our model, as opposed to a purely descriptive empirical one, is that the predictions beyond the observed data range are reasonable, as long as the model assumptions are valid. We suggest that the large range in P_{init} concentrations of the dataset used for the current model contributed to these wide ranges, since some of our low P_{init} concentrated soils had low prediction ranges, similar to those reported by Dodd et al. (2012), with the range widening as P_{init} concentrations increased. In addition, the on-going P_{fert} would have extended some of our periods of decrease.

CONCLUSION

The model developed in this study has provided critical information for our long term management of soil P concentrations, as we found that decreases in soil P are generally slow (14 years (ranging from 11 to 20 years) for Olsen-P concentrations of between 34 and 44 mg/kg to decrease to an agronomic optimum of 17 mg/kg). As a result of our findings, we suggest that policy makers need realistic expectations about achievable P decreases, and land managers can be reassured of the implications of withholding P fertiliser, as soil P concentrations are unlikely to decline rapidly on these Australian pasture soils. Our findings also highlight the risk of using agronomic soil test concentration as a reliable guide to environmental risk, as some soils in the study did not exceed the environmental threshold until Olsen-P concentrations were twice, and Colwell-P concentrations three to four times the agronomic optima, whereas soils with extremely low and very low PBI values exceeded the threshold, despite Olsen-P and Colwell-P concentrations being at or just above agronomic optima.

To achieve more rapid decreases in soil P concentrations, increases in P exports in plant and animal products would need to occur in addition to withholding P fertiliser application. To enable modelling of scenarios where various P exports occur, a P export term needs to be incorporated into future versions of the model. Within the 6 soils examined in the current study, results have shown that soil with inherently very low and extremely low PBIs, require different management than higher PBI soils, in order to limit P losses i.e., application of different quantities and forms of P. However, further work on a greater range of soils types is needed to understand the influence of soil properties such as PBI on extractable soil P decreases, in order to refine this model.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

JT completed all field sampling, laboratory and non-Bayesian data analysis, and presentation, and drafted the manuscript. RC undertook all the Bayesian modelling and assisted JT with the interpretation of these data. LB planned the field experiment and made a substantial intellectual contribution to the research, and the interpretation of data presented in this manuscript. WD made a substantial intellectual contribution in terms of the interpretation and presentation of results presented in this manuscript. All authors contributed to the article and approved the submitted version.

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Sewage Sludge Application in *Eucalyptus urograndis* Plantation: Availability of Phosphorus in Soil and Wood Production

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Phosphate fertilizers used in planted forests mainly come from scarce and non-renewable sources, and there is a need for new sources of phosphorus (P). An alternative is the use of sewage sludge (SS), rich in organic matter, nitrogen, and P. The objectives were to evaluate the soil P availability and its effect on wood production in 22-month-old *Eucalyptus urograndis* plantation treated with SS. A field experiment was set up in a randomized complete block design, four replicates, with the following treatments: control without fertilization; mineral fertilization (MF); 14.5 Mg ha^{-1} of SS + 22 kg ha^{-1} of P (S1P1); 29 Mg ha^{-1} of SS (S2); 29 Mg ha^{-1} of SS + 17.5 kg ha^{-1} of P (S2P2); and 43.5 Mg ha^{-1} of SS (S3), dry base. Of the total P in SS, 65% was in organic form and 42% was in labile + moderately labile forms. S2P2 and S3 positively altered the pattern of soil P distribution, with more P in the labile and moderately labile fractions than in the non-labile fraction, along 0–20 cm depths, than MF and control. There were higher microbial and available P as a function of SS dose. Wood volume and biomass were highly related to soil P availability as a function of SS dose. Within the SS treatments, S2P2 resulted in higher gains of volume and biomass of wood. The SS application at the recommend rate, supplemented with 66% of recommended P fertilizer dose in *Eucalyptus* plantations, can reduce the use of P fertilizer by 33% and N fertilizer by 100% and maintain comparable soil P availability and wood production.

Keywords: alternative fertilizers, biosolids, organic fertilizer, P fractionation, soil P lability

INTRODUCTION

Municipal wastewater treatment plants (WWTPs) have sharply increased worldwide, especially in the industrialized countries as regulated by specific laws (Nascimento et al., 2020). In WWTPs, an important organic residue is generated in huge amounts: the sewage sludge (SS). The management and disposal of SS are complex and expensive, and SS reuse in several fields of application has tremendously increased worldwide (Cieślik et al., 2015). SS application as soil fertilizer/amendment in agriculture is the most promising practice (Abreu-Junior et al., 2005, 2017; Franco et al., 2010; Florentino et al., 2019; Nascimento et al., 2020). Depending on its origin and quality, SS may contain a huge amount of organic matter and essential elements for plants (Bettiol and Ghini, 2011), thus replacing or integrating mineral fertilizers of non-renewable origin widely used for crop cultivation (Alleoni et al., 2012; Ferraz et al., 2016; Abreu-Junior et al., 2017). This means that the use of non-renewable limited resources, such as phosphate fertilizers, can be significantly reduced by the SS agricultural use (Franco et al., 2010; Abreu-Junior et al., 2017), with a circular-economy perspective.

Phosphorous (P) is one of the nutrients present in high concentration in SS (Abreu-Junior et al., 2005; Costa et al., 2014), representing an important resource for agriculture. Agriculture in tropical areas is mainly conducted in highly weathered, leached, and acidic soils where a rapid loss of P can occur because of fixation by soil components such as 1:1 clays and Fe- and Al-oxyhydroxides (Gustafsson et al., 2012; Rodrigues et al., 2016). Such processes increase the content of legacy-P, P in non-labile forms and not available to plants (Withers et al., 2018). For these soils, higher doses of P fertilizers are required to maintain desired agricultural and forest productivity. Since P fertilizers are of non-renewable origin (mainly extracted from phosphate rock), the constant and increasing exploitation may lead to depletion and scarcity of this resource (Van Vuuren et al., 2010). New and non-conventional P sources are strongly required.

Phosphorus in SS, treated and stabilized for agricultural use, is generally in inorganic forms (Carvalho et al., 2015; Kahiluoto et al., 2015; He et al., 2016). In order to be applied to substitute mineral fertilizers in forestry and agricultural areas, assessment of P availability in SS-amended soil is necessary. The sequential extraction method proposed by Hedley et al. (1982) is an analytical tool for understanding the availability of P in tropical soils, since it segregates organic (P_o) and inorganic (P_i) P fractions, relating their affinity with different soil components. Additionally, it also allows grouping P fractions in labile, moderately labile, and non-labile pool, based on their availability to plants.

By determining P availability in soil, together with the microbial biomass, responsible for SS degradation and P cycling, we can improve our knowledge on P chemistry and recycling in a *Eucalyptus* plantation. Studies on P recycling in forests have been already conducted but for different managements and/or after long periods of residue application. To our knowledge, no studies have been conducted to examine the availability and legacy of P in tropical low fertility soil due to SS application for *Eucalyptus* cultivation. Such information is fundamental to characterize and quantify soil P availability for improved management of SS application to increase fertilizer use efficiency and wood production in the tropical regions.

Forest systems in Brazil, despite their high productivity, are usually characterized by soil with high nutrient deficiency, with particular reference to P in *Eucalyptus* commercial plantations (Alvares et al., 2011; Gonçalves et al., 2013; Binkley et al., 2017). This is true despite the low P requirement of this crop; indeed, *Eucalyptus* has been historically planted in the areas with sandy infertile soils (Bazani et al., 2014). As this commercial plantation has currently covered 7.4 million hectares in the whole Brazil (Sistema Ibge De Recuperação Automática - SIDRA, 2017), high amounts of P are required to sustain the Brazilian roundwood industry, one of the most important and productive in the world. In the last few years, roundwood industries and scholars closely collaborated to find new and sustainable P sources and their application and management in tropical soils.

We hypothesized that the application of SS increases the labile P fraction, P microbial biomass, and P availability in soil, improving the wood production of *Eucalyptus urograndis* plantation. The aims of this work were to evaluate soil P availability and wood production in a 22-month-old commercial *Eucalyptus urograndis* plantation treated with sewage sludge, applied 7 months after planting, combined with or without mineral fertilizer.

MATERIALS AND METHODS

Experimental Area and Soil Characterization

The research was conducted in a commercial *Eucalyptus* field at the Suzano S.A. Company, municipality of Boa Esperança do Sul, State of São Paulo, southern Brazil (21°57′05 S e 48°31′ W). The field experiment was conducted in a stand where *Eucalyptus* trees were commercially cultivated in the last 40 years and SS had been never applied before.

The experimental area has humid-temperate climate, with dry winter and hot summer, and with the rare possibility of frost in early winter (Cwa climate type - Köppen classification) (Alvares et al., 2013, 2018). Average annual rainfall is 1,400 mm, and average minimum and maximum temperature ranges from 15 to 31°C. A detailed pedological survey was carried out before the experiment and the soil was classified as Typic Hapludox (Soil Survey Staff, 2014).

Before the experiment, the soil Ap surface horizon (0–40 cm depth) was sampled at 0–5, 5–10, 10–20, and 20–40 cm depths. Each sub-depth of soil was collected by taking 20 subsamples randomly along the lines of the plantation. Samples were then homogenized, air dried, and sieved to <2.0 mm prior to analysis. All soil samples were characterized according to official physical (Camargo et al., 2009) and chemical (Raij et al., 2001) procedures (**Table 1**). The densimeter method was used for particle-size analysis. Soil pH was measured potentiometrically with a glass electrode at the soil/solution mixture of 1:2.5 1 mol L^{-1} $CaCl_2$. Soil organic matter (SOM) content was estimated by the Walkley–Black method. Soil available P, Ca^{2+}, K^{+}, and Mg^{2+} were extracted by an ion-exchange resin procedure. Concentrations of P in the extracts were determined by the colorimetric method, and those of Ca and Mg were determined by atomic absorption spectrophotometer (AAS) using a VARIAN SpectrAA 140 and K using a CORNING 400 flame-photometer. Total acidity (H + Al) was determined by SMP buffer solution at pH 7.0. Cation Exchange Capacity (CEC) was equal to $Ca^{2+} + Mg^{2+} + K^{+} + (H + Al)$. Available Cu, Fe, Zn, and Mn

TABLE 1 | Soil surface Ap physical[1]–chemical[2] characterization according to fixed depths.

Attributes	Unit	Depth (cm)			
		0–5	5–10	10–20	20–40
pH$_{(CaCl2)}$	–	5.3	5.4	5.7	4.9
Organic matter	g dm^{-3}	14	10	7	3
Resin-P	mg dm^{-3}	7	6	3	2
K	mmol$_c$ dm^{-3}	0.3	0.3	0.5	0.4
Ca^{2+}	mmol$_c$ dm^{-3}	23	18	16	9
Mg^{2+}	mmol$_c$ dm^{-3}	2	2	2	1
H + Al	mmol$_c$ dm^{-3}	20	19	15	18
Cation exchange capacity	mmol$_c$ dm^{-3}	46	40	34	29
Base saturation	%	56	51	54	38
B	mg dm^{-3}	0.3	0.3	0.3	0.2
Cu	mg dm^{-3}	0.3	0.3	0.3	0.3
Fe	mg dm^{-3}	32	28	35	28
Mn	mg dm^{-3}	2.0	1.0	2.0	0.7
Zn	mg dm^{-3}	0.6	0.2	0.6	0.8
Sand (>0.05 mm)	g kg^{-1}	900	900	900	900
Silt (>0.002 and <0.05 mm)	g kg^{-1}	10	10	20	20
Clay (<0.002 mm)	g kg^{-1}	90	90	80	80

[1] *Camargo et al. (2009).* [2] *Raij et al. (2001).*

TABLE 2 | Sewage sludge chemical features and PTE characterization.

Attribute	Sewage sludge—CSJ	Attribute	Sewage sludge—CSJ
pH-H_2O	6.8		mg kg^{-1}
	%	B	1.0
Moisture	62	As	3.26 (41)[1]
Volatile solids	49	Ba	629 (1300)
Ashes	51	Cd	2.40 (39)
	g kg^{-1}	Pb	45 (300)
Organic carbon	166	Cu	308 (1500)
Total N	22	Cr	61 (1000)
Total P	30	Hg	<0.05[2] (17)
K	1.7	Mo	8.03 (50)
Na	1.8	Ni	31 (420)
S	24	Se	<0.05[2] (100)
Ca	21	Zn	667 (2800)
Mg	5.0		

[1] *Limits to SS agricultural use established by Resolution 375 (Brasil, 2006).* [2] *ND, not detected (concentrations < 0.1 mg kg^{-1}).*

were extracted by DTPA solution, at pH 7.3, while available B was determined by the hot water method.

Sewage Sludge Characterization

Class B sewage sludge was obtained from the Jundiaí wastewater treatment plant (Jundiaí municipality, São Paulo State). The sludge was generated in a biological system of aerated ponds with a complete mixture followed by sedimentation ponds. The biological sludge was stabilized in the sedimentation ponds for 12 months, which resulted in an OM content of dry solids under 70%. The sludge was then treated with synthetic cationic polymer, centrifuged, and air-dried. Before application, the SS was characterized for both physical properties and potentially toxic elements (PTE) in accordance with method SW-846-3051A (USEPA, 2007) (**Table 2**). The SS contained high concentrations of total OC, N, and P, while PTE contents were below the limits established by Brasil (2006) and those usually observed in SS (Abreu-Junior et al., 2005; Nascimento et al., 2020), thus allowing its reuse for agricultural purposes (Brasil, 2006). Class B sludges contain detectable levels of pathogens and their agricultural use is regulated by CONAMA Resolution No. 375 (Brasil, 2006).

Field Preparation and Experimental Procedure

Minimum tillage started in January 2015, with subsoiling 0.4 m deep between the lines of the previous rotation over the entire area, where the *Eucalyptus* seedlings were planted. Before planting, 1.8 Mg ha^{-1} of limestone, to supply calcium and magnesium, was applied at the soil surface along a continuous band close to the seedling. Seedlings of *Eucalyptus urophylla* S.T. Blake × *Eucalyptus grandis* Hill ex Maiden (clone SP5727 owned by Suzano, *Eucalyptus urograndis*, hereafter *E. urograndis*) were then planted in February 2015. Seedlings were spaced as 3 m × 2.25 m, with an amount of 1,481 plants per hectare.

