

# Pathways of Phosphorous Fraction Dynamics in Field Crop Rotations of the Pampas of Argentina

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The study of labile and nonlabile forms of P might contribute to advances in soil test procedures and provide insights into management strategies to enhance P bioavailability to crops. Our objective was to evaluate the effect of continuous P fertilization on inorganic (Pi) and organic (Po) P fractions to improve the understanding of soil P dynamics. For this purpose, four on-farm experiments were analyzed during a 6-yr period. Two sites followed the corn (*Zea mays* L.)–double cropped wheat (*Triticum aestivum* L.)/soybean [*Glycine max* (L.) Merr.] rotation and two sites followed the corn–soybean–double cropped wheat/soybean rotation. Phosphorus fertilization levels were 0 (Wp treatment) and 34 kg yr<sup>-1</sup> (Fp treatment). The following soil P fractions were analyzed: anion exchange membrane (AEM) Pi, NaHCO<sub>3</sub>-Pi and -Po, NaOH-Pi and -Po, HCl-Pi, and residual P. Path analysis of relationships among all P forms revealed that the main P fertilizer sinks were NaOH-Po and HCl-Pi fractions, accounting for approximately 50% of total applied P. The pathways for the Wp treatment showed the importance of Pi pools for the replenishment of available P, represented by the AEM-Pi and NaHCO<sub>3</sub>-Pi fractions, and the role of NaOH-Pi along with HCl-Pi as major Pi sources. When P fertilizer addition exceeded plant P removal, the pathway analysis showed that the Pi fractions tended to reorganize into more stable Po fractions. Path analysis was a practical tool to elucidate the roles of different Po and Pi pools in the transformations induced by differences in nutrient input and crop removal.

**Abbreviations:** AEM, anion exchange membrane; B-1, Bray-1; C-W/S, corn-double cropped wheat/soybeans; C-S-W/S, corn-full season soybeans-double cropped wheat/soybeans; P, phosphorus; Pi, inorganic phosphorus; Po, organic phosphorus.

The identification of different P forms and their availability to crops is difficult due to the spatial inconsistency and the complexity of soil P chemistry (Webber, 1978; Pierzynski et al., 1990). The bioavailability of soil P could be studied through a fractionation of this element into Po and Pi forms that differ in their availability for plant acquisition. Hedley et al. (1982) proposed a method based on the use of a set of chemical solutions with increasing extracting power that removes P from different soil pools (Cross and Schlesinger, 1995).

The Hedley method was tested under different conditions, from slightly weathered (Tiessen et al., 1983; Richards et al., 1995; Levy and Schlesinger, 1999; Guo et al., 2000; Kuo et al., 2005; Vu et al., 2008; Boschetti et al., 2009) to highly weathered soils (Beck and Sanchez, 1994; Schmidt et al., 1996; Levy and Schlesinger, 1999; Neufeldt et al., 2000; Pheav et al., 2003; Araújo et al., 2004), and has been useful in determining P availability (Tiessen and Moir, 1993). Most researchers seemed to agree that the sum of AEM-Pi or resin-Pi plus NaHCO<sub>3</sub>-Pi is robust to predict the portion of soil P available to plants (equivalent to the plant-available or extractable P by different soil test methods). In addition, the availability of the other fractions was recently studied (He et al., 2008) and compiled (Negassa and Leinweber, 2009).

Only one-quarter to one-third of the total amount of inorganic P fertilizer applied is taken up by crops. The remaining fertilizer P mostly remains in the soil, and only in some circumstances can it migrate from the root exploration zone through runoff or leaching (Haynes and Williams, 1992; Edmeades, 2003; Vadas et

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al., 2008; Ciampitti et al., 2011). In the Pampas region, the soil analysis most frequently used to characterize P available for plant nutrition is the Bray-1, which is recommended for neutral and acid soils (pH < 7.0) but not for alkaline soils (pH > 7.0). Most studies on the fate of fertilizer P in the soil have focused primarily on Bray-1 P, reporting soil P accumulation with increasing amounts of P applications or P depletions with continuous crop P removal under different cropping systems. Additionally, little is known about medium-term (e.g., <10-yr) changes in soil P fractions with different fertilizer inputs and to what extent operationally defined soil P fractions may represent differences in soil P availability in Mollisols under high-yield cropping systems.

Path analysis is a statistical technique that distinguishes between correlation and causation (Wright, 1921; Turner and Stevens, 1959; Afifi and Clark, 1984, p. 235–237), and it has been used recently in studies on P dynamics (Tiessen et al., 1984; Zheng et al., 2002; Zhang et al., 2005). This method can help to unravel the complexity of soil P dynamics and the difficulty of establishing the relationships between labile and stable P components. Tiessen et al. (1984) effectively demonstrated through path analysis the differences in interactions among P pools between Ultisols and Mollisols. They found that much of the labile resin-P originated from  $\text{NaHCO}_3$ - and  $\text{NaOH}$ -Pi in Mollisols, and labile P was accounted for by Po in Ultisols. Zheng et al. (2002) indicated that changes in P forms were strongly related to P transformations, whereas the cause-and-effect relationships between P pools were not identified. In addition, Zhang et al. (2005) reported that path analysis was a very useful tool to reveal the indirect effect of clay content as an important factor in the relationships observed between the P adsorption maximum and Mehlich-3 extractable Ca and Mg. Overall, path analysis has been used to advance the knowledge and understanding of the availability of different P fractions for plant nutrition (Tiessen et al., 1984; Zheng et al., 2002) and to try to comprehend the intercorrelation among the different P pools under contrasting P fertilization strategies. Furthermore, in P fractionation studies, this analysis can be used to describe the directed dependencies among P pools. Ultimately, the use of the path analysis in soil P dynamics might be essential in continuing the progress and understanding of relationships and behavior among P fractions. Nevertheless, this analysis must acknowledge the complexity of mechanistic P transformations and interactions within the diverse soil matrices (different soil types).

The objectives of this study were, therefore, (i) to quantify the changes in soil P fractions after 6 yr of continuous P fertilization, and (ii) to use the path analysis model for a better explanation of soil P dynamics, determining the potential role of each pool acting as a sink or a source for P.

