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

T. Q. Zhang[1]*, Z. M. Zheng[2], Craig F. Drury[1], Q. C. Hu[1] and C. S. Tan[1]

[1] Harrow Research and Development Centre, Agriculture and Agri-Food Canada, Harrow, ON, Canada, [2] Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, ON, Canada

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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Luke Gatiboni,
North Carolina State University,
United States

Reviewed by:
Tadeu Luis Tiecher,
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Djalma Eugênio Schmitt,
Federal University of Santa Catarina,
Brazil

***Correspondence:**
T. Q. Zhang
Tiequan.Zhang@canada.Ca

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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).

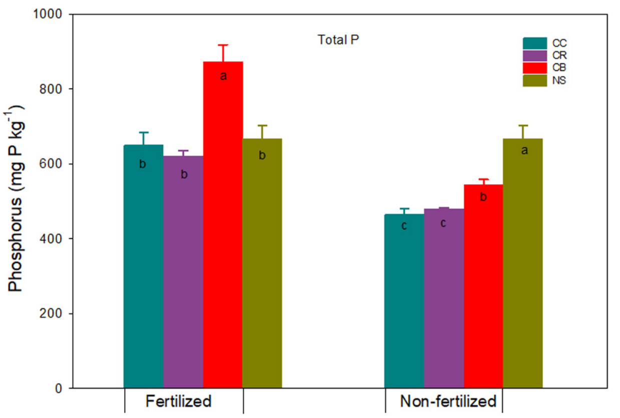

FIGURE 1 | Total P and standard errors ($n = 3$) (mg kg^{-1}) in a Brookston clay loam at Woodslee, ON, Canada, after 45-year consistent cropping with and without P fertilization relative to its native forest soil (NS). CC, continuous corn; CB, continuous bluegrass sod; CR, corn-oats-alfalfa-alfalfa 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