The experimental design consisted in totally randomized blocks with six treatments (**Table 3**) and four replicates for each treatment, totaling 24 experimental plots. Each plot had an area of 675 m^2 planted with 100 trees (10 × 10). For data collection, we used the 36 trees (6 × 6) located in the center of each plot, occupying a useful area of 243 m^2. Mineral fertilizers in the form of triple superphosphate were applied to the planting pit next to the seedling. Seven months after planting, SS was applied in a continuous band of 0.6 m wide, without incorporation into the soil, 0.2 m aside the planting rows.

The studied treatments (**Table 3**) were control, without SS and mineral fertilizers (C); mineral fertilization for high wood production, as recommended by Suzano S.A. Company (MF); 14.5 Mg ha^{-1} of SS + 22 kg ha^{-1} of P (S1P1); 29 Mg ha^{-1} of SS (S2); 29 Mg ha^{-1} of SS + 17.5 kg ha^{-1} of P (S2P2); and 43.5 Mg ha^{-1} of SS (S3). Sludge doses were calculated according to nitrogen criteria, following CONAMA Resolution No. 375 (Brasil, 2006), in order to provide 192 kg ha^{-1} of N, as recommended by the Suzano S.A. Company. Thus, the doses of 14.5, 29.0, and 43.5 Mg ha^{-1} of SS, at dry base, were equivalent to 50, 100, and 150% of the recommended N. Doses of 22 and 17.5 kg ha^{-1} of P were applied to provide 83 and 66% of mineral P recommendation (26 kg ha^{-1}), combined with SS at 50 and 100% of recommended N doses, S1P1 and 100% S2P2 treatment, respectively, in order to obtain wood production equivalent to the mineral NPK fertilizer application, as recommend by Abreu-Junior et al. (2017). All SS treatments were complemented with mineral K (KCl) and B (ulexite).

Soil Sampling and Preparation

Soil samples were collected in September 2016, 15 months after SS application (22 months after planting and first application of mineral fertilizer), at the depths of 0–5, 5–10, and 10–20 cm,

TABLE 3 | Amount of nutrients applied by treatments with mineral fertilizers and sewage sludge.

Treatment	N	P	K	B	Zn	Cu
	kg ha^{-1}					
Control (C)	0	0	0	0	0	0
Mineral fertilization (MF)	**192**	**26**	**137**	**6.5**	**2.8**	**2.8**
Planting	30	26	25	1.5	1.5	1.5
Fertilization 1[3]	45	0	112	1.8	1.3	1.3
Fertilization 2[4]	54	0	0	1.5	0	0
Fertilization 3[5]	63	0	0	1.7	0	0
Sludge (50%)[2] + P (83%) + B + K (S1P1)	**96**[6] (319)	**22** (435)	**137** (290)	**6.5** (0.015)	**0** (9.7)	**0** (4.5)
Planting[1]	0	22	25	3.2	0	0
Fertilization 1[3]	0	0	112	3.3	0	0
Sludge (100%)[2] - P + B + K (S2)	**192**[6] (638)	**0** (870)	**137** (855)	**6.5** (0.03)	**0** (19.3)	**0** (9)
Planting[1]	0	0	25	3.2	19.3	9
Fertilization1[3]	0	0	112	3.3	0	0
Sludge (100%)[2] + P (66%) + B + K (S2P2)	**192**[6] (638)	**17.5** (870)	**137** (855)	**6.5** (0.03)	**0** (19.3)	**0** (9)
Planting[1]	0	17.5	25	3.2	0	0
Fertilization 1[3]	0	0	112	3.3	0	0
Sludge (150%)[2] - P + B + K (S3)	**288**[6] (957)	**0** (1,305)	**137** (870)	**6.5** (0.045)	**0** (29)	**0** (13.5)
Planting[1]	0	0	25	3.2	0	0
Fertilization 1[3]	0	0	112	3.3	0	0

Values in parentheses represent the total amount of nutrients supplied by sewage sludge. [1]Planting fertilization (triple superphosphate, KCl, and ulexite) in 02/12/2015. [2]Sewage sludge application in 09/12/2015, 207 days after transplantation, in doses of 14.5, 29, and 43.5 Mg ha^{-1} (dry base) corresponding to 50, 100, and 150% of N recommendation (192 kg ha^{-1}). [3]First fertilization, performed in 11/05/2015, 261 days after transplantation. [4]Second fertilization, performed in 12/21/2015, 307 days after transplantation. [5]Third fertilization, performed in 02/02/2016, 350 days after transplantation. [6]Bold values represent the total amount of nutrients applied by mineral fertilizers, except for N values that show the dose of available nitrogen applied by sewage sludge, considering the N mineralization rate of 30% for aerobic SS (Brasil, 2006).

in order to assess differences on P pools, considering superficial SS application, and that labile inorganic P is rapidly adsorbed and organic P can move along 0–20 soil depth. Each soil sample consisted of 18 subsamples collected per plot, nine in the planting rows, where mineral fertilizers were applied, and nine in the interrow, where SS was applied, after carefully removing superficial fallen organic material. At the laboratory, samples were air-dried and sieved at 1.0 mm prior to P analysis. To avoid biomass degradation, soon as the 0–5 cm depth samples were taken, they were homogenized and a fresh 1/4 subsample of each sample was collected, transferred to a plastic flask, and stored in a polystyrene thermal box with ice until transported to laboratory, where the subsamples were stored in a freezer until P microbial analysis.

Wood Production Assessment

To estimate the volume of wood and biomass of wood, barks, branches, and leaves, the height of 12 trees and circumference at breast height (CBH) of all trees in the inner plot were measured 22 months after planting. The height of all trees was estimated by the regression model proposed by Curtis (1967) and modified by Ribeiro et al. (2010), using the equation $\ln H = 2.87875 - 4.28769^{***}/CBH$; $R^2_{aj.} = 0.55$; $n = 431$. After that, Schumacher and Hall's logarithmic model (Schumacher and Hall, 1933) was chosen to adjust the data and estimate tree biomass at the age of 22 months (**Supplementary Data**).

Phosphorus Analysis

Phosphorus Fractionation in Sewage Sludge

Phosphorus fractionation in the SS was performed according to the method proposed by Hedley et al. (1982) and modified by Sui et al. (1999). The SS sample was air-dried and sieved to <2 mm, and then portions of 0.5 g in triplicates were placed in 50-ml centrifuge tubes and sequentially extracted with 30 ml of deionized water (water-soluble inorganic P; fraction 1, F1); 0.5 mol L^{-1} $NaHCO_3$ (both inorganic and organic extracted P; fraction 2, F2); 0.1 mol L^{-1} NaOH (both extracted P_i and P_o; fraction 3, F3); 1 mol L^{-1} HCl (extracted P_i; fraction 4, F4); and 0.5 mol L^{-1} NaOH (both extracted P_i and P_o; fraction 5, F5). After the final extraction, residual P (residual P_i; fraction 6, F6) was extracted with $H_2SO_4 + H_2O_2 + MgCl_2$ and determined colorimetrically according to Murphy and Riley (1962). From the alkaline extracts, an aliquot was taken to determine total P (P_t) by digestion with ammonium persulfate [$(NH_4)_2S_2O_8$] and sulfuric acid (H_2SO_4) in an autoclave. Inorganic P was determined according to Dick and Tabatabai (1977). Organic P was determined as the difference between P_t and P_i. After quantification of each fraction, they were grouped according to predicted lability: the labile P included F1 + F2; moderately labile P was composed of F3 + F4; and non-labile P was F5 + F6.

Soil P Availability

Soil available P was estimated by a resin-extraction method with a mixture of Amberlite IRA-400 anion-exchange resin and P was determined using a spectrophotometer colorimetry at 882 nm. Weighted soil samples from the 0–20 cm depth of each plot were used for the analysis (Raij et al., 2001) and interpretation of resin-P availability was referred to Raij et al. (1997).

Microbial Biomass Phosphorus

The Brookes et al. (1982) and Hedley and Stewart (1982) methods were applied. Microbial biomass P is calculated from the difference between the amount of inorganic P extracted by 0.5 mol L^{-1} $NaHCO_3$ (pH 8.5) from fresh soil fumigated with $CHCl_3$ and the amount extracted from unfumigated soil. Additionally, a known amount of P was added to the sample to prevent adsorption of released P by soil colloids (Morel et al., 1996).

P Fractionation in Soil

Phosphorus fractionation was performed according to the method proposed by Hedley et al. (1982) and modified by Condron et al. (1985). Portions of 0.5 g in triplicates were placed in 50-ml centrifuge tubes and sequentially extracted with 2 cm^2 of anion-exchange resin saturated with $NaHCO_3$ and immersed in distilled water (extracted P_i), 0.5 mol L^{-1} $NaHCO_3$ (both extracted P_i and P_o), 0.1 mol L^{-1} NaOH (both extracted P_i and P_o), 1 mol L^{-1} HCl (extracted P_i), and 0.5 mol L^{-1} NaOH (both extracted P_i and P_o). Assessment of inorganic and organic phosphorus, as well as its lability fractions, was conducted as described for SS analysis. P extracted from the soil was evaluated as orthophosphate with a spectrometry (colorimetry) method.

Plant Samples

Plant samples were washed with distilled water and then dried in a forced air oven at 60°C until constant weight is obtained. Samples were weighed for moisture determination and digested by an open system nitric-perchloric digestion. P concentration in the digested solution was determined in a spectrophotometer (colorimeter) with wavelength at 420 nm (Malavolta et al., 1997).

Statistical Analysis

Statistics were carried out using the software program R (R Core Team, 2013). Differences among treatments were compared using a one-way analysis of variance by the *F* test on each measured variable and, when a significant effect was observed, multiple comparisons were assessed by a Duncan's *post hoc* test ($p < 0.05$). When reported, values in this study indicate the mean ± standard error. Single correlations were also performed.

RESULTS

Phosphorus Fractionation in the Sewage Sludge

The fractionation of P in SS showed that 0.5% of total P (30 g kg^{-1}, **Table 2**) was extractable to water; 14% to 0.5 mol L^{-1} $NaHCO_3$; 13% to 0.1 mol L^{-1} NaOH; 14.5% to 1 mol L^{-1} HCl; and 57% to 0.5 mol L^{-1} NaOH; and that 1% represented the residual P (**Figure 1**). The SS contained 15% of P in labile form (4.5 g kg^{-1}); 27% in moderately labile form (8.1 g kg^{-1}); and 58% as non-labile (17.4 g kg^{-1}). The SS had predominant organic P forms (65% of total P), which, in turn, represents 40, 42, and 82%, respectively, of P contained in labile, moderately labile, and non-labile P.

Soil P Lability

In 15 months after application, SS treatments at the dose of 100 and 150% of N criterion, with or without mineral P (S2, S2P2, and S3) promoted a higher level of P_i (39–68 mg kg^{-1}) in the 0–20 cm soil depth, as compared to S1P1 treatment (P_i, 28–40 mg kg^{-1}) or control (P_i, 16–20 mg kg^{-1}) (**Figures 2A–C**). In general, labile P_o concentration was similar among the treatments (2–18 mg kg^{-1}).

The effect of SS application, in increasing P_i/P_o ratio in P fraction, was higher for moderately labile P, especially for S3 (P_i = 84–126 mg kg^{-1}; P_o = 3–17 mg kg^{-1}), along 0–20 cm soil horizon depth. The S2P2 and S2 treatments promoted higher concentration of moderately labile P (P_i = 71–92 mg kg^{-1}; P_o = 4–36 mg kg^{-1}), at the upper 0–10 cm soil horizon depth, than mineral fertilizer, S1P1, and control (P_i = 25–47 mg kg^{-1}; P_o = 2–22 mg kg^{-1}) (**Figures 2D,E**); however, these treatments were similar at 10–20 cm soil horizon depth (P_i = 24–47 mg kg^{-1}; P_o = 6–23 mg kg^{-1}) (**Figure 2F**).

Non-labile fraction, which contained the most of total P in soils of mineral fertilizer and control treatments, and same P_i/P_o ratio in all treatments (**Figures 2G–I**), was just increased by S3 treatment, at very upper 0–5 soil horizon depth (P_i = 89 mg kg^{-1}; P_o = 13 mg kg^{-1}), compared with other treatments (P_i = 56–73 mg kg^{-1}; P_o = 7–16 mg kg^{-1}) (**Figure 2G**).

Soil P in the Microbial Biomass and Availability

Sewage sludge increased P in the microbial biomass, at 0–5 cm depth, and soil available P, at 0–20 cm depth, estimated by resin extraction (**Figure 3**), as assessed 15 months after application. With the increasing dose of SS, the concentration of MB-P was raised from 0.20 mg kg^{-1}, at MF, to 0.95 mg kg^{-1}, at S3. Mineral fertilizer application had no effects on MB-P concentration (**Figure 3A**), but S1P1, S2 and S2P2 had intermediate values (0.45–0.67 mg dm^{-3}). Soil resin-P concentration was higher for S2P2 application (43 mg dm^{-3}), than MF, S1P1, S2 and S3 treatments (20–31 mg dm^{-3}), and the control (7 mg dm^{-3}) (**Figure 3B**).

Production and P Nutrition in *E. urograndis* Plantation Treated With Sewage Sludge

Twenty-two months after *E. urograndis* planting, the highest production of wood volume (62 m^3 ha^{-1}) and biomass of wood (22.6 Mg ha^{-1}), bark (2.4 Mg ha^{-1}), branches (7.0 Mg ha^{-1}), and leaves (4.0 Mg ha^{-1}) were achieved by mineral fertilizer application (**Table 4**). However, SS application at the recommended dose (29 Mg ha^{-1}) supplemented with triple superphosphate resulted in similar results, with wood volume of 60.5 m^3 ha^{-1} and biomass of wood, bark, branches, and leaves of 22, 2.4, 6.8, and 3.8 Mg ha^{-1}, respectively. Both treatments had a higher production than the control.

Mineral fertilizer combined with SS application increased P concentration and accumulation in branches and total P accumulation in *Eucalyptus* trees (**Table 5**). However, there were no differences among the treatments for P concentration and accumulation in wood, bark, and leaves. For the branches, there was a 60% higher P concentration in the MF treatment relative to the control, remaining equal to the S2P2 treatment. The compartments with higher P concentration followed the order: leaves > bark > branches > wood. There was greater P accumulation in branches due to mineral fertilizer application, with an average of 4.5 kg ha^{-1}, being 71% higher than the control and statistically equal to the SS treatments. The accumulation of P in the tree components followed the order wood > leaves > branches > bark.

Production of *E. urograndis* Plantation in Relation to Soil P Availability and as a Function of SS Application

Production of wood volume and biomass of wood, bark, branches, and leaves were correlated ($p < 0.01$) with soil P availability, expressed in terms of soil resin-P, in the control and SS application treatments (**Figure 4**).