## MATERIALS AND METHODS

The Pampas is the most productive region of Argentina. Some features that characterize this excellent region are its temperate climate, its

humid rainfall regime to the east and semiarid regime to the west area, and Mollisol soils (from sandy Entic Hapludolls and *Quarzipsammets* in the west to clayey Vertisols and Vertic Argiudolls in the east).

In 2000, a long-term fertilization study was established at 11 farms of the Regional Consortium for Agricultural Experimentation (CREA), Southern Santa Fe Region, a consortium of farmers from the Southern Santa Fe region in the northern Pampas of Argentina ( $32^{\circ}11'50.68''$ – $34^{\circ}11'55.04''$  S and  $63^{\circ}17'43.7''$ – $61^{\circ}20'0''$  W). After the first year, the farms were divided into two groups: five sites continued under a corn–double-cropped wheat/soybean (C-W/S) rotation and six sites under a corn–full-season soybean–double-cropped wheat/soybean (C-S-W/S) rotation. From this network, four experimental sites (two sites under C-W/S and two sites under C-S-W/S rotations) under stable no-till systems were selected for this study, with variation across sites in soil type, soil Bray-1 P, and soil management history (Table 1). At each site, the experimental design was a randomized complete block design with three replicates. The plots were 25 to 30 m wide and 65 to 70 m long. Treatments included a control without P (Wp) and annual P fertilization (Fp) by adding inorganic fertilizer (monoammonium phosphate, 12–52–0 N– $\text{P}_2\text{O}_5$ – $\text{K}_2\text{O}$ ). For both treatments, N and S were applied at equivalent rates based on crop demand. Each experimental site included only one rotation and each crop phase was not present every year. In addition, only the fertilizer treatments were replicated within each rotation. The P rate was decided each year at the beginning of the crop season, according to the expected crop yield and by estimation of P removal by the crop to be planted. For each year and experimental site, a constant grain P concentration (calculated to 0% moisture content) was assumed for the different crops: 4.0, 5.0, and 7.0 g P uptake  $\text{kg}^{-1}$  grain yield for maize, wheat, and soybean, respectively (Ciampitti and Garcia, 2007). The final P rate for each crop was 5 to 10% higher than the estimated P removal. The goal of the fertilization rate was to obtain a slightly positive P budget to gradually build up soil P levels. Fertilizers were incorporated at seeding 5 cm below and to the side of the seed. The cumulative Pi applied by the fertilizer, the P removed, and the balance for both rotations during the 6 yr of the experiment is presented in Table 1. Further details on P application, removal, and balances are available in Ciampitti et al. (2011).

At the beginning of the experiment, in 2000, soil samples (0–20-cm depth) were collected to perform a complete soil test analysis (Table 1). Soil samples were air dried at  $40^{\circ}\text{C}$  and sieved to 2 mm. Soil properties measured were: total organic C (Walkley and Black, 1934; Nelson and Sommers, 1982), pH (1:2.5 soil/distilled water suspension), Bray-1 P (Bray and Kurtz, 1945), and exchangeable cations (pH 7  $\text{NH}_4\text{OAc}$  method; Chapman, 1965). The soil bulk density was determined according to the method of Grossman and Reinsch (2002). The soil texture was determined by the pipette method (Gee and Bauder, 1986).

A P fractionation analysis was performed in 2006, before the maize growing season and 6 yr after establishment of the treatments. For P fractionation, soil samples (0–20 cm) were collected before applying P at each experimental site. Each sample consisted of 10 to 12 cores (1.9-cm diameter), and three soil samples, one per block, were taken from each treatment. Samples were air dried and sieved to <2 mm; a 50-g subsample was ground, sieved to <0.5 mm, and stored at room temperature until analyzed.

**Table 1. Experimental sites in corn-double cropped wheat/soybean (C-W/S) and corn-full season soybean-double cropped wheat/soybean rotation (C-S-W/S) without addition P fertilization (Wp) and with 34 kg P ha<sup>-1</sup> yr<sup>-1</sup> added (Fp), plot management history, soil properties before experimental P application, cumulative Pi applied, P removal, and P balance from 2000 to 2006.**

Soil property	Unit	C-W/S Rotation		C-S-W/S Rotation	
		La Marta	San Alfredo	La Blanca	La Hansa
Soil classification	–	Entic Haplustolls	Typic Argiudolls	Typic Hapludolls	Aquic Argiudolls
Years of Agriculture	–	40	8	6	+20
Previous crop	–	Wheat/Soybean	Wheat/Soybean	Wheat/Soybean	Soybean
B-1	mg kg <sup>-1</sup>	11.2	18.3	16.2	45.5
TOC	g kg <sup>-1</sup>	12.1	19.8	13.3	12.2
Soil bulk density	g cm <sup>-3</sup>	1.40	1.41	1.31	1.25
pH	–	6.3	6.0	6.6	5.5
Ca	mg kg <sup>-1</sup>	1380	2200	1440	1520
Mg	mg kg <sup>-1</sup>	252	252	240	192
K	mg kg <sup>-1</sup>	936	663	741	663
Na	mg kg <sup>-1</sup>	115	92	92	92
B	mg kg <sup>-1</sup>	1.1	0.8	1.1	0.9
Cu	mg kg <sup>-1</sup>	0.9	1.8	1.3	1.4
Fe	mg kg <sup>-1</sup>	54	105	71	86
Mn	mg kg <sup>-1</sup>	28	103	36	54
Textural class		sandy loam	silt loam	silt loam	silt loam
Cumulative P Balance (0- to 20-cm soil depth)					
<u>Fp treatment</u>					
P applied		204.0	204.0	204.0	184.0
P removal	kg P ha <sup>-1</sup>	194.3	204.9	166.4	141.2
P balance		9.7	-0.9	37.6	42.8
<u>Wp treatment</u>					
P removal	kg P ha <sup>-1</sup>	139.2	168.4	160.8	122.2
P balance		-139.2	-168.4	-160.8	-122.2

