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Effect of long-term phosphorus fertilization on soil phosphorus fractions

Efecto a largo plazo de la fertilización fosfatada en las fracciones de fósforo del suelo
Efeito a longo prazo da adubação fosfatada nas frações de fósforo do solo

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ABSTRACT

A limitation to crop production in the southeast of the Buenos Aires province (Argentina) is the low phosphorus (P) availability. P fertilization is required for high yields. The objective of this work was to quantify the forms of soil P as affected by different P fertilization strategies after 8 years under wheat. The combination of high rate (H; 176 kg P ha^{-1}), low rate (L; 88 kg P ha^{-1}), single application (S; at the beginning of the experiment), and fractionated application (F; annual fertilization of 22 and 11 kg P ha^{-1}) were evaluated. Soil total P, total inorganic P, total organic P, organic (Po) and inorganic (Pi) extractable with $NaHCO_3$ and NaOH, were determined, plus available P (P-Bray 1). Fertilization did not change the total P or the total Po (344 and 412 mg P kg^{-1}, respectively). Fertilized treatment, compared with a non-fertilized one, increased the concentration of Pi-$NaHCO_3$ (14.49 and 7.62 mg P kg^{-1}) and Pi-NaOH (47.13 and 28.37 mg P kg^{-1}). The H rate increased the Pi extracted with $NaHCO_3$ (13.16 mg P kg^{-1}) and with NaOH (53.82 mg P kg^{-1}) compared with the L rate (9.82 and 40.43 mg P kg^{-1}, respectively). The FH rate increased the concentration of Pi-NaOH (59.55 mg P kg^{-1}) compared to SH (48.10 mg P kg^{-1}), while the low rate produced no changes in this fraction. In summary, when the amount of P added was fractioned and exceeded the quantity removed by crop, the excess was converted mainly to Pi-NaOH. A positive and significant correlation ($r^2 = 0.95$; $P < 0.001$) between the sum of Pi-$NaHCO_3$ and Pi-NaOH vs. P-Bray 1 was established.

RESUMEN

Una de las limitaciones para la producción de cultivos en el sudeste de la provincia de Buenos Aires (Argentina) es la baja disponibilidad de fósforo (P), que es cubierta con la fertilización fosfatada. El objetivo de este trabajo fue cuantificar las formas de P en el suelo después de 8 años de monocultivo de trigo con diferentes dosis y estrategias de aplicación. Se determinó: P orgánico (Po) e inorgánico (Pi) extractable con $NaHCO_3$ *y con NaOH, y P-Bray 1. Se evaluó la combinación de dosis alta (H; 176 kg P ha*$^{-1}$*), dosis baja (L; 88 kg P ha*$^{-1}$*), aplicación única (S; al inicio del ensayo) y aplicación fraccionada (F; fertilizaciones anuales de 22 y 11 kg P ha*$^{-1}$ *durante 8 años). La fertilización no produjo cambios en el P total ni en el Po total (412 y 344 mg P kg*$^{-1}$*, respectivamente), e incrementó el Pi-*$NaHCO_3$ *(14,49 y 7,62 mg P kg*$^{-1}$*) y el Pi-NaOH (47,13 y 28,37 mg P kg*$^{-1}$*). La dosis H aumentó el Pi-*$NaHCO_3$ *(13,16 mg P kg*$^{-1}$*) y Pi-NaOH (53,82 mg P kg*$^{-1}$*) comparado con la dosis L (9,82 mg P kg*$^{-1}$ *y 40,43 mg P kg*$^{-1}$*, respectivamente). La aplicación HF incrementó el Pi-NaOH (59,55 mg P kg*$^{-1}$ *) respecto a HS (48,10 mg P kg*$^{-1}$*), mientras que la dosis L no produjo cambios en esta fracción. Cuando el P agregado fue fraccionado y excedió lo eliminado por el grano, el exceso de P se convirtió principalmente en Pi-NaOH. Se estableció una correlación positiva y significativa (*$r^2 = 0,95$*,* $P < 0,001$*) entre la suma de Pi-*$NaHCO_3$ *y Pi-NaOH vs. P-Bray 1.*