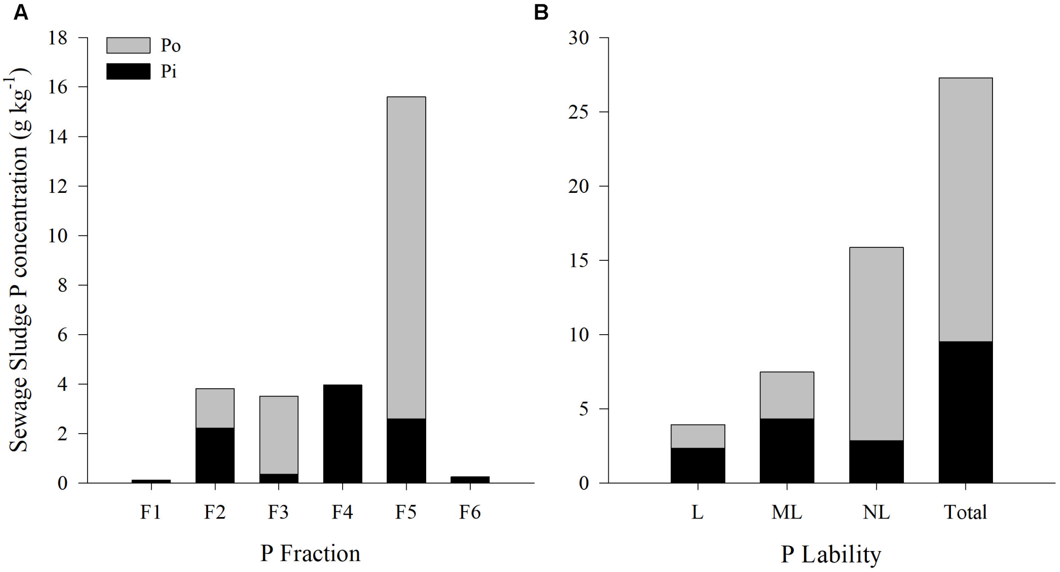

FIGURE 1 | Fractionation **(A)** and lability **(B)** of phosphorus in the class B sewage sludge used in the *Eucalyptus* planting area. P_o, organic phosphorus; P_i, inorganic phosphorus; F1, P extracted by water; F2, P extracted by 0.5 mol L^{-1} sodium bicarbonate; F3, P extracted by 0.1 mol L^{-1} sodium hydroxide; F4, P extracted by 1 mol L^{-1} hydrochloric acid; F5, P extracted by 0.5 mol L^{-1} sodium hydroxide; F6, residual P; L, Labile (F1 + F2); ML, moderately labile (F3 + F4); NL, non-labile (F5 + F6) (*n* = 4).

Based on wood production of 62 m^3 ha^{-1} for *E. urograndis* after 22 months of planting under mineral fertilizer application, corresponding to 100% of the expected relative production (**Table 6**), an equivalent relative wood production given by the application of SS at recommend dose supplement with 66% of mineral P recommend (S2P2 treatment) can be observed. Consequently, this treatment reduces the use of mineral P and N fertilizer by 33 and 100%, respectively.

DISCUSSION

Phosphorus Availability in the Sewage Sludge and Soil

Our results showed that the class B sewage sludge had 65% of total P as organic compounds, which is slightly higher than previously reported (Carvalho et al., 2015; Kahiluoto et al., 2015; He et al., 2016). The SS also had 15 and 27% of total P in labile and moderately labile compounds, which agrees with literature (Kahiluoto et al., 2015; He et al., 2016). Such properties justify the forest and agricultural use of SS as a potential source of P fertilizer. For a *Eucalyptus* plantation, the application of the recommend dose by the N criteria (29 Mg ha^{-1}, dry base) of SS, with 30 g kg^{-1} of total P (**Table 2**), was expected to add 566 kg ha^{-1} of organic P, including 50 kg ha^{-1} of labile organic P, plus 75 kg ha^{-1} of labile inorganic P, including 4.35 kg ha^{-1} of water-soluble P (equivalent to that supplied by a soluble mineral source of P, e.g., triple superphosphate), while suppling 192 kg ha^{-1} of N, equivalent to the recommend mineral N dose. Labile and moderately labile P are mainly responsible for the short- and medium-term soil P supply, since SS contained both P_i and P_o fractions and the mineralization of P_o in sludge after application is governed by a combination of physicochemical and biological processes in soil (Condron and Newman, 2011; Weihrauch and Opp, 2018).

Non-labile P fraction in SS might play an important role in a long-term release of P in the soil, since it has a higher proportion of P_o (F5 P_o fraction, **Figure 1A**) than P_i (F5 P_i fraction, **Figure 1A**). Higher P_o in this fraction should be attributed to organic phospholipids, innermost adsorbed P, and other organic contaminants derived from the organic urban sludge and from runoff infiltration/derivation and few illegal sewage pipes. Inorganic P adsorbed to Fe and Al could be enhanced by the chemical treatments in the sewage sludge using metal salts (e.g., $FeCl_3$) (García-Albacete et al., 2012), but not by treatment with synthetic polymer (Carvalho et al., 2015). These treatments can modify the proportions of P-Fe and P-Al of the SS and subsequently in the soil (Alleoni et al., 2012). In the present study, the SS was treated with synthetic cationic polymer, and most of Al_2O_3 and Fe_2O_3/FeO were derived from soil particles (kaolinite and oxides) from runoff infiltration/derivation and few illegal sewage pipes, so the F5 P_i fraction was low (**Figure 1A**). The non-labile P_o in the SS is likely an important source for the formation of legacy P in soil, due to long-term mineralization (Munhoz et al., 2011; Alleoni et al., 2014; Foltran et al., 2019).

Considering P lability in highly weathered tropical soil untreated with organic fertilizer, there is a lower level of labile P compared with moderately labile and non-labile fractions, caused by the high P adsorption capacity (Alleoni et al., 2014; Lopes and Guilherme, 2016; Withers et al., 2018). The SS application changed this pattern and improved soil P lability (**Figure 2**). Fifteen months after application, SS at recommended dose

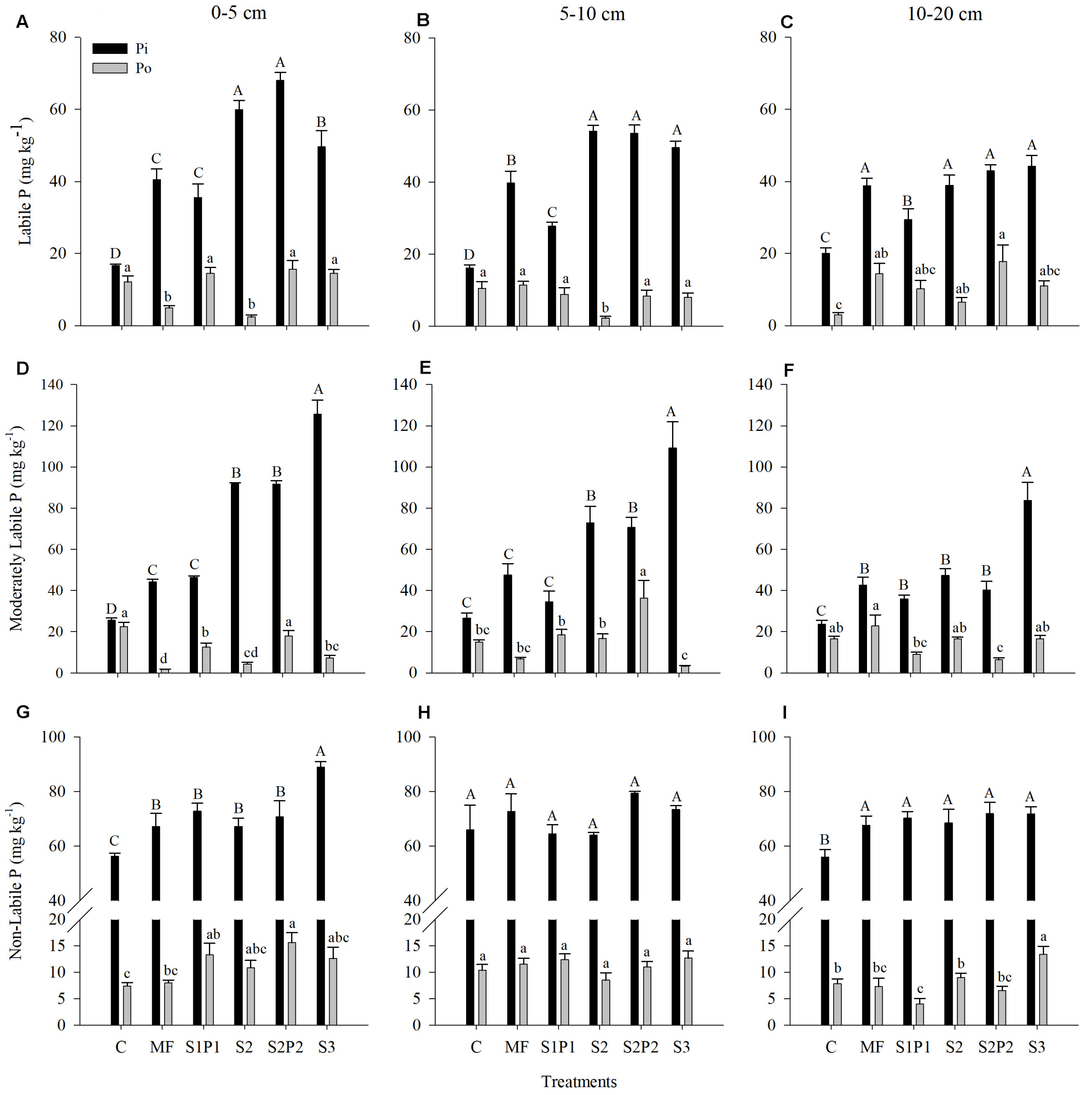

FIGURE 2 | Inorganic (P_i) and organic (P_o) labile **(A–C)**, mederately **(D–F)** and non-labile **(G–I)** phosphorus in soil, 15 months after sewage sludge application (SS was applied 7 months after *E. urograndis* planting). C, control; MF, mineral fertilizer; S1P1, sludge (50%) + P (83%) + B + K; S2, sludge (100%), P + B + K; S2P2, sludge (100%) + P (66%) + B + K; S3, sludge (150%) - P + B + K. Bars followed by the same letter, uppercase to P_i and lowercase to P_o, do not differ by the Duncan test ($p \leq 0.05$). Vertical lines on bars represent standard error ($n = 4$).

with or without soluble triple superphosphate P (S2 and S2P2 treatments), and SS at 150% of dose (S3 treatment) positively altered the pattern of soil P distribution, with more P in the labile and moderately labile fractions than non-labile fraction, at 0–5, 5–10, and 10–20 cm horizon depths, compared with mineral fertilizer, with soluble triple superphosphate P.

For short to medium term, 15 months after SS application, there were higher concentrations of P in labile and moderately labile pools in S2, S2P2, and S3 treatments, mostly in inorganic P forms, up to 20 cm depth (**Figure 2**), explained with a very high positive correlation ($r = 0.97$, $p < 0.01$) with amount of SS-P applied to soil in organic forms, corresponding to 566 and 848 kg ha^{-1} of organic P. Application of organic matter to highly weathered soils generally decreases P adsorption and increases P lability (Novais and Smyth, 1999; Laboski and Lamb, 2003), as some absorbing sites are occupied by organic ligands with

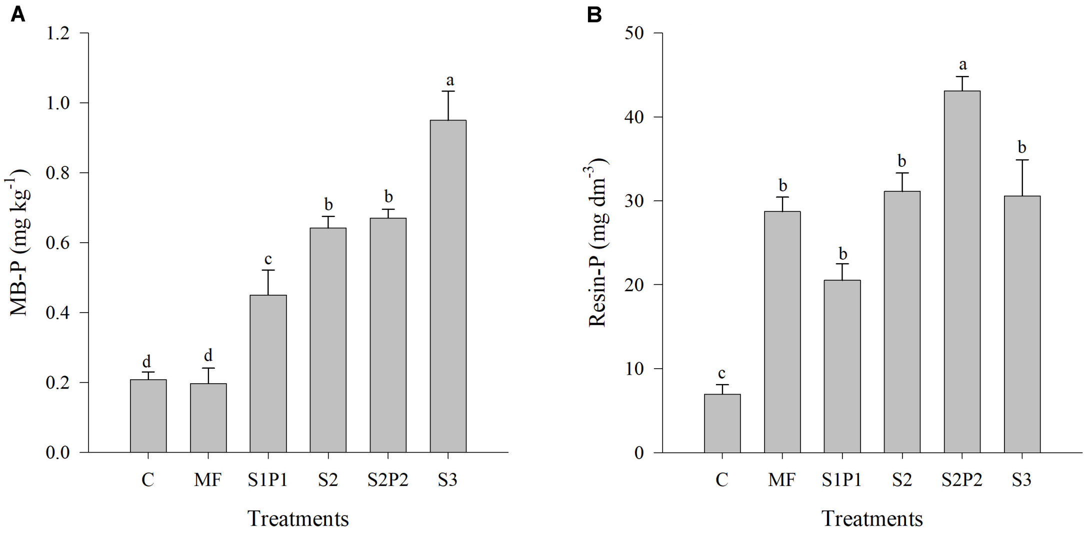

FIGURE 3 | Phosphorus in the microbial biomass **(A)**, at 0–5 cm horizon depth, and P availability, at 0–20 cm horizon depth, assessed by resin **(B)** in soil, 15 months after sewage sludge application (SS was applied 7 months after *E. urograndis* planting). C, control; MF, mineral fertilizer; S1P1, sludge (50%) + P (83%) + B + K; S2, sludge (100%) - P + B + K; S2P2, sludge (100%) + P (66%) + B + K; S3, sludge (150%) - P + B + K. Bars followed by the same letter do not differ by Duncan test ($p \leq 0.05$). Vertical lines on bars represent standard error ($n = 4$).

TABLE 4 | Production of 22-month-old *E. urograndis* plantation in a soil treated with class B sewage sludge 7 months after planting (mean ± standard error, $n = 4$).