A total of seven P fractions was measured to quantify the total soil P concentration in the different soil samples. The AEM-Pi fraction measured the soluble and rapidly exchangeable P (Cooperband et al., 1999). The NaHCO<sub>3</sub> extracts the Pi adsorbed on surfaces of more crystalline P compounds, sesquioxides, or carbonates (Tiessen et al., 1984) and the readily mineralizable Po associated with ribonucleic acids and glycerophosphate (Bowman and Cole, 1978). The NaOH extracts the Pi adsorbed on amorphous and some crystalline Al and Fe phosphates (Williams et al., 1980) and the Po associated with humic and Fe and Al components (Schoenau et al., 1989). The HCl extracts Pi bound to Ca and soil minerals, such as apatite and octocalcium (Williams et al., 1980; Frossard et al., 1995). Finally, the residual P, extracted by H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub>, is the insoluble and recalcitrant Pi and Po, occluded Pi covered with sesquioxides, P associated with minerals, and unextracted Po (Syers et al., 1967; Hsu, 1977; Tiessen et al., 1984).

A modified version of the Hedley et al. (1982) procedure was used to sequentially fractionate the soil P. In brief, two AEM strips were added to 0.5 g of soil and shaken for 16 h at constant temperature (24°C) with 30 mL of deionized water and saturated with NaHCO<sub>3</sub>. When the process was completed, the AEM strips were shaken with 15 mL of 1 mol L<sup>-1</sup> NaCl for 1 h according to the method of Cooperband and Logan (1994), which desorbed the P from the AEM strips. The soil suspension was then centrifuged at 25,000 × g at constant temperature (0°C). The residual soil was extracted in sequence with 30 mL of 0.5 mol L<sup>-1</sup> NaHCO<sub>3</sub> (pH 8), 0.1 mol L<sup>-1</sup> NaOH, and 1.0 mol L<sup>-1</sup> HCl by shaking for 16 h at constant temperature (24°C). The soil suspension was then centrifuged for 10 min

at 25,000 × g at constant temperature (0°C). A 10-mL soil extract was taken from the NaHCO<sub>3</sub> and NaOH fractions; in addition, these extracts were acidified to precipitate organic matter (pH 1.5, adding 0.9 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>), and were kept for 1 h inside a refrigerator. In the next step, the extracts were centrifuged for 10 min at 25,000 × g at constant temperature (0°C). After this step, an extract of the supernatant was analyzed for Pi and another extract (5 mL) to quantify total P. For the last determination, the extract of the supernatant was digested in an autoclave (121°C) with acidified ammonium persulfate [(NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub>] (0.5 and 0.6 g were added to determine the NaHCO<sub>3</sub> and NaOH fractions, respectively) and 10 mL of 0.9 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>. The residual P was not differentiated between Pi and Po and was determined by H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> digestion. The total P in each fraction was quantified after digestion in an autoclave by an acidified ammonium persulfate oxidation (Tiessen and Moir, 1993). The difference between total P and Pi was assumed to be Po. Total soil P (TP) was determined by H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> digestion (Gasparatos and Haidouti, 2001). The P concentration in the extracts was determined colorimetrically by the molybdate–ascorbic acid procedure of Murphy and Riley (1962) after pH adjustment using β-nitrophenol as an indicator.

The mean P change between fertilization treatments (Fp minus Wp), as presented in Table 3, was used to estimate the total P recovery from P applied as fertilizer. From this calculation, P changes between treatments were expressed on a mass per area basis (kg ha<sup>-1</sup>) after previous correction by the bulk density values (0–20-cm soil depth), which are shown in Table 1 for each field site.

## Statistical Analysis

The fulfillment of the assumptions of variance homogeneity and normal distribution of the P fractions was evaluated. The data were analyzed using the PROC MIXED statement of the SAS statistical package (SAS Institute, 1996). The experimental sites and fertilization treatments were considered as fixed effects of the model, while blocks and interactions were considered random effects. Medians and significant interactions were obtained using the LSMEAN/PDIFF procedure. The best fixed parameters of the covariance structure for each model were the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC).

Regression analysis was conducted with the SAS PROC REG procedure to study the relationships between the sum of all P fractions and TP, the sum of AEM-Pi plus NaHCO<sub>3</sub>-Pi fractions and Bray-1 P, and the soil P balance and the sum of all P fractions. Correlation analyses were performed to determine the intensity of the relationships among the various P fractions, and the significance of the relationships was tested at  $P < 0.05$ .

The PROC CALIS procedure (SAS Institute, 1996), using the sample covariances of the P pool variables, was used to derive path coefficients among the pools in a conceptual model representing the soil P cycle. Path coefficients are linear regression coefficients (e.g., a coefficient of 0.8 means that if the source changes by one unit, the sink changes by 0.8 unit). The AIC and BIC were the criteria selected to determine the fit. For the simple correlation analysis and the pathway transformations, the data of all sites were pooled to simplify the analysis of the information.

## RESULTS AND DISCUSSION

### Soil Phosphorus Fractions

Phosphorus fertilization increased all Pi fractions ( $P < 0.05$ ) (Table 2). In relative terms, this increase was greater in the AEM-Pi fraction (60%), while in absolute terms the HCl-Pi form presented the greatest variation between fertilization treatments (12 mg P kg<sup>-1</sup>). The largest AEM-Pi values were observed for the C-S-W/S sites (8.0–8.5 mg P kg<sup>-1</sup> for Fp treatments at La Hansa and La Blanca, respectively). At the C-W/S sites,

**Table 2. Inorganic (AEM, NaHCO<sub>3</sub>, NaOH and HCl), organic (NaHCO<sub>3</sub>, NaOH), residual (H<sub>2</sub>SO<sub>4</sub>) P and the sum of all fractions for the P fertilized and non-fertilized treatments at the 0- to 20-cm soil layer under two crop rotations, corn-soybean-wheat/soybean (C-S-W/S) and corn-wheat/soybean (C-W/S), in four experimental sites.**