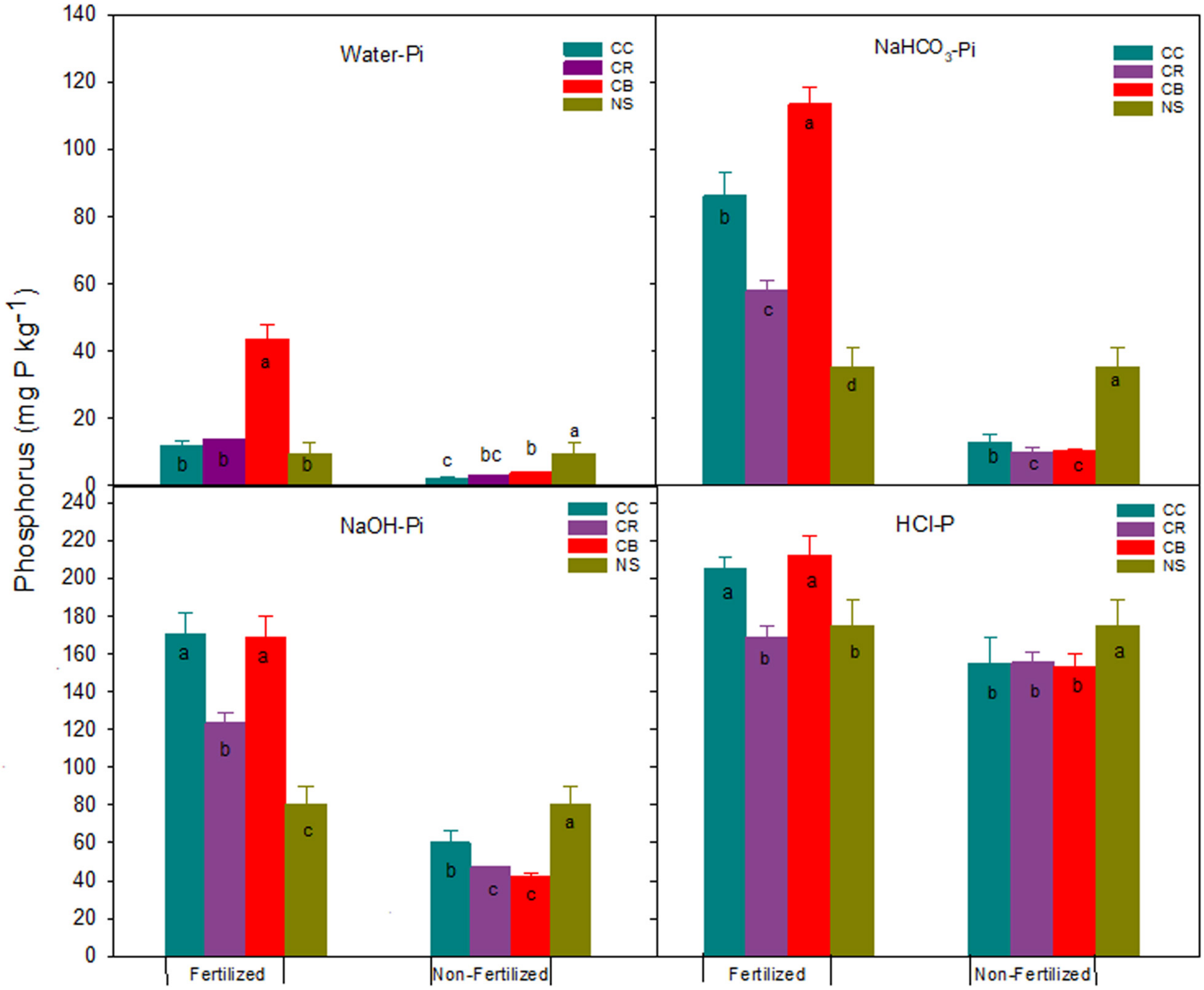

FIGURE 2 | Water-P$_i$, NaHCO$_3$-P$_i$, NaOH-P$_i$, HCl-P, and standard errors (n = 3) (mg kg^{-1}) in a Brookston clay loam at Woodslee, ON, Canada, after 45-year consistent cropping with and without P fertilization relative to its native forest soil (NS). CC, continuous corn; CB, continuous bluegrass 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