Treatment	Volume (m^3 ha^{-1})	Wood (Mg ha^{-1})	Bark (Mg ha^{-1})	Branch (Mg ha^{-1})	Leaves (Mg ha^{-1})	Total (Mg ha^{-1})
C	55.7 ± 1.2 c	20.1 ± 0.5 c	2.2 ± 0.05 c	6.2 ± 0.15 c	3.5 ± 0.09 c	32.0 ± 0.7 c
MF	62.0 ± 1.1 a	22.6 ± 0.4 a	2.4 ± 0.05 a	7.0 ± 0.15 a	4.0 ± 0.09 a	36.0 ± 0.7 a
S1P1	57.9 ± 1.8 bc	21.0 ± 0.7 bc	2.3 ± 0.08 bc	6.5 ± 0.23 bc	3.6 ± 0.14 bc	33.4 ± 1.1 bc
S2	58.2 ± 0.7 bc	21.1 ± 0.3 bc	2.3 ± 0.03 bc	6.5 ± 0.09 bc	3.7 ± 0.05 bc	33.6 ± 0.4 bc
S2P2	60.5 ± 1.0 ab	22.0 ± 0.4 ab	2.4 ± 0.04 ab	6.8 ± 0.13 ab	3.8 ± 0.08 ab	35.0 ± 0.6 ab
S3	58.0 ± 0.9 bc	21.0 ± 0.4 bc	2.3 ± 0.04 bc	6.5 ± 0.12 bc	3.7 ± 0.07 bc	33.5 ± 0.6 bc
CV (%)	3.6	3.9	3.9	4.2	4.3	4.3

C, control; MF, mineral fertilizer; S1P1, sludge (50%) + P (83%) + B + K; S2, sludge (100%) - P + B + K; S2P2, sludge (100%) + P (66%) + B + K; S3, sludge (150%) - P + B + K. Mean values followed by different letters, in the column, are different by Duncan test ($p \leq 0.05$).

high binding energy. In addition, the organic compounds are rapidly mineralized, releasing more P_i to soil solution (Novais et al., 2007). Thus, when SS is applied, there was a greater contribution to the increment of soil P_i in labile and moderately labile pools (**Figure 2**) when there was a greater labile plus moderately labile P input.

Indeed, 15 months after application, the greater contribution of SS application, except at the lower dose (S1P1), was the buildup of a soil moderately labile P pool (**Figures 2D–F**), which has an important nutritional and ecological advantage (Rodrigues et al., 2016; Withers et al., 2018). However, the greater or lesser contribution to soil available P is dependent on the characteristics of soil and applied organic material, and its dose (Abreu-Junior et al., 2005; García-Albacete et al., 2012; Borges et al., 2019).

The microbial community is responsible for the conversion of organic to its inorganic forms, by solubilization and mineralization processes (Shi et al., 2019), so microbial P concentration would be an indicator of short- to long-term potential of soil in maintaining P availability to plant nutrition. There was also a close relation between organic P and soil microbial P concentration ($r = 0.89$, $p < 0.01$), as SS dose increased, with no mineral fertilizer effect, decreasing the following order of treatments: S3 > S2P2 = S2 > S1P1 > MF = C (**Figure 3A**). Rheinheimer et al. (2000) reported a flow from 7 to 26 kg ha^{-1} per year of P in no-tillage system on three different soils of the tropical region, showing the importance of soil microbiota for P availability to plants and cycling. Also, some microorganisms, already present in the soil and/or supplied by SS, have the ability to solubilize P that is chemically bonded to Fe and Al oxides, playing a fundamental role in soil P maintenance and availability, particularly in the tropical and degraded soils (Wu et al., 2007). Despite the great contribution of SS to soil microbial activity, microbial biomass P concentrations were lower than those reported in the literature, for clays and organic soils of temperate regions (Rheinheimer et al., 2000; Wu et al., 2007; Sugito et al., 2010; Shi et al., 2019). So, the lower MB-P values in our study may be explained by field conditions, with soil sampling at the 0–5 cm depth of a superficial sand

TABLE 5 | Concentration and accumulation of P in 22-month-old *E. urograndis* plantation in a soil treated with class B sewage sludge 7 months after planting (mean ± standard error, $n = 4$).

P concentration (g kg^{-1})

Treatment	Wood	Bark	Branches	Leaves
C	0.36 ± 0.009 a	0.81 ± 0.03 a	0.40 ± 0.02 c	1.47 ± 0.05 a
MF	0.33 ± 0.015 a	0.83 ± 0.04 a	0.64 ± 0.06 a	1.38 ± 0.14 a
S1P1	0.39 ± 0.011 a	0.78 ± 0.02 a	0.60 ± 0.05 a	1.41 ± 0.06 a
S2	0.37 ± 0.007 a	0.77 ± 0.03 a	0.57 ± 0.05 ab	1.46 ± 0.10 a
S2P2	0.35 ± 0.015 a	0.82 ± 0.03 a	0.44 ± 0.05 bc	1.43 ± 0.03 a
S3	0.36 ± 0.013 a	0.76 ± 0.03 a	0.56 ± 0.03 ab	1.54 ± 0.11 a
CV (%)	7.3	7.7	17.0	5.1

P accumulation (kg ha^{-1})

Treatment	Wood	Bark	Branches	Leaves	Total
Control	7.2 ± 0.15 a	1.8 ± 0.03 a	2.5 ± 0.17 c	5.1 ± 0.16 a	16.6 ± 0.43 b
MF	7.4 ± 0.30 a	2.0 ± 0.08 a	4.5 ± 0.46 a	5.4 ± 0.28 a	19.3 ± 0.39 a
S1P1	8.1 ± 0.10 a	1.8 ± 0.09 a	3.8 ± 0.19 ab	5.1 ± 0.21 a	18.9 ± 0.18 a
S2	7.8 ± 0.19 a	1.8 ± 0.07 a	3.7 ± 0.35 ab	5.4 ± 0.23 a	18.7 ± 0.66 a
S2P2	7.7 ± 0.21 a	1.9 ± 0.09 a	3.0 ± 0.35 bc	5.5 ± 0.07 a	18.3 ± 0.31 a
S3	7.5 ± 0.35 a	1.7 ± 0.05 a	3.7 ± 0.25 ab	5.6 ± 0.21 a	18.5 ± 0.64 a
CV (%)	6.9	8.2	18.1	6.4	5.4

C, control; MF, mineral fertilizer; S1P1, sludge (50%) + P (83%) + B + K; S2, sludge (100%) - P + B + K; S2P2, sludge (100%) + P (66%) + B + K; S3, sludge (150%) - P + B + K. Mean values followed by different letters, in the column, are different by Duncan test ($p \leq 0.05$).

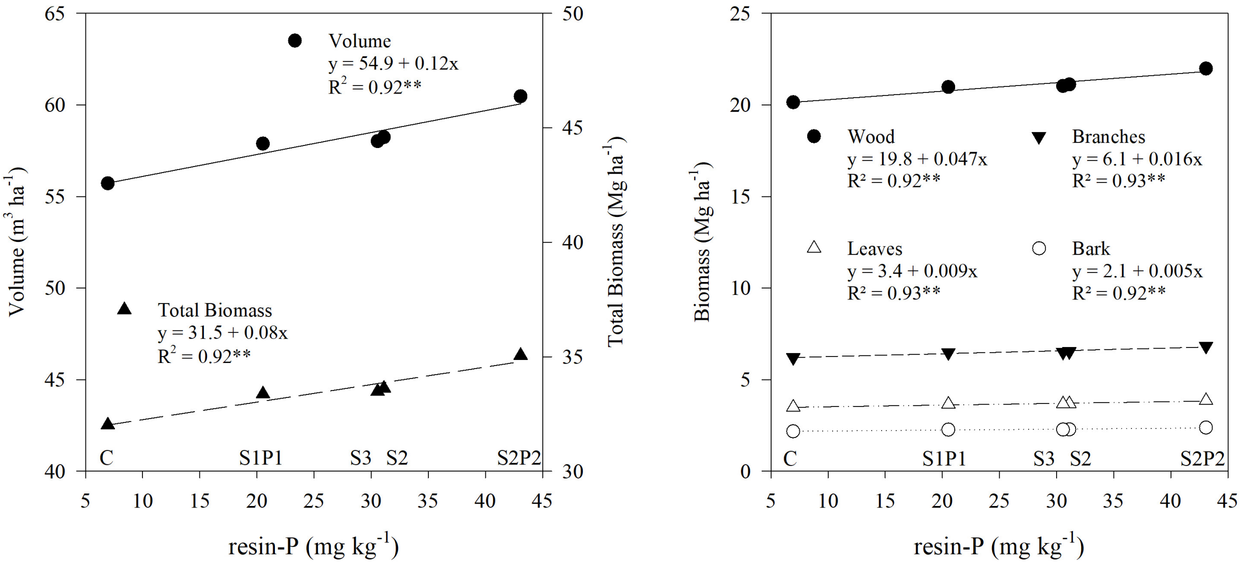

FIGURE 4 | Production of wood volume and biomass of wood, bark, branches and leaves in 22-month-old *E. urograndis* plantation related with soil P availability (resin-P), 15 months after sewage sludge application (SS was applied 7 months after *Eucalyptus* planting) ($n = 5$). C, control; MF, mineral fertilizer; S1P1, sludge (50%) + P (83%) + B + K; S2, sludge (100%) - P + B + K; S2P2, sludge (100%) + P (66%) + B + K; S3, sludge (150%) - P + B + K. $^{**}p < 0.01$.

horizon (90% of sand, **Table 1**), in a very dry season, in September 2016, following 2–3 months with no rain. The influence of seasonality was observed by Haripal and Sahoo (2014), where the rainy season had higher soil P-MB values than the dry season, due to drought stress. This also might have resulted from the pretreatment of our soil samples before analysis (freezing rather than cold storage).

Concerning soil P availability, after 15 months of application, all treatments with SS had resin-P at a high concentration (>16 mg dm^{-3}) (**Figure 3B**) for forest plantation (Raij et al., 1997), mainly because of the mineralization of exogenous organic P added to soil by SS (Condron and Newman, 2011; Weihrauch and Opp, 2018), while the control still kept resin-P at an average level (from 6 to 8 mg dm^{-3}), due to

TABLE 6 | Doses of nitrogen, phosphate, potassium, and boron mineral fertilizer applications for *E. urograndis* planting, as a function of the sewage sludge doses, based on the N criterion.

Dose of SS application[1] (Mg ha^{-1})	Dose of mineral fertilizer (kg ha^{-1})				Estimated relative wood volume productivity[2] (%)
	N	P	K	B	
0	192	26	137	6.5	100 a
29	0	17.5	137	6.5	98 ab
29	0	0	137	6.5	94 bc
43.5	0	0	137	6.5	94 bc
14.5	0	22	137	6.5	93 bc
0	0	0	0	0	90 c

[1] *The dose of sewage sludge (dry base) based on the N criterion (Brasil, 2006).*
[2] *Relative production of 100% is equivalent to a wood volume estimated at 62 m^3 ha^{-1} for E. urograndis grown for 22 months under minimum tillage and conventional application of lime and NPK fertilizers, at the recommended dose. Relative productivity means followed by different letters are different by Duncan test ($p \leq 0.05$).*

P-litter recycling (Vargas et al., 2019) and low P adsorption capacity attributed to low clay content in the soil (**Table 1**). However, the application of SS, at the recommend dose by N criterion complemented with 66% of the triple superphosphate P recommended dose (S2P2 treatment) had the best improvement on soil P availability. This was attributed to the SS organic P mineralization and the positive effect of the interaction between the organic and mineral sources of P on soil P availability, higher than when they are applied alone (Munhoz et al., 2011; Borges et al., 2019), besides lowering P adsorption in soil as Al and Fe are complexed by exogenous organic matter from the SS (Kahiluoto et al., 2015).

Sludge applied at the 50% of recommended dose supplemented with 83% of triple superphosphate P recommended dose (S1P1 treatment), at the recommend dose (S2 treatments), and at 150% of the recommend dose (S3 treatment) were as effective as mineral fertilization (after 22 months of application, at *Eucalyptus* planting) in increasing soil P availability (**Figure 3B**). This effect of mineral fertilizer is also explained by the soil low P adsorption capacity at the superficial sand mineral horizon. There was higher P availability as indicated by a larger amount of P-resin in the soil after the application of phosphate fertilizer in sandy soils as compared to the highly weathered clay soils (Sugihara et al., 2016; Martins et al., 2018). SS application at the recommended dose (29 Mg ha^{-1}) added 75 kg ha^{-1} of labile P_i to the soil, corresponding to 75 mg dm^{-3} of labile P_i, at the 0–10 cm soil depth (**Figures 2A,B**). Moreover, the SS P_o could be slowly mineralized to contribute to the labile P pool (**Figure 2**), thus reducing P fixation by adsorption (Novais and Smyth, 1999; Laboski and Lamb, 2003) and increasing soil P_i availability (**Figure 3B**) even in 15 months after SS application.

As most of soil inorganic P in the SS treatments was in the moderately labile fraction, with higher P_i/P_o ratio (**Figures 2D–F**), it is expected that there will be a continuous long-term release of available inorganic P into the soil solution and taken up by plants, thus reducing the risk of loss by transforming to non-labile forms. This result was corroborated by Florentino et al. (2019) who reported high soil resin-P concentration even after more than 17 years of sewage sludge application in four fields of *Eucalyptus* plantations, as compared to the control and mineral fertilization treatments. These findings reinforce the importance of soil inorganic moderately labile P fraction for the long-term effect of SS application on legacy P.

Our results (**Figures 1–3**) and those obtained by Florentino et al. (2019) provide evidences that labile, moderately labile, and organic non-labile P in the SS are important sources for short- to long-term soil P_i supply and building up legacy P in soil. Considering that soil P availability is estimated by resin extractable P and the length of *Eucalyptus* rotation, of 6 years, it is reasonable to suggest the following soil P dynamic after SS application: (1) labile SS P continuously supplied the short-term available P to plants (e.g., in the 12 months after SS application); (2) moderately labile SS P fraction maintained the labile plus moderate labile soil P pools, as the medium-term available P to plants (e.g., from 12 to 36 months of SS application); and (3) non-labile SS P compounds, specially organics through the biological soil cycles, might supply the non-labile, moderately labile, and labile soil P pools as the long-term plant available P sources (e.g., after 36 months of SS application), in other words, the legacy P in soil. Such P dynamics for tropical soils treated with SS agrees with the reports by Munhoz et al. (2011); Alleoni et al. (2014), and Foltran et al. (2019).