P fraction	Experimental sites							
	C-S-W/S Rotation				C-W/S Rotation			
	La Blanca		La Hansa		La Marta		San Alfredo	
Wp	Fp	Wp	Fp	Wp	Fp	Wp	Fp	
mg P kg <sup>-1</sup>								
	<b>Inorganic P (0 to 20 cm)</b>							
AEM	3.4	8.5	3.6	8.0	1.2	2.0	1.4	5.7
NaHCO <sub>3</sub>	8.9	12.6	4.6	8.5	4.1	5.0	3.7	8.1
NaOH	9.6	13.0	6.3	12.7	8.0	11.8	6.7	11.1
HCl	60.5	68.5	69.0	86.9	32.7	46.6	37.0	44.8
	<b>Organic P (0 to 20 cm)</b>							
NaHCO <sub>3</sub>	10.5	9.7	9.3	9.1	32.7	44.6	9.5	13.8
NaOH	104.4	116.8	82.8	97.3	35.6	44.7	45.7	60.6
	<b>Residual P (0 to 20 cm)</b>							
H <sub>2</sub> SO <sub>4</sub>	302.4	311.8	283.7	305.2	312.1	320.4	283.7	288.1
Sum of P fractions	499.7	540.9	459.3	527.7	426.4	475.1	387.7	432.2

La Marta showed a slight difference between P treatments (0.8 mg P kg<sup>-1</sup>), but San Alfredo presented similar differences to those observed for the C-S-W/S rotation. In absolute terms, the changes in NaHCO<sub>3</sub>-Pi due to P application followed the same pattern shown for the AEM-Pi fraction (3.7 and 3.2 mg P kg<sup>-1</sup> for AEM- and NaHCO<sub>3</sub>-Pi, respectively).

Nonsignificant differences were observed in the two-way site × fertilization interaction for almost all fractions at the C-S-W/S sites (Table 3). The only exception was the NaOH-Pi form ( $P < 0.05$ ). At the C-W/S sites, only the NaOH-Pi, -Po, and residual P fractions resulted in nonsignificant two-way interactions (Table 3).

The differences between fertilization treatments for the NaOH-Pi were similar in both rotations (4.5 mg P kg<sup>-1</sup>). La Hansa and San Alfredo, however, presented greater changes in P concentration (5.4 mg P kg<sup>-1</sup>; Fp minus Wp treatments) than La Blanca and La Marta (3.6 mg P kg<sup>-1</sup>; Table 2). A similar pattern was observed for the HCl-Pi fraction, for which La Blanca, La Marta, and San Alfredo sites resulted in smaller changes in the available P concentration values (10.0 mg P kg<sup>-1</sup>) than La Hansa location (17.9 mg P kg<sup>-1</sup>; Table 2).

Among the Pi fractions, the HCl-Pi form had the highest P concentration values (average 55.8 mg P kg<sup>-1</sup> across treatments and locations), probably because the Pampean soils possess a high base saturation and exchangeable Ca percentage. In a similar manner, Boschetti et al. (2003) reported a linear and direct relationship between the HCl-Pi fraction and the Ca exchangeable percentage. Regarding the chemistry of the HCl-P form (Pi or Po), recently some researchers concluded that some concentration of Po can be found in this pool and recommended quantifying the organic concentration in this HCl-P fraction (He et al., 2003, 2006; Negassa and Leinweber, 2009). In the future, research should incorporate the measurement of Po within the HCl-P fraction to accurately delimit and quantitatively determine the magnitude and fates of P fractions in different soils under different management practices.

The sum of the AEM- and NaHCO<sub>3</sub>-Pi fractions was highly and significantly related to the soil extractable P test usually used in this Pampean region (Bray-1 P) ( $P < 0.0001$ ;  $R^2 = 0.65$ ) (Fig. 1). Other researchers have also reported that the sum of AEM-Pi or resin-Pi and NaHCO<sub>3</sub>-Pi contributes to the available P pool (Mattingly, 1975; Hedley et al., 1982; Tiessen et al., 1984; Cross and Schlesinger, 1995). The Bray-1 P values of the soils used in the current study (Ciampitti et al., 2011), however, were greater than those obtained from the sum of AEM-Pi and NaHCO<sub>3</sub>-Pi, suggesting that a significant fraction of the soil extractable P came from other fractions considered as moderately labile. Our results suggest that the soil test analysis commonly used for extractable P considers not only the labile fractions but also the moderately labile forms identified by the Hedley extraction procedure. One good example to visualize this phenomenon was reported by Ciampitti et al. (2011): for soils presenting low Bray-1 P values (<10 mg P kg<sup>-1</sup>), the Bray-1 P concentration did not change or changed only slightly

under continuous crop P removal without P fertilizer applications after 6 continuous yr of evaluation.

Among the organic P fractions, P fertilization affected the  $\text{NaHCO}_3\text{-Po}$  form at only one of the four sites evaluated (La Marta site; Tables 2 and 3). Conversely, P fertilization exerted a large effect on the  $\text{NaOH-Po}$  fraction. In this case, differences between P treatments varied from 9.1 to  $14.9 \text{ mg P kg}^{-1}$  across sites (Table 2). Interestingly, the site where the P effects were greater for the  $\text{NaHCO}_3$  fraction was the one with the lower effect for the  $\text{NaOH}$  fraction (i.e., La Marta site). These differences among experimental sites could be explained by the sand content of the soil, which was highest at La Marta location ( $566 \text{ g kg}^{-1}$ ; Table 2). This would have generated a low soil P sorption capacity and a predominance of the more labile  $\text{NaHCO}_3\text{-Po}$  fraction as a sink for fertilizer P. Blake et al. (2003) found consistent changes in the  $\text{NaHCO}_3$  fraction during long periods of P addition or depletion in soils with high sand contents ( $713\text{--}751 \text{ g kg}^{-1}$ ), presumably because the soil contained only a little clay and had a low sorption capacity, compared with soils with a low sand content ( $180\text{--}280 \text{ g kg}^{-1}$ ). In the C-S-W/S rotation, represented by La Blanca and La Hansa sites, the absence of a response to P application in the  $\text{NaHCO}_3\text{-Po}$  pool (Table 2) could be associated with the buildup of the  $\text{NaOH-Po}$  fraction, which seems to act as a “buffer” P sink for the other soil P fractions, labile and moderately labile (Zheng et al., 2002). In fact, the changes observed in the  $\text{NaOH-Po}$  fraction at La Blanca and La Hansa sites were greater than in the  $\text{NaHCO}_3\text{-Po}$  fraction, as it represented the largest extractable P fraction for most soils (without considering the residual P fraction).