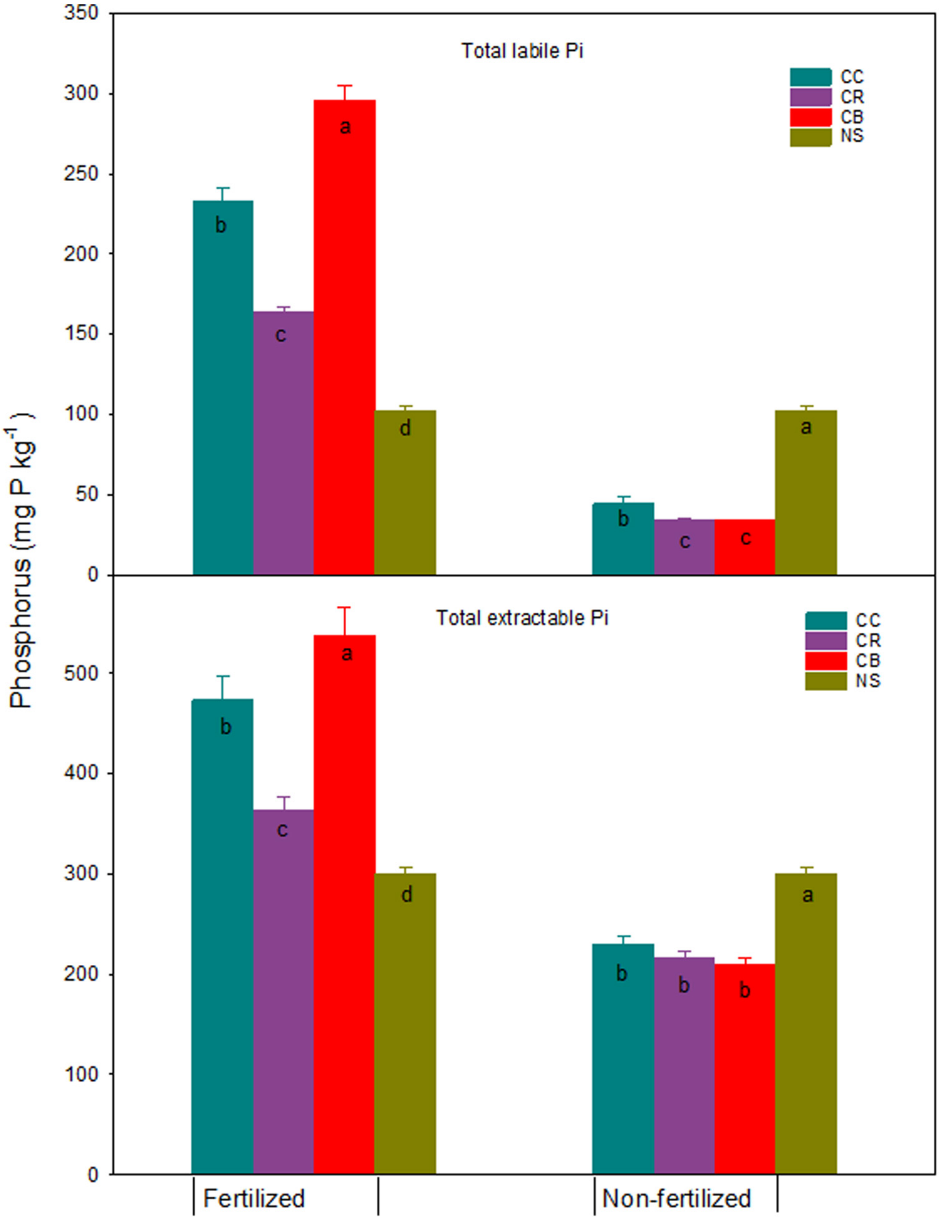

FIGURE 3 | Total extractable P_i and labile P_i and standard errors (*n* = 3) (mg kg^{-1}) in a Brookston clay loam at Woodslee, ON, Canada, after 45-year consistent cropping with and without P fertilization relative to its native forest soil (NS). CC, continuous corn; CB, continuous bluegrass sod; CR, 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

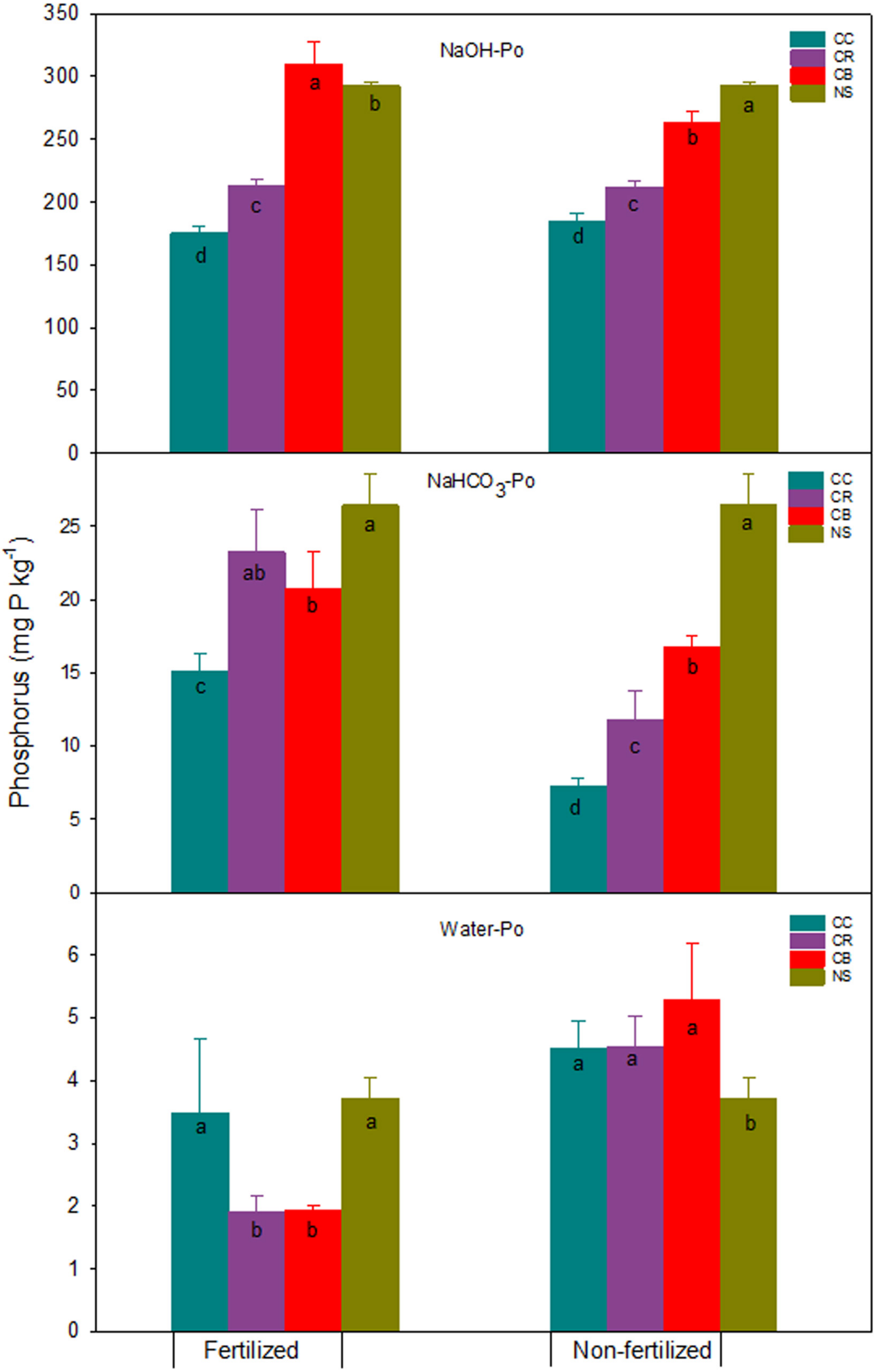

FIGURE 4 | Water-P$_o$, NaHCO$_3$-P$_o$, NaOH-P$_o$, and standard errors (n = 3) (mg kg^{-1}) in a Brookston clay loam at Woodslee, ON, Canada, after 45-year consistent cropping with and without P fertilization relative to its native forest soil (NS). CC, continuous corn; CB, continuous bluegrass sod; 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.