Production and P Concentration and Accumulation in *E. urograndis* Plantation as a Function of Sewage Sludge Application

In the present study, for a 22-month-old *E. urograndis* trees, higher production of wood volume and biomass was achieved by application of (**Table 4**) (i) SS at the recommended dose supplemented with mineral P fertilizer (S2P2; 60.5 m^3 ha^{-1}) and (ii) mineral fertilizers for high yield (MF; 62 m^3 ha^{-1}). This is in accordance with a 23-month-old *E. grandis* with a wood volume production of 62 m^3 ha^{-1}, when SS was applied according to N criterion and supplemented with 50% of P recommendation (Abreu-Junior et al., 2017). However, in our study, 56 m^3 ha^{-1} of wood was achieved by the control treatment (**Table 4**), while, in the 23-month-old *E. grandis* experiment (Abreu-Junior et al., 2017), 30 m^3 ha^{-1} of wood was recorded by the control, corresponding to 90 and 48% of the maximum production observed (**Table 6**).

The low growth difference of *E. urograndis* up to 22 months between SS and mineral fertilizer treatments with the control (**Tables 4**, **6**) may have been caused by two reasons, among the various biotic and abiotic factors that influence plant development (Chen et al., 2011). The first is related to the soil resin-P concentration, which was 7 mg dm^{-3} at the control, indicating a medium level of P (6–8 mg dm^{-3}) for *Eucalyptus* growth (Raij et al., 1997). Thus, contributing to medium response of *E. urograndis* to the fertilization. Ferraz et al. (2016) observed a 75% lower wood volume production at the control (without fertilization) in relation to SS treatment. In this case, soil available P concentration was 2 mg dm^{-3}, corresponding to low level of P (0–2 mg dm^{-3}) in the surface mineral horizon (0–5 cm) for *Eucalyptus* growth, thus contributing to high response of *E. grandis* to the fertilization. The second point is related to the interaction of *E. urograndis* genotype with precipitation (soil moisture) and nutrient availability in the soil environment. Precipitation during the first and second year after plantation was 1,442 and 926 mm, respectively; they were mainly concentrated between November and March, providing low soil water availability. This may lead to reduced absorption and nutrient efficiency as well as to issues in light use by plants, thus limiting the full expression of potential production of *Eucalyptus* plantation (Stape et al., 2008; Elli et al., 2019). Water stress may lead to physiological and metabolic disorders in the *Eucalyptus* plants, thus compromising its development (Tariq et al., 2019). The planted material (clone SP5727 owned by Suzano, *E. urograndis*) was genetically selected for wood production on dry and infertile soil. Consequently, this clone has low fertilizer response under dry periods, differently from other *Eucalyptus* genotypes that have higher dry matter on roots instead of canopy when growing in dry soil, especially with no P fertilization (Tariq et al., 2019).

According to Abreu-Junior et al. (2017), as time goes by and SS mineralization continuously releases N and P to the soil, the application of SS on 100% of the N supplemented with 66% of the P had an increase of 7% in the relative wood volume production for 44-month-old *E. grandis* trees (178 m^3 ha^{-1}), compared to mineral NPK fertilization (166 m^3 ha^{-1}). For a 42-month-old *E. urograndis* trees, at the same experiment of this study, application of SS on 100% of the N supplemented with 66% of the P (S2P2; 210 m^3 ha^{-1}) had an increase of 5 and 11% in relative wood production compared to MF and control treatments (199 and 188 m^3 ha^{-1}), respectively (Mandu, 2020). These indicated a high potential for wood productivity in *Eucalyptus* plantations, growing in infertile tropical soils treated with sewage sludge, with yield comparable with those of mineral fertilization for high production.

The soil fertility, *E. urograndis* genotype, and precipitation, as explained above, also reflected in the P concentration and accumulation in *Eucalyptus* tree compartments. It was noticed that SS and MF treatments generally had the same pattern for P concentration and accumulation in the wood, bark, branches, leaves, and total biomass. The P concentration in leaves was higher (mean value of 1.45 g kg^{-1}) than those usually representing the appropriate range (0.9–1.3 g kg^{-1}; Raij et al., 1997). This can be related by both available soil P and by the higher volume of soil explored by deep fine *Eucalyptus* roots. However, these values refer to mature fully developed plants, with leaves sampled at 22 months of age, when there is still high demand and absorption of nutrients by plants (Laclau et al., 2010; Ferraz et al., 2016; Foltran et al., 2019). As a matter of fact, Crous et al. (2019) observed a reduction in *Eucalyptus* foliar P concentration with increasing planting age in medium P availability soil.

The most important result, besides the relative low growth rate, is that the sludge application, 7 months after *Eucalyptus* planting, based on N criterion and complemented with mineral P, brought production gains up of 4.8 m^3 ha^{-1} of wood volume, 1.9 Mg ha^{-1} of wood, 0.2 Mg ha^{-1} of bark, 0.6 Mg ha^{-1} of branches, and 0.3 Mg ha^{-1} of leaves biomass, with a total of 3.0 Mg ha^{-1} of biomass on a 22-month-old *E. urograndis* trees (**Table 4**). Additionally, SS application (i) increased concentration of available P from 7 to 43 mg kg^{-1}, in soil surface Ap horizons (0–20 cm; **Figure 4**), while (ii) reducing by 33 and 100% the use of mineral P and N fertilizer, respectively (**Table 6**). These results demonstrated that the use of sewage sludge in commercial *Eucalyptus* plantations can improve soil P availability and wood yield by combining sludge and mineral P fertilizer doses, as compared to conventional NPK fertilization. This outcome strongly reinforced previous results and recommendations proposed by Abreu-Junior et al. (2017).

CONCLUSION

The sewage sludge contained different pools of labile and non-labile P that can be used to support the short- to long-term soil P availability to plants and to build up a legacy P in soil, when applied at the N criterion dose. Application of SS at the recommended dose with or without triple superphosphate P and at the 150% of recommended dose positively altered the pattern of soil P distribution, with more P in the labile and moderately labile fractions than non-labile fraction, along the 0–20 cm depths (corresponding to the mineral Ap surface horizon), as compared with triple superphosphate application alone. Consequently, there were higher P concentration in the microbial biomass and soil available P pools, indicating that P cycling in the system and microbial community is enhanced by SS application and soil P availability is improved. Production of wood volume and biomass of tree compartments were highly correlated with soil P availability, as a function of SS dose. SS application at the recommend dose and supplemented with 66% of mineral P resulted in the highest gains of volume and biomass of wood, while reducing by 33 and 100% the use of mineral P and N fertilizer, respectively. The application of SS combined with mineral P fertilizer in commercial *E. urograndis* plantations can increase soil P availability and subsequent wood production, comparable to NPK-micronutrient fertilization for high yield.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

AF, CA-J, FO, and MO: conception and design of the work. AF, MO, and TM: acquisition of data. AF, CA-J, JR, MO, and

PC: analysis and interpretation of data. CA, CA-J, FO, GC, JR, JS, PC, TN, and ZH: drafting and revising the work. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2020.00116/full#supplementary-material

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Distribution of Soil Phosphorus Fractions as a Function of Long-Term Soil Tillage and Phosphate Fertilization Management

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The forms in which phosphorus (P) accumulates in soils are dependent on management practices, fertilizer sources, and methods of application, which may promote distinct P solubility and plant uptake. We aimed here to evaluate how soil tillage and phosphate fertilization strategies affected soil P fractions over 17 years and to identify best management practices for improving labile P fractions. The experiment was conducted in a very clayey Rhodic Ferralsol (Oxisol) with initially very low P availability, during 17 years under soybean and corn, fertilized with 35 kg P ha^{-1} $year^{-1}$. Treatments were two soil management systems (CT-conventional tillage and NT-no-till) and four phosphate fertilization strategies (TSP – triple superphosphate or RRP – reactive rock phosphate, applied to the crop furrow or broadcast). Soil samples were taken at five depth layers, and organic (Po), inorganic (Pi), and total P (Pt) were determined by Hedley's sequential fractionation. CT resulted in a more homogeneous distribution of Pi fractions throughout the soil profile, while under NT there was a steep depth gradient characterized by Pi accumulation in the fertilizer application zone. NT resulted in accumulation of Pi in more labile fractions and higher accumulation of Po physically protected by aggregation, both compared to CT. Also, under NT with RRP, there was a great accumulation of Pi associated with calcium (HCl Pi) compared to TSP, especially when the fertilizer was broadcast applied. An accumulation of Po down to 20 cm (CT) and 10 cm (NT) was also detected, compared to Cerrado natural soil. NT and RRP positively affected legacy P fractions and can be recommended as strategies to improve P fertilizer use efficiency.

Keywords: no-tillage, P fractionation, legacy P, P source, P distribution

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INTRODUCTION

Brazilian Cerrado (savanna) soils originally present low phosphorus (P) availability for cropping, making the addition of phosphate fertilizers mandatory, firstly to build up P availability to acceptable levels before cropping and secondly to maintain the available levels during subsequent growing seasons, through periodic fertilization to replace the P exported by harvesting

(Kurihara et al., 2016). The reason is that Cerrado soils have a high solid phase P retention capacity, due to their high Fe and Al sesquioxide constitution, capable of retaining phosphate ions in less labile forms (Fink et al., 2014, 2016a). Consequently, a high fertilizer P input, at a rate above that exported by the crop harvested products, is demanded when establishing the cropping system under these low initial soil test P (STP) soils, to improve P contents and compensate the soil solid phase P sink (de Oliveira et al., 2020a), which is significantly higher than plant demand (Novais et al., 2007). This high P input is usually called corrective P fertilization, and P rates depend on STP and soil buffer capacity (Sousa et al., 2010). Excessive fertilization after the P correction phase or incorrect agricultural practices, as observed for soybean monoculture under conventional tillage (de Oliveira et al., 2019) and/or in the absence of cover crop in the rotation system (Sousa et al., 2010), may promote even more legacy P accumulation, with very low P use efficiency under these production systems.

The soil P dynamics are interdependent and related to an organic and inorganic equilibrium and are under the influence of various factors related to the climate, soil type, and management practice. Regarding climate, the main factors are those related to fertilizer solubilization and SOM mineralization, such as the water regime and temperature (Sharpley, 1985). The soil's mineralogy and texture define the kind of P chemical reaction after fertilizer addition, as well as the intensity of accumulation under distinct fractions (Sharpley, 1985; Huffman et al., 1996; Rheinheimer and Anghinoni, 2001, 2003; Pavinato et al., 2009). In terms of management practices, soil tillage system (Zheng et al., 2001; Tiecher et al., 2012a,b), the chemical and physical nature of the fertilizer (Zheng et al., 2001; Pavinato et al., 2017), the degree of fertilizer–soil contact (Santos et al., 2008), the rate applied (Zhang et al., 2004; Gatiboni et al., 2007), and the contribution of plant biomass (Tiecher et al., 2012a,b) are the most influential factors. All of these factors, combined, may affect soil legacy P characteristics.

The minimal soil disturbance under no-till (NT) has promoted the accumulation of nutrients in the top surface, especially those of low mobility, such as P from fertilizers and amendment residues (Selles et al., 1997; De Maria et al., 1999; Santos and Tomm, 2003). This phenomenon is intensified when the phosphate fertilizer is broadcast applied and/or slow-release sources are used, such as natural reactive rock phosphates (RRP) (Nunes et al., 2011b). Otherwise, the conventional tillage system (CT) results in the homogenization of P and SOM in the plowed layer (Selles et al., 1997; De Maria et al., 1999; Santos and Tomm, 2003), which exposes P to new adsorption sites (Sousa and Volkweiss, 1987a) and accelerates fertilizer sorption (Miranda et al., 2005), as well as SOM mineralization (Nunes et al., 2011a).

Soil P fractions have been successfully grouped and quantified (although not identified) by sequential chemical extractions, with the widely used Hedley's P fractionation procedure, by its ability to concomitantly determine Pi and Po fractions (Hedley et al., 1982). The procedure involves subjecting the P in the soil to successive extractions in solutions of increasing extraction strength. Accordingly, each fraction of P relates to a chemical form of P in the soil (Hedley et al., 1982; Cross and Schlesinger, 1995), resulting in estimates of its lability. In this way, this procedure helps assess how management practices may transform P dynamics in the soil.

There are many reasons for adopting the NT system in tropical weathered soils, and many studies have evaluated its benefits in soil biology, physics, and diverse aspects of soil chemistry and fertility. However, the dynamics of P in soil and the relative contribution of its fractions to crop nutrition have not yet been specifically related to the positive benefits of this conservationist system in tropical soils. In addition, the use of rock phosphates has been restricted to corrective fertilization or low-cost fertilizations, due to concerns related to calcium-bound phosphate source efficiency, especially in NT, even though there is a lack of experiments assessing these concerns.

This study aimed to evaluate how soil tillage, phosphate source, and mode of application affected soil P fractions after 17 cropping years in an Oxisol considered highly adsorptive for P. These three management factors are directly related to the different reactions of P in soil and consequently may significantly affect legacy P characteristics, including depth distribution and lability and, ultimately, potential bioavailability to plants. This information is useful for orientating P management decisions in agricultural systems where significant legacy P occurs.

MATERIALS AND METHODS

Description of the Experimental Area

The experiment was conducted at the experimental area of Embrapa Cerrados, Planaltina-DF, Brazil (15°36′S; 47°42′W; 1014 m elevation). The regional climate is classified as Cwa according to Köppen (Alvares et al., 2013), with an average annual precipitation of 1570 mm and temperature ranging between 15.9 and 26.4°C. The soil is classified as Latossolo Vermelho Distrófico according to the Brazilian Soil Classification System (EMBRAPA, 2013), or Rhodic Ferralsol (IUSS Working Group WRB, 2006), containing 64% clay, with a predominance of kaolinite (634 g kg^{-1}), gibbsite (195 g kg^{-1}), and hematite (108 g kg^{-1}) in the clay fraction. Naturally, it is a nutrient-poor soil (**Table 1**), whose initial P content in the 0–20 cm layer (0.9 mg kg^{-1}; Mehlich-1) is very low (Sousa and Lobato, 2004). The original vegetation was Cerrado, with smooth undulating relief. For experiment establishment, all deficiencies besides P were corrected with the application of dolomitic limestone to increase the base saturation to 50%, agricultural phosphogypsum (3,000 kg ha^{-1}), potassium (42 kg K ha^{-1}), and micronutrients to obtain high annual crop yields, according to recommendations for the region (Sousa and Lobato, 2004). According to these authors, phosphate fertilizer recommendation, for the clay content and initial Mehlich-1 P levels found in the experimental area, would be 183 kg P ha^{-1} as corrective fertilization plus 35 kg ha^{-1} as maintenance fertilization.