The residual P was affected by P fertilization only in the C-S-W/S rotation ( $P < 0.05$ ), suggesting that the continuous P fertilization enhanced the accumulation of the stable soil P fraction (Tables 2 and 3). Vu et al. (2008) reported an increase in residual P with increasing P fertilization rates.

The sequential P extraction accuracy was assessed by correlating TP with the sum of all the P forms obtained by the fractionation method ( $P_\Sigma$ ). The association was linear but had a slope different from the 1:1 line and an intercept different from 0. The value of  $P_\Sigma$  was 91% of the TP. Similarly, Blake et al. (2003) reported a high correlation ( $R^2 = 0.95$ ) between  $P_\Sigma$  and TP, presenting a slope different from 1, and noted that the  $P_\Sigma$  was around 86% of the TP. The difference between the sequential fractionation procedure ( $P_\Sigma$ ) and the TP measurement might be caused by solubility differences in the extraction or by the lack of quantification of the Po in the  $\text{HCl-P}$  fraction (He et al., 2003, 2006; Negassa and Leinweber, 2009). Nevertheless, we do not have enough information to draw conclusions about this result, which is a limitation of this research.

Summation of the difference in extractable P ( $W_p - F_p$ ) from Table 3 showed that the soil P content changed after 6 yr of fertilization, accounting for 85% of the cumulative P balance

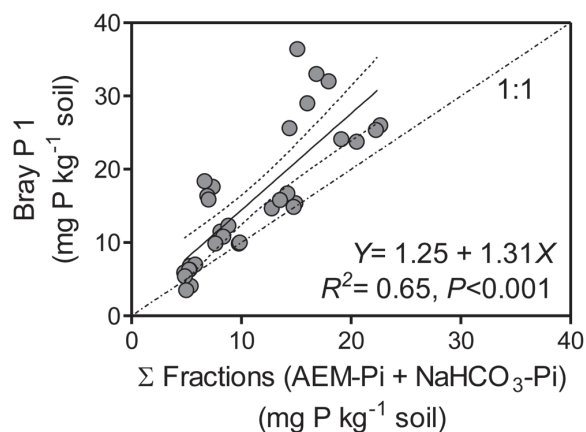
**Table 3. Significance P values from analysis of variance (ANOVA) and mean change due to fertilization at the 0- to 20-cm soil layer under two crop rotations, corn-soybean-wheat/soybean (C-S-W/S) and corn-wheat/soybean (C-W/S), in four experimental sites.**

	P fractions						
	AEM-Pi	$\text{NaHCO}_3\text{-}$		$\text{NaOH-}$		$\text{HCl-Pi}$	$\text{H}_2\text{SO}_4\text{-P}$
		Pi	Po	Pi	Po		
Significance level from ANOVA, $P > F\#$							
C-S-W/S Rotation							
S	NS	0.0001	NS	0.0030	0.0002	0.0001	NS
F	0.0001	0.0001	NS	0.0001	0.0050	0.0001	0.0294
S x F	NS	NS	NS	0.008	NS	NS	NS
C-W/S Rotation							
S	0.0001	0.0001	NS	NS	0.0090	NS	0.0048
F	0.0001	0.0001	0.0001	0.0001	0.0003	0.0001	NS
S x F	0.0001	0.0001	0.0004	NS	NS	0.0430	NS
Mean change between fertilization treatments ( $F_p$ minus $W_p$ ), $\text{kg P ha}^{-1}$ (0 to 20-cm soil depth)							
C-S-W/S Rotation							
La Blanca	13.5	9.7	-0.5	9.1	38.3	47.6	24.7
La Hansa	11.7	10.1	-2.1	16.9	32.8	47.4	57.0
C-W/S Rotation							
La Marta	2.3	2.4	47.9	10.9	18.6	39.4	23.5
San Alfredo	12.0	12.2	11.9	12.3	41.9	21.9	12.3

S = Site; F = Fertilization.  $W_p$  = without P added and  $F_p$  = with P fertilizer applied.

#NS, not significant at  $P < 0.05$ .

(Table 2). Blake et al. (2003) reported that 88% of the net P (P fertilizer input minus crop removal) balance was accounted for by the sum of resin,  $0.5 \text{ mol L}^{-1} \text{NaHCO}_3\text{-Pi}$ ,  $0.1 \text{ mol L}^{-1} \text{NaOH}$ ,  $1.0 \text{ mol L}^{-1} \text{NaOH}$ , and  $0.5 \text{ mol L}^{-1} \text{H}_2\text{SO}_4$  P fractions, and there was no further improvement in the average P accounted for by relating the P balance to the sum of all the P fractions. There was a full recovery of the applied P at the 0- to 20-cm soil depth at La Hansa and La Marta sites, while it was only 76 and 72% at the San Alfredo and La Blanca locations, respectively. Because the fractionation method had a good accuracy to determine the soil P fractions, one of the reasons for the incomplete recovery of P could be that part of the applied P moved into the subsoil, as was



**Fig. 1. Relationship between the sum of anion exchange membrane (AEM) plus  $\text{NaHCO}_3$  inorganic P ( $P_i$ ) fractions and the soil extractable P (Bray-1). The broken lines represent the confidence interval of 95% of the data set. For further details about P Bray-1 values, see Ciampitti et al. (2011).**

observed by Blake et al. (2003) on cropped soils. In agreement, the Bray-1 P concentration in the 20- to 40-cm sampling depth for the fertilized treatments increased by 6.9 and 11.5 mg P kg<sup>-1</sup> at the San Alfredo and La Marta sites, respectively (Ciampitti, 2009). Downward transport of P can occur as inorganic phosphate ions in solution, as low-molecular-weight organic compounds in solution, or as occluded Pi or Po (Blake et al., 2003). Phosphorus transport could occur due to preferential flow paths created by root growth, leading to percolation of surface P into the deeper subsoil layers, and promoting a mobilization of P from the surface to deeper layers (Blake et al., 2000, 2003; Zhang et al., 2006; Wang et al., 2007).