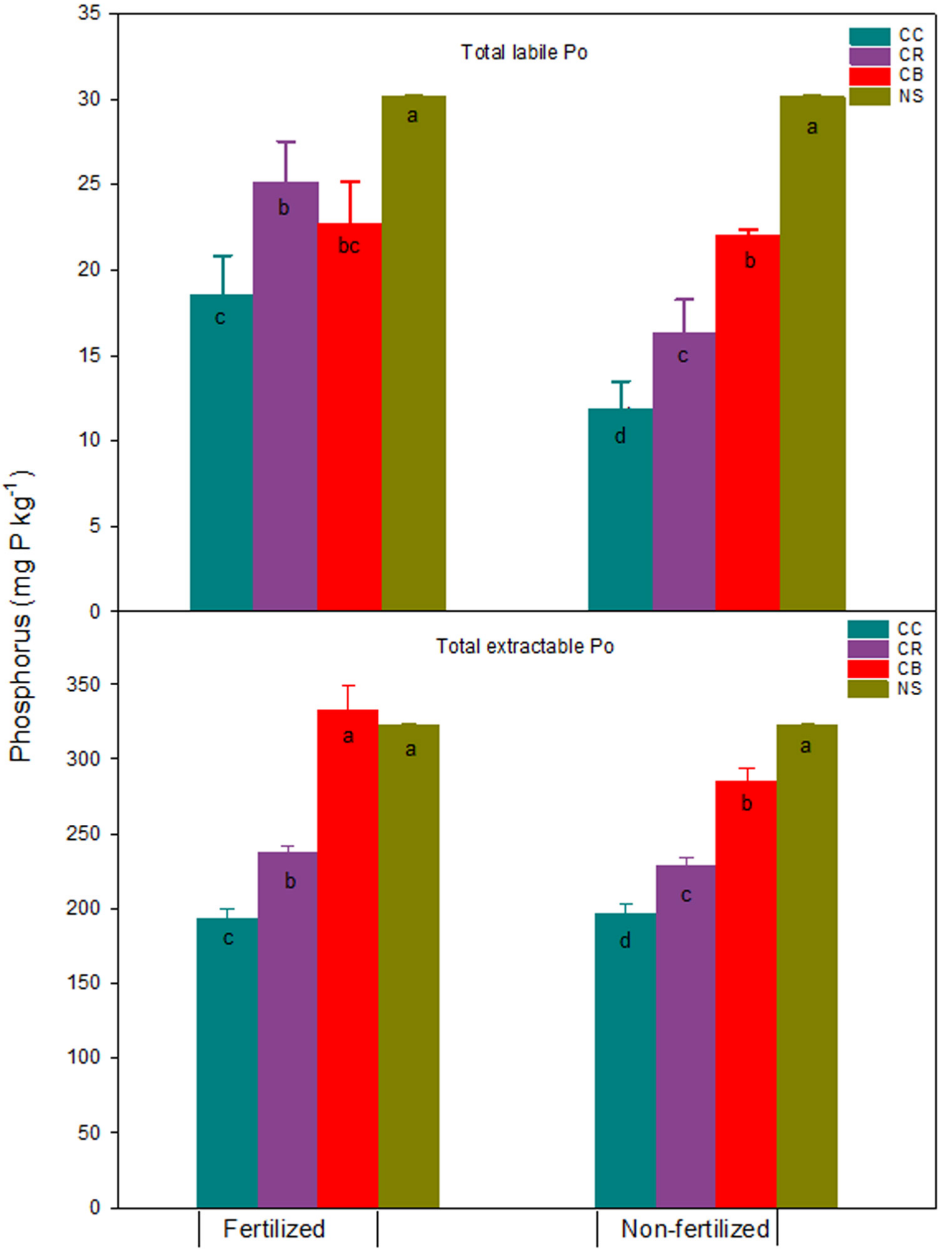

FIGURE 5 | Total extractable P_o and labile P_o and standard errors (*n* = 3) (mg kg^{-1}) in a Brookston clay loam at Woodslee, ON, Canada, after 45-year consistent cropping with and without P fertilization relative to its native forest soil (NS). CC, continuous corn; CB, continuous bluegrass sod; CR, 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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