Experimental Design

A randomized split-plot block design was used, with two soil management systems, CT and NT, as main plots and four strategies of phosphate fertilization management, combining

TABLE 1 | Soil chemical parameters[a] (0–20 cm layer) in two moments prior to the experiment establishment, before and after liming.

Year	pH_{H2O}	P	K	Ca	Mg	Al	H + Al	CEC[b]	V[c]	OC[d]	Clay[e]	Silt[e]	Sand[e]
		$mg\ kg^{-1}$	$cmol_c\ kg^{-1}$						%	$g\ kg^{-1}$			
1985	4.5	0.9	0.10	0.82	0.20	1.40	9.13	10.25	10.9	17.0	640	90	270
1994	5.4	1.0	0.11	2.41	2.01	0.08	5.27	9.80	46.2	16.2	–	–	–

[a]*According to procedures later summarized in Embrapa (1997), mean values, n = 3.* [b]*Cation exchange capacity at pH 7.* [c]*Base saturation.* [d]*Organic carbon by the Walkley–Black procedure.* [e]*Parameters from the 40–60 cm layer.*

two sources of P (TSP and RRP) and two placement methods (crop furrow and broadcast application) as subplots. The trial, therefore, consisted of eight treatments, with three replicates, all fertilized annually with 35 kg ha^{-1} of total P. This P dose was chosen so that it would allow a small but continuous soil P stock gain according to the expected yields. Plots measured 16 × 8 m (128 m^2) and were split into subplots of 8 × 4 m (32 m^2). In the center of each subplot, a useable area of 15 m^2 for soybean and 12 m^2 for maize was delimited for harvest procedures.

The experiment was initiated in the 1994/95 season, with the cultivation of soybean/corn as main (summer) crops in the following chronological order: soybean for the first nine years, corn in the 10th and 11th years, and then rotation of soybean and corn until the 17th crop (corn). After 2003 (9th soybean cultivation), millet (*Pennisetum glaucum*) was cultivated as a winter cover crop, with fertilization of 45 kg ha^{-1} of N in the form of urea. Plots under CT were plowed annually with a disk plow (to a depth of up to 20 cm) and harrow leveled before sowing the summer crop. Plots under NT were always directly sown over the crop residues. Soybeans and corn grains were harvested, while crop residues remained in the respective plots. The cover crop plant (millet) residues remained as mulch in NT and were incorporated in the soil in CT. These crop sequences are similar to those adopted by farmers in the region, involving a sequence of soybean crops after clearing a native vegetation area, followed by eventually introducing the corn crop.

The TSP used contained 20.8% total P, 92% of which was soluble in a 2% citric acid solution. The RRP consisted of 12.3% total P, 44% of which was soluble in 2% citric acid (1:100 phosphate:solution ratio, RRP particles analyzed at <0.063 mm). Thus, the RRP used in this study was highly reactive, as a consequence of the high substitution rate of carbonate to phosphate in the crystal structure (Zapata and Roy, 2004). Most RRP particles applied to the field were between 0.5 and 2.8 mm.

Maintenance fertilization was done annually in the summer crop with 66.7 kg ha^{-1} of K in the form of KCl, 30 kg ha^{-1} of S as phosphogypsum, and in the case of corn, 30 kg ha^{-1} of N was applied in the planting furrow along with two posterior applications with 60 kg ha^{-1} of N at 4–5 and 7–8 open leaves in the form of urea, according to Sousa and Lobato (2004).

Soil Sampling

Soil samples were taken from the 0–2.5, 2.5–5.0, 5–10, 10–20, 20–30, and 30–40 cm layers after the 17th cultivation, using a soil core sampler auger to remove intact samples for the three first layers and a Dutch auger for the two deeper layers. 15–20 subsamples were randomly taken within the useable area plots to make up the composite sample of each plot in the treatments with broadcast spread fertilization. For the treatments with band-applied fertilization in the furrow, the composite samples of each plot were formed by subsampling according to Nicolodi et al. (2002) and CQFS-RS/SC (2004). This method involves sampling from seven points across a crop row: one right over the row, and three equally spaced to each side, up to the center of the corn inter-row. The soil from each layer of these seven positions was then mixed to form a subsample. This procedure was repeated three times per plot, which were mixed to form the plot sample. This methodology was adopted because it better considers the localized effects of band-applying P fertilizers. An area under natural Cerrado vegetation located 50 m from the experiment was chosen as reference and was sampled in three replications with 20 subsamples each.

Soil Analysis

Total digestion P (Pt_{dig}) was determined in soil samples by acid digestion with H_2SO_4 and H_2O_2 in the presence of an $MgCl_2$ saturated solution (Brookes and Powlson, 1981; Hedley et al., 1982), analyzed by spectrophotometry at 820 nm (Murphy and Riley, 1962).

Total organic P (Po) in the soil was determined according to the ignition method (Hance and Anderson, 1962; Olsen and Sommers, 1982). Two subsamples were submitted to extraction for 16 h with H_2SO_4 2.0 mol L^{-1}, in the 1:8 soil:solution ratio. One subsample was previously submitted to ignition at 550°C for 1.5 h, in order to obtain total ignition P (Pt_{ign}) and the other not. Total Po was then obtained by the difference in P content between the two subsamples, analyzed by spectrophotometry at 820 nm (Thermo Fisher Scientific model Helios Omega) (Murphy and Riley, 1962).

Chemical fractionation of P was performed using the procedure originally proposed by Hedley et al. (1982) with modifications, as described below. Samples of 1.5 g soil were subjected to successive extractions with $NaHCO_3$ 0.5 mol L^{-1}, NaOH 0.1 mol L^{-1}, HCl 1.0 mol L^{-1}, and NaOH 0.5 mol L^{-1}. After extraction, the remaining soil was oven-dried at 40°C, and one part was subjected to digestion with $H_2SO_4 + H_2O_2 + MgCl_2$ (Brookes and Powlson, 1981; Hedley et al., 1982) to obtain the residual Pt (Pt_{res}). To obtain the residual Po (Po_{res}), the soil remaining after fractionation was subjected to the ignition method described above (Hance and Anderson, 1962; Olsen and Sommers, 1982). The residual Pi (Pi_{res}) was obtained by subtracting Po_{res} from Pt_{res}.

In Hedley's fractionation scheme of alkaline extracts ($NaHCO_3$ and NaOH fractions), Pi was obtained by acidifying the extract and centrifuging to precipitate the organic compounds; acidified extract Pi concentration was then determined by spectrophotometry at 820 nm (Murphy and Riley, 1962). Total P for these Hedley's fractions, on the other hand, was obtained by digesting the extract with $H_2SO_4 + (NH_4)_2S_2O_8$ in an autoclave (103.4 kPa, 121°C, 2 h) (USEPA, 1971) and determined by inductively coupled plasma atomic emission spectrometry (ICP/AES; model iCAP 6000, Thermo Fisher Scientific). The Po from these extracts was calculated by subtracting Pi from total P. The P fractions from Hedley's fractionation scheme were then categorized as geochemical (P_{geo}) and biological P (P_{bio}), according to Cross and Schlesinger (1995); the first was obtained by summing all fractions of Pi and the second by summing all fractions of Po. The sum of Pi and Po obtained by the fractionation procedure was denoted $Pt_{\Sigma\ frac}$.

Soil P Balance and P Recovery

Total P applied over the 17 years consisted of fertilizer P (17 years × 35 kg ha^{-1} $year^{-1}$ P = 595 kg P ha^{-1}) plus P provided by phosphogypsum application (13 kg P ha^{-1}), totaling 608 kg P ha^{-1}. To estimate the soil's input–output residual P, total P applied was discounted by the quantity of P exported in the grains, assessed by wet digestion using $HNO_3 + HClO_4$ (3:1 v/v) (EMBRAPA, 1999).

The variation in P stocks in the 0–40 cm layer, compared to the natural Cerrado soil, was also assessed. To perform these soil P balance calculations, soil bulk densities were evaluated in all treatments and the reference area of Cerrado in the 0–2.5, 2.5–5, 5–10, 10–15, 15–20, 20–30, and 30–40 cm layers, after the 17th cultivation according to the volumetric ring method (EMBRAPA, 2005). To calculate the inventories and consequent P balance, the methodology of mass equivalence was applied (Ellert and Bettany, 1995). P balance was determined for both Pt_{dig} and Pt_{ign} fraction contents. It was considered a discount in mass in the last sampled layer (30–40 cm), which was not affected by soil tillage or P fertilizer management. The equivalent mass in the 30–40 cm layer was calculated as follows:

$$Eq_{mass(30-40)} = M_{t(30-40)} - \left[\left(M_{t(0-40)} - M_{r(0-40)}\right)\right]$$

where $M_{t(30-40)}$ is the real soil mass in a given treatment in the 30–40 cm layer, given by its soil density multiplied by the volume occupied in a hectare; $M_{t(0-40)}$ is the sum of soil mass in the different layers evaluated down to 40 cm in the given treatment; and $M_{r(0-40)}$ is the sum of soil mass in the different layers down to 40 cm in the reference area (natural Cerrado vegetation soil).

Soil P stocks for each layer were then calculated, multiplying its P content by soil mass in the respective layer, considering the equivalent mass in the case of the 30–40 cm layer. The sum of P stocks in all evaluated layers minus that found in the reference area of the Cerrado is the final $P_{balance}$. Soil bulk densities, P contents, and mass equivalence for the different layers and treatments are shown in **Supplementary Table S5**.

P recovery calculated for a given total P fraction (Pt_{dig} or Pt_{ign}) was defined as follows:

$$P\ recovery\,(\%) = (P_{ex} + P_{balance})/(P_{applied})$$

where P_{ex} is the amount of P exported in grains after 17 crops (kg ha^{-1}); $P_{balance}$ is the gain of P in soil in the 0–40 cm layer compared to native vegetation soil (kg ha^{-1}), for a given soil P fraction analysis method; and $P_{applied}$ is the total amount of P applied as fertilizer (608 kg P ha^{-1}).

Statistical Analysis

To analyze the variance in grain yield and recovery of P, the following model was used:

$$\begin{aligned} Y_{ijk} = \mu &+ B_i + S_j + error(ij) + F_k + M_l + FM_{kl} \\ &+ SF_{jk} + SM_{jl} + SFM_{jkl} + error(ijkl) \end{aligned}$$

To analyze the variance in P levels at the different dependent variable depth, the following model was used:

$$\begin{aligned} Y_{ijklm} = \mu &+ B_i + S_j + error(ij) + F_k + M_l + FM_{kl} \\ &+ SFjk + SM_{jl} + SFM_{jkl} + error(ijkl) + C_m \\ &+ SC_{jm} + FC_{km} + MC_{lm} + FMC_{klm} + SFC_{jkm} \\ &+ SMC_{jlm} + SFMC_{jklm} + error(ijklm) \end{aligned}$$

where μ = overall data mean; B = block (i = 1, 2, 3); S = management system (j = 1, 2); F = source of P (k = 1, 2); M = type of application (l = 1, 2); C = layer (m = 1, 2, 3, 4, 5); and error = experimental error.

Analysis of variance was carried out using SAS PROC MIXED, and when significant, the Fischer's least significant difference method ($p < 0.05$) was used to differentiate the means. Moreover, a paired t-test was applied at the 5% significance level to compare differences in P stocks in the weighted 0–30 cm layer mean between each treatment and the reference area under natural vegetation. This analysis was applied using the software R (version 3.4.0) (R Core Team, 2017).

RESULTS AND DISCUSSION

Phosphorus Balance and Residual Effect

The soil and fertilization management methods, as well as the phosphate sources used over the 17 years of cropping, resulted in differences in crop yield, grain P export, and consequently residual P stocks in the soil (**Table 2**). Under NT, total grain yield was higher for soybean and maize using TSP (mean of 97.2 Mg ha^{-1} using TSP compared to 89.0 Mg ha^{-1} for RRP, mean of both application methods) (**Table 2**). Treatments with lower values of grain yield resulted in higher values of calculated (input–output) residual P. Average soybean production throughout the experiment varied between 1.06 Mg ha^{-1} in the first year, when P stocks in soil were still low, and 3.76 Mg ha^{-1} in the 14th year, which is similar to regional yields. For corn, the average yield was 11.5 Mg ha^{-1}, which is in line with high technology systems in the region.

P stocks gain in the soil in **Table 2** was calculated considering soil P contents determined by each extraction method, and soil densities, adjusting stocks by the methodology of mass equivalence (Ellert and Bettany, 1995). Total P by the ignition

TABLE 2 | Soil P balance in a clayey Oxisol cultivated for 17 years under distinct soil management systems, phosphate fertilization sources, and application methods.

Management system	Source	Method	Accumulated grain yield	P exported in grains^a (B)	Input-output residual P in soil^b	P balance in soil^c		P recovery^f	
						Pt_{ign}^{d}	Pt_{dig}^{e}	Pt_{ign}	Pt_{dig}
			kg ha^{-1}	kg P ha^{-1}				%	
CT	TSP	Broadcast	91,370	300	308	111	272	68 c	94 a
		Furrow	88,609	272	336	135	317	67 c	97 a
	RRP	Broadcast	90,142	297	311	97	279	65 c	95 a
		Furrow	86,776	269	339	172	302	73 c	94 a
NT	TSP	Broadcast	97,832	385	223	198	234	96 ab	102 a
		Furrow	96,547	368	240	183	243	91 b	101 a
	RRP	Broadcast	90,183	314	294	300	307	101 a	102 a
		Furrow	87,904	306	302	242	279	90 b	96 a

^{a}Grain yield multiplied by its P content, for each year. ^{b}Values are relative to P additions through phosphate fertilizers plus P contained in phosphogypsum (0.2% P Mg^{-1}), over 17 years, totaling 608 kg P ha^{-1}, minus P exported in grains. ^{c}Values correspond to the differences in P stocks in the 0–40 cm layer between each treatment and the Cerrado reference area, for the different P analysis methods. ^{d}Total P by the ignition method (Hance and Anderson, 1962; Olsen and Sommers, 1982). ^{e}Total P by acid digestion (Brookes and Powlson, 1981; Hedley et al., 1982). ^{f}Values correspond to P exported in grains plus P gain in soil by each soil analysis method, divided by total P applied. Different letters indicate different P recoveries according to Fischer's test ($P < 0.05$).

method (Pt_{ign}) consists in extraction with a relatively dilute H_2SO_4 solution (2.0 mol L^{-1}) of soil samples subjected to ignition at 550°C, according to Hance and Anderson (1962) and Olsen and Sommers (1982), while total P by acid digestion (Pt_{dig}) uses a concentrated H_2SO_4 solution (18 mol L^{-1}) plus H_2O_2 under heating (Hedley et al., 1982), allowing for more complete access to total soil P. As a result, P gain in the soil by the ignition method resulted, on average, in a P recovery of 67% under CT, with no difference between fertilizer sources and management methods. In contrast, the same balance performed using acid digestion (Pt_{dig}) resulted in an average 95% P recovery under the same tillage system.