The sum of the AEM-Pi, NaHCO<sub>3</sub>-Pi, and NaHCO<sub>3</sub>-Po fractions represented 24% of the excess Pi applied to the soil (Fp minus Wp treatments), whereas the moderately labile pools, NaOH-Pi and -Po and HCl-Pi forms, made up 57%, and the residual P comprised 19% of the total P applications. On average, 50% of the difference between fertilization treatments was distributed in the HCl-Pi and NaOH-Po pools. Consistent with our results, the main sink of excess Pi applied through inorganic fertilizers was previously identified as the 0.1 mol L<sup>-1</sup> NaOH fraction in both a sandy clay loam soil (Zhang and MacKenzie, 1997) and a Typic Paleudult (Beck and Sanchez, 1994).

## Correlation Analysis

A simple correlation analysis among soil P fractions was performed, separating Wp and Fp treatments as two different groups, because as shown in Table 2, greater differences resulted from P fertilization than from management systems. The correlation coefficients among the seven P pools and the exogenous Pi (inorganic fertilizer) differed between treatments (Table 4). Within the check (Wp) group, AEM-Pi was positively related to the NaHCO<sub>3</sub>-Pi, HCl-Pi, and NaOH-Po fractions. Similar trends resulted within the Fp group between the AEM-Pi and NaHCO<sub>3</sub>-Pi, HCl-Pi, and NaOH-Po fractions. Nevertheless, the AEM-Pi was negatively related to NaHCO<sub>3</sub>-Po (Table 4). In the Wp group, the NaHCO<sub>3</sub>-Po and -Pi fractions were positively correlated with the NaOH-Po and -Pi pools, respectively, suggesting that both organic and inorganic P fractions decreased when P removal by crops increased over the years. In the Fp group, the relationship between the two organic pools (NaHCO<sub>3</sub>- and NaOH-Po) was negative, which implies that when NaHCO<sub>3</sub>-Po decreased, NaOH-Po increased, or vice versa (Table 4). In addition, within the Fp group, the NaHCO<sub>3</sub>-Pi form was positively related to the HCl-Pi fraction (Table 4). Furthermore, the NaHCO<sub>3</sub>-Pi fraction was inversely associated with the NaHCO<sub>3</sub>-Po form (Table 4). In the Wp group, the HCl-Pi was positively and closely related with the AEM-Pi, NaHCO<sub>3</sub>-Po, and NaOH-Po forms. The added Pi was not significantly related to any P fractions within the Fp group (Table 4).

As mentioned by Zheng et al. (2002), correlation is descriptive and does not necessarily indicate a cause-and-effect relationship, and a significant correlation coefficient could be a

noncausal or unauthentic relationship (Johnson and Wichern, 1988, p. 273–333). Therefore, path analysis is specifically suggested to accurately analyze cause-and-effect relationships and split these relationships into direct and indirect effects (Johnson and Wichern, 1988, p. 273–333).

## Pathways of Phosphorus Fractions in Soil

The NaOH-Po, HCl-Pi, and residual P were the fractions that showed the largest changes due to fertilization (Table 2). Similar results were reported by Beck and Sanchez (1994), who showed that the NaOH-Po and residual P forms were the major P pools. The path relationships between soil P pools under the two fertilization treatments followed different trends; therefore they are discussed separately.

## Check Treatment

The path analysis showed that 97% of the variability in the AEM-Pi fraction was derived from changes in the different soil Pi and Po fractions (Fig. 2). The NaHCO<sub>3</sub>-Pi form derived mainly from NaOH-Pi and HCl-Pi, as shown by the positive and linear correlation with NaOH-Pi ( $\beta = 0.71$ ) and HCl-Pi ( $\beta = 0.85$ ) fractions. In contrast to the correlation analysis, the path coefficient between NaHCO<sub>3</sub>-Pi and NaHCO<sub>3</sub>-Po was significant ( $P < 0.05$ ) but weak, suggesting that NaHCO<sub>3</sub>-Po mineralized into the NaHCO<sub>3</sub>-Pi pool. Furthermore, it appears that NaHCO<sub>3</sub>-Po mineralized as the NaHCO<sub>3</sub>-Pi pool increased. The NaOH-Po and -Pi fractions acted as sources of labile NaHCO<sub>3</sub>-Po and -Pi, respectively. This clearly indicates that the labile Pi pool resulted from mineralization of NaOH-Po through NaHCO<sub>3</sub>-Po and mobilization of NaOH-Pi. Therefore, NaHCO<sub>3</sub>-Pi is an important reservoir of P that acted as a main sink of NaOH-Po, NaHCO<sub>3</sub>-Po, NaOH-Pi, and HCl-Pi (P sources). The NaOH-Po fraction also acted as a source for the AEM-Pi labile pool by mineralization. Similar results were reported by Beck and Sanchez (1994), who found that NaOH-Po was the primary source of plant-available P in unfertilized soils.

Conversely to the correlation analysis, a negative relationship was found between the AEM-Pi and NaHCO<sub>3</sub>-Pi and the NaOH-Pi fractions (Fig. 2). Similarly, Zheng et al. (2002) observed that resin-P and NaHCO<sub>3</sub>-Pi had a negative path coefficient correlation because both fractions are part of the same P pool, which is considered as labile P adsorbed on surfaces of more crystalline P compounds, sesquioxides, or carbonates (Mattingly, 1975). The negative relationship between AEM-Pi and NaOH-Pi was reported earlier by Tiessen et al. (1984) for Mollisols, by Beck and Sanchez (1994) for an Ultisol without fertilization, and by Zheng et al. (2002) in two monoculture systems. The AEM-Pi fraction significantly contributed to P mobilization from NaHCO<sub>3</sub>-Pi ( $\beta = -0.41$ ) and NaOH-Pi ( $\beta = -0.51$ ), and the accumulation of AEM-Pi tended to reduce the formation of NaHCO<sub>3</sub>-Pi and NaOH-Pi. The residual P was positively related to NaHCO<sub>3</sub>-Pi ( $\beta = 0.37$ ) and NaOH-Po ( $\beta = 2.10$ ) and negatively related to HCl-Pi ( $\beta = -2.20$ ). A decrease

in  $\text{NaHCO}_3\text{-Pi}$  and  $\text{NaOH-Po}$  and an increase in  $\text{HCl-Pi}$  accompanied decreases in the residual P in this system. The path analysis also showed that residual P was indirectly affected by  $\text{NaOH-Pi}$  through  $\text{HCl-Pi}$  (Fig. 2).