Under NT, the P recovery by Pt_{ign} resulted in an average of 95% of the P applied, with a better response under broadcast application by both phosphate fertilizer sources (**Table 2**). Considering Pt_{dig}, P recovery was 100% under NT, with no difference ($p > 0.05$) between the two systems, phosphate sources and fertilization methods.

The difference between the two methods in P recovery noticed for CT but not for NT indicates an accumulation of P in more labile forms under the conservationist system, where the soil is not revolved. Under CT, some 28% of the P applied over the 17 years was in chemical forms beyond the absorption capacity of the plants, a P form accessed by the digestion method (Pt_{dig}) but not the ignition method (Pt_{ign}). In addition, through the digestion method, it can be seen that practically all the P applied to the soil over the 17-year cropping period and not taken up by the grains remained in the soil under both systems.

P Fraction Distribution Throughout the Soil Profile According to Soil Tillage and Fertilizer Management

The variance analysis (**Supplementary Table S1**) showed that inorganic fractions, rather than organic, were the most influenced by the treatments and their interaction, but residual P fractions were less influenced than the others. Also, depending on the soil layer, the most common order in which the factors influenced P fractions was first the soil management system, then the source of phosphate fertilizer, and finally the method of application. The interaction of phosphate fertilizer management systems with the soil management system is probably related to the fact that CT and NT may provide different degrees of soil/fertilizer contact, possibly modifying the dynamics of the fertilizer solubilization, especially for slowly soluble sources like RRP, which requires more time and greater soil surface contact for dissolution (Pavinato et al., 2017; de Oliveira et al., 2019). In NT, the fertilizer application method directly influences P contents in the different soil layers, while CT minimizes the effect of the placement of the phosphate applied in previous growing seasons.

The various Pi and Po fraction contents throughout the different soil layers are shown in **Supplementary Figures S1–S5**. Here summarized as geochemical (P_{geo}) and biological P (P_{bio}), respectively, it was noted that the accumulation in these distinct compartments was influenced by soil tillage and fertilizer management (**Figure 1**). Tillage and fertilizer management did not affect P fractions in the 30–40 cm soil layer, because tillage was limited to about 20 cm deep. For this reason, data is shown up to the 20–30 cm layer, with fairly similar P_{geo} and P_{bio} values for all treatments.

The highest P_{geo} and P_{bio} levels were found in the 0–2.5 cm layer, especially when RRP was broadcast applied under NT. Under CT, P contents presented a more homogeneous distribution over the soil profile, whereas under NT there was a steep depth gradient, with layers down to 5 cm showing higher values than under CT, similar values in the 5–10 cm layer, and lower values under NT compared to CT in the 10–20 cm layer. The tillage system adopted was thus the main factor affecting P_{geo} and P_{bio} contents at depth. Dynamics of fertilizer solubilization and SOM mineralization are factors closely related to tillage that may explain these differences.

For the weighted average of all layers down to 30 cm, geochemical fractions (P_{geo}) represented some 64% and biological fractions (P_{bio}) represented 36% of soil $Pt_{\Sigma\ frac}$, with a

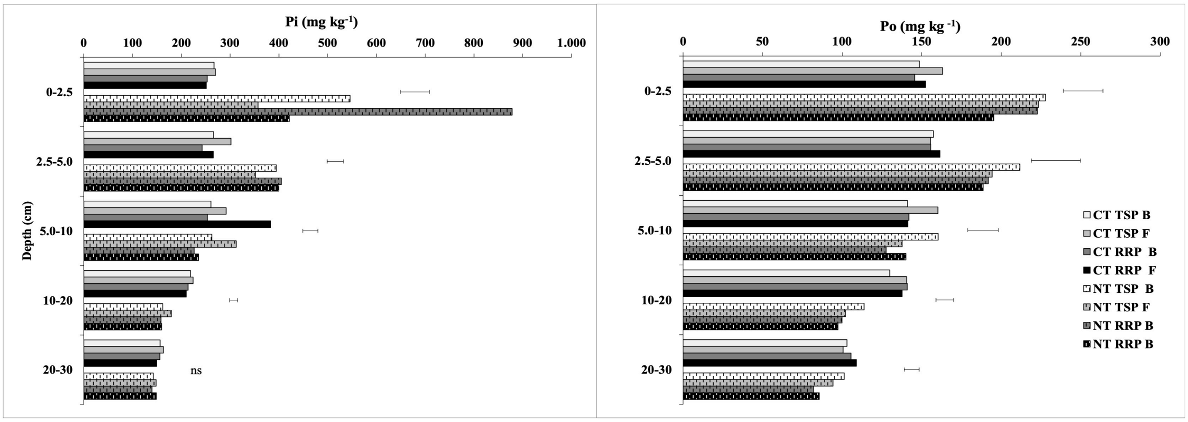

FIGURE 1 | Distribution of geochemical P (Pi) and biological P (Po) in five layers of a very clayey Oxisol after 17 years of cultivation in different soil tillage systems and phosphate fertilizer sources and application methods. CT, conventional tillage; NT, no-tillage; TSP, triple superphosphate; RRP, reactive rock phosphate; B, broadcast; F, furrow. The bars represent the LSD at 5% probability by Fischer's test.

mean of 348 mg kg^{-1}, and very similar values for the different soil and fertilization management systems. However, there were some punctual variations: for instance, where the RRP fertilizer was applied under NT by broadcast application, the amount of P_{geo} was 80% of $Pt_{\Sigma\ frac}$ in the 0–2.5 cm layer. Otherwise, in the Cerrado soil, 53 and 47% of $Pt_{\Sigma\ frac}$ were respectively present as P_{geo} and P_{bio}, with a $Pt_{\Sigma\ frac}$ mean of 268 mg kg^{-1} (**Supplementary Table S4**). Tiecher et al. (2012a), working in an area cropped for 23 years under NT and annual fertilization, found a reduction in the proportion of P_{geo} (from 57 to 49%) and consequently an increase in the proportion of P_{bio} (from 43 to 51%) in $Pt_{\Sigma\ frac}$. These authors ascribed the Po gains to a balanced P fertilization, with no excessive application beyond plant demand, and the adoption of management systems with high potential for boosting SOM using cover crops. In their work, like in Rheinheimer et al. (2019), increasing total P (Pt) and SOM contents were found to contribute to the transformation of Pi into Po under NT. In contrast, in our work, the nine years of initial soybean cultivation without cover crop rotation, when P offtake was significantly greater than P offtake (data not shown), resulted in a high accumulation of Pt (**Table 2**) and a small plant biomass input that contributed to maintaining the fertilizer P especially in inorganic fractions.

Proportional Alterations in Soil P Fractions

The adoption of NT soil management was able to promote more P accumulation in labile + moderately labile forms compared to CT in the top surface layers (**Figure 2**). Under CT, the labile + moderately labile fractions with the average weighted by the most influential fertilized layer (0–20 cm) represented around 62% of $Pt_{\Sigma\ frac}$ (average of 393 mg kg^{-1}). Otherwise, under NT with the average weighted by the most influential fertilization layer in this system (0–10 cm), this value was 69% of $Pt_{\Sigma\ frac}$ (average of 539 mg kg^{-1}), reaching 75% in the 0–2.5cm layer with broadcast TSP and 82% in the same layer with broadcast RRP.

Many studies account for the increase in P lability under NT in terms of gains in labile Po fractions (Rheinheimer and Anghinoni, 2003; Tiecher et al., 2012a). However, in our study, the greater proportion of labile + moderately labile fractions in $Pt_{\Sigma\ frac}$ evaluated under NT was resultant from Pi fraction increase, not Po. This can be seen in **Supplementary Figure S6A**, where the Pi levels of each fraction are shown in terms of the relative proportion of total Pi obtained in fractionation (P_{geo}), and in **Supplementary Figure S6B**, where the Po levels of each fraction are shown in terms of the relative proportion of the total fractionation scheme Po (P_{bio}). Under NT, Pi was present in greater amounts as labile and moderately labile Pi fractions in P_{geo} in comparison to CT (**Supplementary Figure S6A**). However, the proportion of labile and moderately labile Po fractions in P_{bio} was even slightly lower for NT when compared to CT (**Supplementary Figure S6B**). This means that NT has preferably accumulated available Pi instead of labile Po.

Under CT, the fertilizer incorporation when plowing the soil resulted in a homogeneous distribution of Pi fractions. When fertilizer was broadcast in this system, due to thorough mixing with the soil, we hypothesize that differences in solubilization speed between TSP and RRP were minimized, resulting in complete solubilization of RRP. This is shown by the similarity between these two sources in the fractions of $NaHCO_3$ Pi and HCl Pi when in CT, but not in NT (**Supplementary Figure S6**).

In NT, TSP showed a greater proportion of Pi and Po in the labile fraction NaOH 0.1 M in relation to RRP (**Supplementary Figure S6A**). Moreover, fertilizer furrow application resulted in more Pi in the region of fertilizer placement (between 2.5 and 10 cm) compared to the broadcast application. The application on soil surface has limited the solubilization of RRP, shown by the high levels of HCl Pi in the 0–2.5 cm layer (**Supplementary Figure S6A**), related to unsolubilized rock phosphate.

In CT, since the soil was completely homogenized every year together with plant root and shoot waste, the differences that may exist in the dynamics of P under CT are ephemeral, and merely the results of the year in question. Also, CT helps expose Pi to

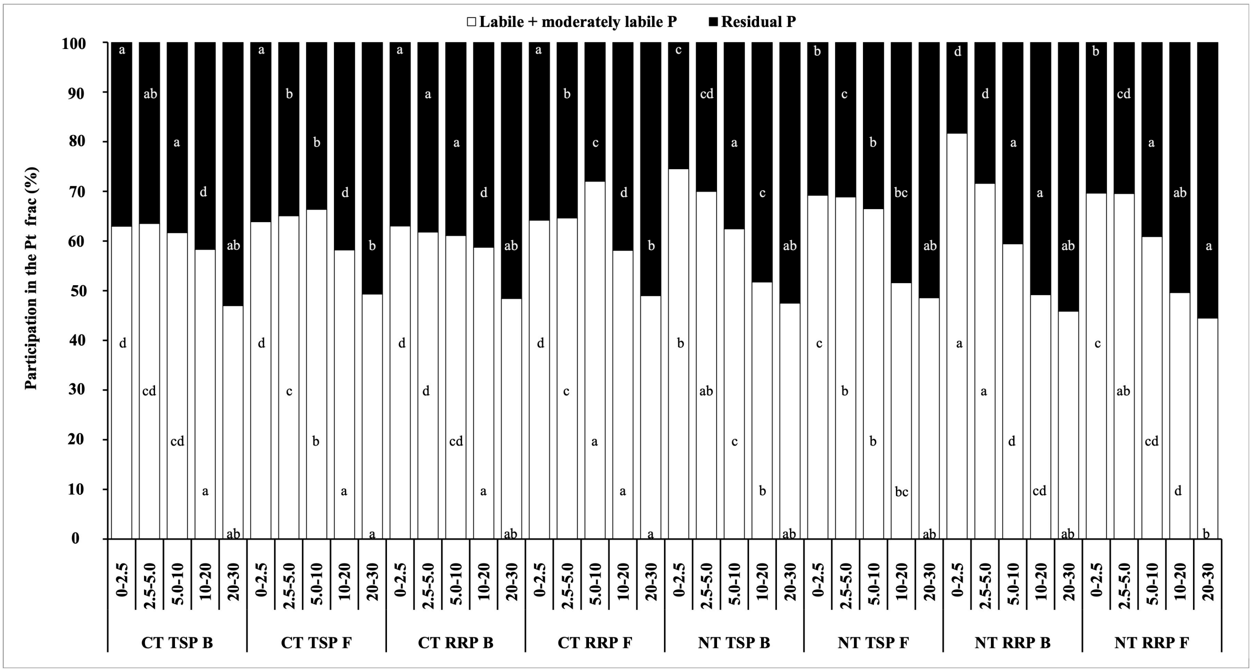

FIGURE 2 | Participation of labile + moderately labile and residual fractions of P in relation to $Pt_{\Sigma\,frac}$ in different layers of a very clayey Oxisol after 17 years of cultivation under distinct soil management systems, phosphate fertilizer sources, and application methods. CT, conventional tillage; NT, no-tillage; TSP, triple superphosphate; RRP, reactive rock phosphate; B, broadcast fertilizer application; F, furrow fertilizer application. Different letters represent significant differences between treatments in a given soil layer and P fraction, according to Fischer's LSD test ($P < 0.05$).

new adsorption sites (Sousa and Volkweiss, 1987a), favoring the transformation of more labile forms of Pi, such as $NaHCO_3$ Pi and NaOH 0.1 M Pi, into forms of lower lability (Pi_{res}), impairing their lability in the system, especially in soils with an elevated sink effect (Novais et al., 2007).

Under NT, the differences between fertilizer management methods were more intense in the application layers (0–5.0 cm for broadcast and 5.0–10 cm for furrow) and are more evident than under CT. There was a superficial accumulation of Pi under NT, especially under broadcast application, due to the preservation of the fertilized layer and due to P cycling through the crops over the years (Santos and Tomm, 2003) through the decomposition of plant tissue waste deposited on the surface or absorbed by the roots (Costa et al., 2010). This Pi is of higher lability in these layers compared to the subsurface layers. This could be due to the higher accumulation of SOM on the surface under NT (Zhou et al., 2019), reducing Pi retention in the soil due to organic ions competing for adsorption sites (Sibanda and Young, 1986; Hue, 1991; Mesquita Filho and Torrent, 1993; Ohno and Erich, 1997; Nziguheba et al., 1998; Fink et al., 2016b), and the accumulation of P in these layers partially saturates adsorption sites (Guertal et al., 1991), so that the remaining Pi may be adsorbed with lower energy, possibly facilitating subsequent desorption, as observed by Barrow et al. (2018) and Tiecher et al. (2012b). In fact, this was observed by de Oliveira et al. (2020b) after cultivating this area without P input during successive corn crops, where despite about 100% of crop nutrition was supplied by the 0–5.0 cm layer under broadcast P application, compared to 70% under band application, P offtake and grain yield were similar between both application methods. Continuous broadcast P fertilization under NT could even increase yields in highly adsorptive weathered soils (de Oliveira et al., 2020a).

The reaction of the fertilizer itself is usually slower under NT (Kunishi et al., 1982). This might have resulted in the accumulation of non-reacted forms of calcium phosphate in our experiment (Sousa and Volkweiss, 1987b), and the possible formation of secondary calcium phosphates (Magid, 1993) observed in HCl Pi. It may protect P under this system, helping to maintain P in more labile forms. In the superficial layer under NT, the neoformation of calcium phosphates could be boosted by the high levels of Ca and high pH resulting from superficial liming, as also reported by Guo et al. (2000); Rheinheimer and Anghinoni (2001), and Tiecher et al. (2012b). RRP results in a high accumulation of these calcium phosphates and also P forms adsorbed on Fe and Al oxides and clay minerals of lower lability compared to TSP, evidenced by both the higher levels of HCl Pi and NaOH 0.5 M Pi, as previously reported by Soltangheisi et al. (2018) in a similar Oxisol. Although these fractions are considered to be of lower lability, an elevated residual effect and P use efficiency was observed from RRP fertilization after cultivating this area without P input during successive corn crops (de Oliveira et al., 2019).

As a result of this complex P dynamics in soil under the different tillage systems and phosphate fertilization strategies, it is noticed that NT and RRP use could be recommended as strategies to improve legacy P bioavailability in highly P adsorptive Oxisols in the tropics. This is new in the way which previous studies in CT systems were not able to observe the special importance of rock phosphates in highly adsorptive acid soils when soil is minimally disturbed. NT, combined with RRP applications, promotes efficient P nutrition while probably protecting P from adsorption due to calcium-bound forms.

No-Tillage Effect on Organic P Fractions

While CT stimulates fertilizer reaction with colloids in the soil and speeds up SOM decomposition (Nunes et al., 2011a), favoring the accumulation of Pi_{res}, there is less accumulation of Po_{res} under this system for the same reasons. Tillage and fertilization management little influenced the proportion of the Po_{res} fraction in P_{bio}, with values around 30% of P_{bio} (**Supplementary Figure S6**). Furthermore, the proportion of the $NaHCO_3$ Po fraction was fairly stable across the treatments at around 8% of P_{bio}. Under NT, even the more labile fraction of Po did not accumulate, probably due to rapid turnover (Bowman and Cole, 1978).

In general, a greater proportion of the less labile fraction NaOH 0.5 M and a smaller proportion of the more labile NaOH 0.1 M under NT when compared to CT were observed (**Supplementary Figure S6B**). This was especially evident when the P source was RRP. Although counterintuitive, this fact might be related to the intensified microbial activity under NT (Mendes et al., 2003), responsible for SOM chemical stabilization reactions and improved soil structure (Bertol et al., 2004). This physical protection (Nunes et al., 2011a; Figueiredo et al., 2013) might boost Po stability in the soil, evidenced by the NaOH 0.5 Po fraction, and therefore results in Po accumulation. This is a new observation which may add to the positive benefits of the NT system that generally results in increased crop yields. Labile and moderately labile fractions of Po under CT, on the other hand, are more easily mineralized and may constitute the main source of P for cropping under this system (Tiecher et al., 2018).

Otherwise, the accumulation of Po in more stable forms observed by RRP fertilization is intriguing. One hypothesis proposed by Santos et al. (2008) is that the replenishment of labile P, previously made by less labile Po fractions, could be made by the slow RRP P release in soils fertilized with this source. This would leave stable Po fraction contents relatively unaltered and able to accumulate.

P Budget Compared to the Soil Under Natural Vegetation

Compared to the soil under natural Cerrado vegetation (**Supplementary Table S4**), gains in P_{geo} were observed throughout the soil profile, irrespective of soil tillage and fertilizer management, ranging from 14 to 700 mg kg^{-1} (**Figure 3**). On the other hand, gains in P_{bio} did not occur in all soil layers and were significantly less than those of P_{geo}. Even so, gains in P_{bio} in some layers were considerable, reaching 75 mg kg^{-1}.

Under CT, intermediate layers (2.5–5, 5–10, and 10–20 cm) exhibited net gains in P_{bio}, mostly due to the NaOH 0.1 M

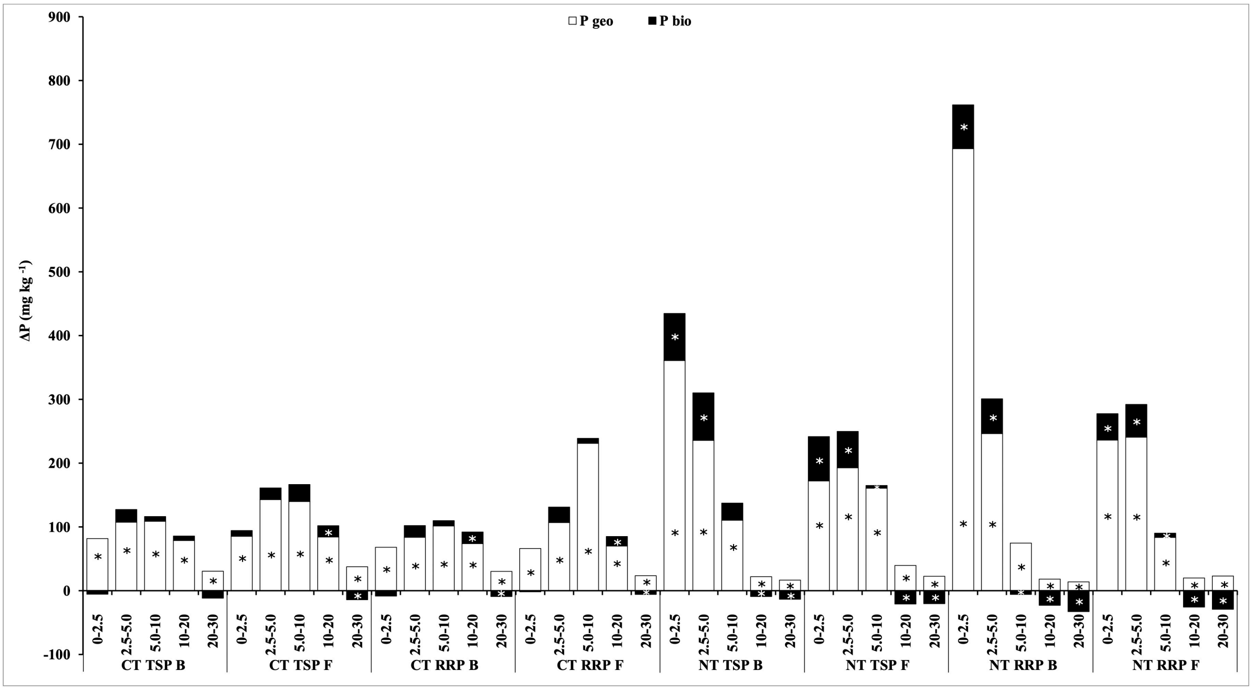

FIGURE 3 | Variation in geochemical and biological P in relation to natural Cerrado in five layers of a very clayey Oxisol after 17 years of cultivation under distinct soil management systems and phosphate fertilizer sources and application methods. CT, conventional tillage; NT, no-tillage; TSP, triple superphosphate; RRP, reactive rock phosphate; B, broadcast; F, furrow. *Significant according to the Student's *t*-test at $P < 0.05$.

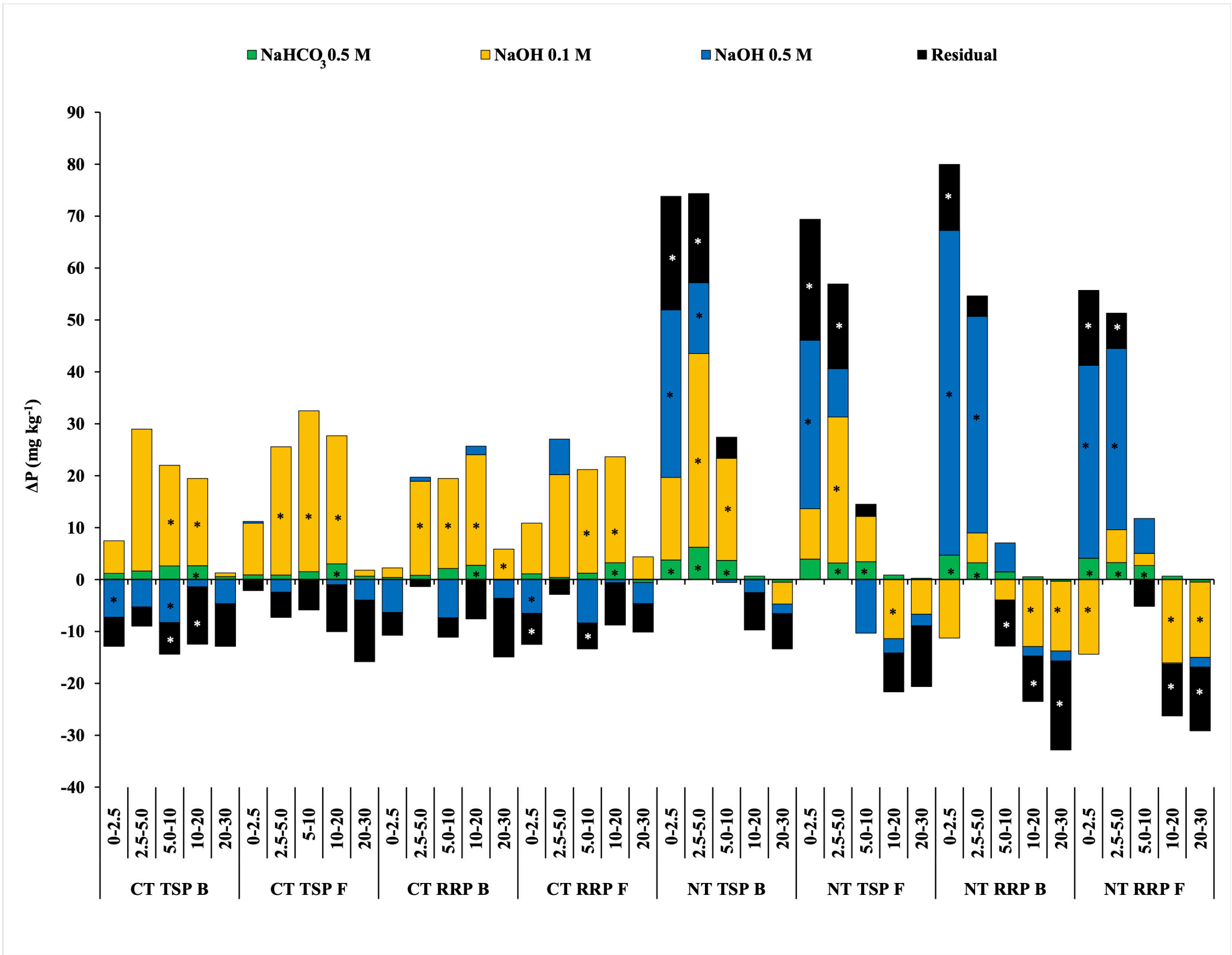

FIGURE 4 | Variation in the Po pool in relation to natural Cerrado in five layers of a very clayey Oxisol after 17 years of cultivation under distinct soil management systems and phosphate fertilizer sources and application methods. CT, conventional tillage; NT, no-tillage; TSP, triple superphosphate; RRP, reactive rock phosphate; B, broadcast; F, furrow. *Significant according to the Student's t-test at $P < 0.05$.

fraction, whereas NaOH 0.5 M Po and residual Po presented a negative balance (**Figure 4**). Under NT, in most treatments and layers down to 10 cm, there were expressive gains in P_{bio} compared to Cerrado, especially in the NaOH 0.5 M fraction. Below 10 cm, there were losses of P_{bio}, especially in the NaOH 0.1 M and residual fractions.

These differences in P gains between both tillage systems are related to the soil depth affected by the management practices in each system: under CT, the soil is revolved up to 20 cm deep, while in NT soil disturbance was limited to about 10 cm deep due to sowing operations and fertilization, in the case of furrow application. This way, some losses of Po below 20 cm under CT and below 10 cm under NT are explained by no effect of the management system on these layers, whereas in the Cerrado soil the deep root systems of the various species make a steady contribution of Po at deeper layers in the profile.

These results challenge the idea that Po is not altered by phosphate fertilization in highly weathered clayey soils, as reported by Conte et al. (2003) under NT after 5 years of cropping, and Pavinato et al. (2009) under CT and NT after 13 years of cropping. Possibly, management system duration plays a major role in producing this effect on Po, as reported by Costa et al. (2010), who observed the effect of the phosphate fertilizer application method on Po in an Argisol cropped for 18 years under CT and NT.

Considering the layers influenced by the soil management system (0–20 cm under CT and 0–10 cm under NT), it can be seen that P_{bio} gains under both CT and NT become fairly significant. Taking into account the fact that the superficial layer, especially under NT, exhibits the highest accumulation of nutrients (De Maria et al., 1999), roots (Costa et al., 2010), and microbial activity (Mendes et al., 2003), the effect of the soil and fertilization management system on Po has significant potential to influence the dynamics of P nutrition for plants, corroborating to Redel et al. (2007).

CONCLUSION

After 17 years of grain crop cultivation and fertilizer use, legacy P accumulated in the soil was influenced by soil tillage and phosphate fertilization management.

There was an accumulation of Pi and Po under both tillage systems compared to Cerrado natural soil. CT resulted in a more homogeneous distribution of Pi fractions throughout the profile, minimizing the effects of P fertilizer management.

Despite a strong P content stratification through the soil profile, with Pi accumulation in the fertilizer application zone, the NT system promoted P lability and net Po accumulation in layers where roots predominantly develop (0–10 cm). This is especially relevant considering that the conservationist system increased P exports in grains by 21% when compared to CT, after the 17 crops. This is a NT system benefit not previously observed in depth in tropical soils.

There was a high accumulation of Pi associated with calcium (HCl Pi) in soil under NT with RRP application, especially when this fertilizer was broadcast applied. This P fraction may act as a source of slow-release legacy P, which could not be observed in previous experiments involving CT only.

NT and RRP positively affected legacy P fractions and can be recommended as strategies to improve P fertilizer use efficiency.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

RN: substantial work elaboration, writing, and data interpretation. DS: experimental design elaboration and laboratorial analyses. WG: contribution in experimental design elaboration and writing. LO: critical analysis of content and contributions in writing and statistics. PP: corrections in writing and significant scientific approach insights. TP: data interpretation and figure making. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/feart.2020.00350/full#supplementary-material

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