### Fertilized Treatment

After 6 yr of continuous P applications, path analysis showed that 91% of the variability in AEM-Pi was explained by changes in the other soil P fractions. An important difference compared with the check treatment was the positive relationship between the  $\text{HCl-Pi}$  and residual P fractions ( $\beta = 0.76$ ; Fig. 3). Beck and Sanchez (1994) and Zhang and MacKenzie (1997) also observed a positive correlation between these two fractions for fertilized systems. In addition, the  $\text{NaOH-Pi}$  was mainly the direct effect of the applied Pi ( $\beta = 0.86$ ). Similarly, Simard et al. (1995) and Zheng et al. (2004) suggested that  $\text{NaOH-Pi}$  was an important sink for added or mineralized P in agricultural soils. The negative coefficient of  $-1.65$  between  $\text{HCl-Pi}$  and  $\text{NaHCO}_3\text{-Pi}$  indicated that the accumulation of  $\text{NaHCO}_3\text{-Pi}$  reduced the formation of  $\text{HCl-Pi}$  or vice versa; however, the increases in  $\text{NaOH-Pi}$  replenished the  $\text{HCl-Pi}$  ( $\beta = 0.57$ ) and  $\text{NaHCO}_3\text{-Pi}$  fractions ( $\beta = 0.36$ ). Furthermore, the  $\text{NaOH-Pi}$  fraction acted indirectly as a source for residual P through the transient  $\text{HCl-Pi}$  pool (Fig. 3). This observation was also reported by Beck and Sanchez (1994), who observed a positive correlation coefficient between  $\text{NaOH-Pi}$  and residual P.

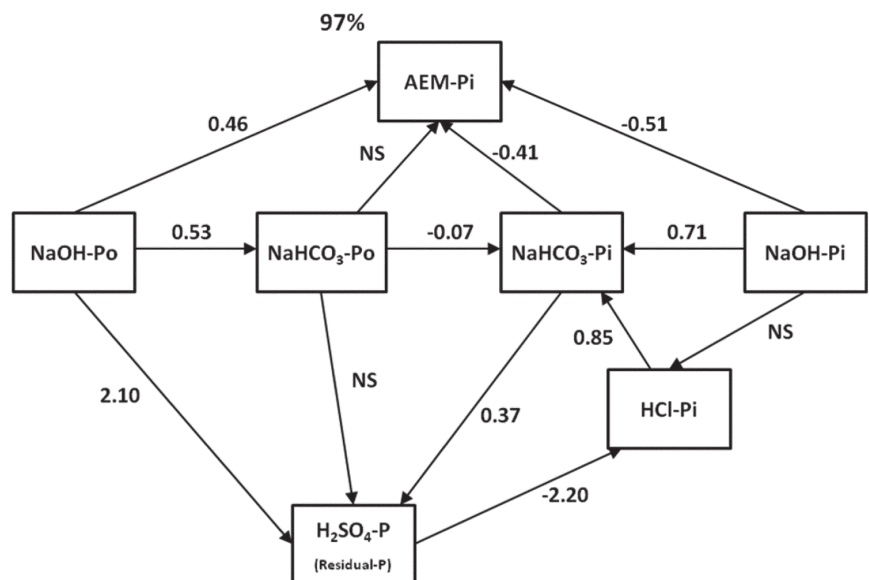
The residual P was not significantly related to  $\text{NaOH-Po}$  or  $\text{NaHCO}_3\text{-Po}$  or  $\text{-Pi}$ , as indicated by the lack of significant correlation among these pools. In accordance with the observed trends and paths, similar findings were also reported by Zheng et al. (2004). The AEM-Pi contributed significantly to the formation of  $\text{NaHCO}_3\text{-Po}$  ( $\beta = -0.85$ ) and  $\text{-Pi}$  ( $\beta = -0.89$ ) but acted as a sink for  $\text{NaOH-Pi}$ . Thus, the effect of inorganic P fertilization on AEM-Pi was mainly indirectly through the formation of  $\text{NaOH-Pi}$ . Similarly, Beck and Sanchez (1994) found that  $\text{NaHCO}_3\text{-Po}$  was the sink for the resin-Pi. Furthermore, Zheng et al. (2004) reported that  $\text{NaHCO}_3\text{-Pi}$  was the main sink for the resin-Pi. The path coefficients between the  $\text{NaOH-Po}$  and  $\text{NaHCO}_3\text{-Po}$  fractions ( $\beta =$

**Table 4.** Pearson's correlation coefficients for P fractions in the 0–20 cm soil layer in two fertilization treatments, with or without P, after 6 yr of continuous cropping. Coefficients significant at  $P < 0.05$  are shown.

	Inorganic P				Organic P		$\text{H}_2\text{SO}_4$	Added Pi
	AEM	$\text{NaHCO}_3$	NaOH	HCl	$\text{NaHCO}_3$	NaOH		
<u>0–20 cm soil layer, without P fertilizer (Wp)</u>								
$\text{H}_2\text{SO}_4$	–	–	–	–	–	–	1.00	
NaOH-Po	0.83	–	–	0.97	0.60	1.00		
$\text{NaHCO}_3\text{-Po}$	–	–	–	0.61	1.00			
HCl-Pi	0.89	0.47	–	1.00				
NaOH-Pi	–	0.68	1.00					
$\text{NaHCO}_3\text{-Pi}$	0.69	1.00						
AEM-Pi	1.00							
<u>0–20 cm soil layer, with P fertilizer (Fp)</u>								
Added Pi	–	–	–	–	–	–	–	1.00
$\text{H}_2\text{SO}_4$	–	–	–	–	–	–	1.00	
NaOH-Po	0.75	0.63	–	0.92	-0.77	1.00		
$\text{NaHCO}_3\text{-Po}$	-0.85	-0.77	–	-0.74	1.00			
HCl-Pi	0.77	0.61	0.61	1.00				
NaOH-Pi	–	–	1.00					
$\text{NaHCO}_3\text{-Pi}$	0.77	1.00						
AEM-Pi	1.00							

Pi = inorganic P; Po = organic P.

$-0.56$ ) and the  $\text{NaHCO}_3\text{-Po}$  and  $\text{-Pi}$  fractions ( $\beta = -0.78$ ) were negative (Fig. 3). The  $\text{NaOH-Po}$  and  $\text{NaHCO}_3\text{-Po}$  fractions appeared to be the sink for the  $\text{NaHCO}_3\text{-Pi}$  labile pool in this system, increasing these organic fractions as a consequence of P mobilization from  $\text{NaHCO}_3\text{-Pi}$ . In our experiment, however, the  $\text{NaHCO}_3\text{-Po}$  fraction was lower under the Fp treatment than the Wp treatment at La Blanca and La Hansa sites; consistent increases were reported, however, for all four sites in the  $\text{NaOH-Po}$  fraction under continuous P fertilization compared with the unfertilized P plots. Nevertheless, the path analysis seems to indicate that Pi pools tended to reorganize into more stable Po



**Fig. 2.** Relationships among P pools in the 0- to 20-cm soil layer under continuous cropping during 6 yr without P added to the soil. Path coefficients are non-normalized. The percentage indicates the partial correlation between connected pools. Po = soil organic P, Pi = soil inorganic P, NS = not significant at  $P < 0.05$ .

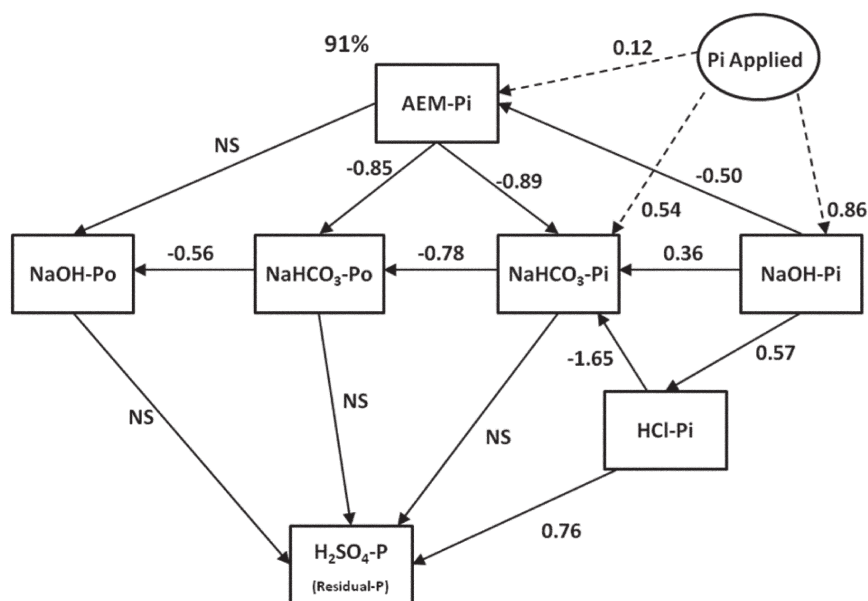


Fig. 3. Relationships among P pools in the 0- to 20-cm soil layer under continuous cropping during 6 yr with continuous P applied to the soil. Path coefficients are non-normalized. The percentage indicates the partial correlation between connected pools. Po = soil organic P, Pi = soil inorganic P, NS = not significant at  $P < 0.05$ .

fractions. The same finding was previously reported by Schmidt et al. (1996), Zhang and MacKenzie (1997), and Zheng et al. (2002). This is probably related to the excess of Pi fertilizer compared with plant uptake (Ciampitti et al., 2011), which might reduce the phosphorylase activity and consequently diminish the mineralization of NaOH-Po (Schmidt et al., 1996). In agreement, Hedley et al. (1982) showed increases in the Po fractions when soils were incubated with Pi additions.

## CONCLUSIONS

Fertilizer P itself is partly resin extractable, partly bicarbonate extractable, and largely acid extractable. The main sinks of fertilizer P were the NaOH-Po and HCl-Pi fractions, which, on average, accounted for 50% of the total P applied. Therefore, our results indicate that these fractions would have an active role in the soil P cycle.

The NaOH-Pi and -Po, HCl-Pi, and residual P fractions accounted for >76% of the TP. Our results suggest that the availability of inorganic P for plant nutrition could be limited not because of the soil P concentration but by the processes that regulate the release of P from these sources.

Despite the fact that the initial P values might exert some influence over some of the trends observed in our experiment, our main focus was the comparison between unfertilized and fertilized plots. The effect of the initial P values in a medium- to long-term trend is an appropriate question, however, for future research.

The pathway analysis for the unfertilized treatment showed (i) the importance of the Po pools for the replenishment of soil available P, represented by the AEM-Pi and NaHCO<sub>3</sub>-Pi fractions, and (ii) the role of NaOH-Pi along with HCl-Pi as major inorganic P sources of the NaHCO<sub>3</sub>-Pi fraction (Fig. 2).

For the fertilized plots, when the amount of P added was in excess of that removed by plants, the pathway analysis showed that the Pi fractions tend to reorganize into more stable Po fractions, increasing these fractions as a consequence of mobilization from NaHCO<sub>3</sub>-Pi. The organic P fraction seems to replenish the available P (as represented by AEM-Pi and NaHCO<sub>3</sub>-Pi), and the NaOH-Pi along with HCl-Pi acted as source of NaHCO<sub>3</sub>-Pi in situations with P deficiencies.

Finally, considering that the P fertilization treatments exerted significant and quantitatively relevant effects across the whole range of the Hedley fractions, we might assert that the classification of these fractions as labile or recalcitrant seems not to completely apply to their response to soil P enrichment through fertilizers. Considering the continuous nature of P forms in soils, the functional interpretation of operationally defined P fractions should be made with care.

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