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RESUMO

Uma das limitações à produção vegetal no sudoeste da provincia de Buenos Aires (Argentina) é a baixa disponibilidade de fósforo (P) no solo, compensada pela adubação fosfatada. O objectivo do presente estudo foi quantificar, após 8 anos da monocultura de trigo, as formas de P no solo, recorrendo a diferentes doses e modalidades de aplicação. Determinou-se: P orgânico (Po) e P inorgânico (Pi) extraíveis com $NaHCO_3$ *e com NaOH, e P-Bray 1. Avaliou-se o efeito isolado e combinado de uma dose elevada (H; 176 kg P* ha^{-1}*), uma dose baixa (L; 88 kg P* ha^{-1}*), uma aplicação única (S; no início do ensaio) e uma aplicação fracionada (F; adubações anuais de 22 e 11 kg P* ha^{-1} *durante 8 anos). A adubação não conduziu a alterações do P total e do Po total (412 e 344 mg P* kg^{-1} *respetivamente), mas provocou um aumento do Pi-*$NaHCO_3$ *(14,49 y 7,62 mg P* kg^{-1}*) e do Pi-NaOH (47,13 e 28,37 mg P* kg^{-1}*). Por sua vez, a dose H aumentou o Pi-*$NaHCO_3$ *(13,16 mg P* kg^{-1}*) e o Pi-NaOH (53,82 mg P* kg^{-1}*) comparativamente à dose L (9,82 mg P* kg^{-1} *y 40,43 mg P* kg^{-1}*, respetivamente). A modalidade HF produziu um aumento do Pi-NaOH (59,55 mg P* kg^{-1} *) relativamente à modalidade HS (48,10 mg P* kg^{-1}*), enquanto a dose L não conduziu a alterações desta fração. Quando o P total foi fracionado e excedeu o P removido pelo grão, o excesso de P converteu-se essencialmente em Pi-NaOH. Obervou-se uma correlação positiva e significativa (*$r^2 = 0,95$*, P* $< 0,001$*) entre o somatório Pi-*$NaHCO_3$ *mais Pi-NaOH vs. P-Bray 1.*

KEY WORDS
residual P,
P balance,
P fractions

PALABRAS CLAVE
P residual,
balance de P,
fracciones de P

PALAVRAS-CHAVE
P residual,
balanço de P,
P fracionado

1. Introduction

One of the principal limitations to crop production in the southeast of Buenos Aires province (Argentina) is the low phosphorous (P) availability in the soils, which is frequently less than 10 mg kg^{-1} of Bray-extractable P (Echeverría and Ferrari 1999). In recent years, intensification in crop production has increased crop yields, and therefore phosphate fertilizers have had to be added more frequently to maximize yields.

The usual way to calculate the amount of phosphate fertilizer to be applied to a sequence of crops is to add the amount that would replace the P removed by crops in a cycle as a function of the expected yield, plus an amount for the partial reconstruction of the soil P level. Another fertilization strategy in crop rotation is to apply to the first crop an amount of P that exceeds its requirements so that the surplus could be used by the next crop in the rotation. The latter method results in an initial increment of soil extractable P that later diminishes in a magnitude that varies according to rates and the frequency of fertilization (Berardo et al. 1997). P availability in the soil decreases over the time and tends to stabilize (Halvorson and Black 1985), although the mechanisms accounting for this process are not fully understood.

Without fertilization, the flux between the different P reservoirs in the soil is regulated by plant absorption. The P extracted by crops is replaced in the soil solution by the dissolution of primary and secondary minerals, desorption of Pi from the mineral surface, and mineralization of Po. The increase in P concentration in the soil solution by fertilizer addition enhances the phosphate ions absorbed by roots, microbial biomass, or retained through sorption–desorption–precipitation–dissolution and immobilization–mineralization mechanisms (Havlin et al. 2005).

It has been widely agreed that P fertilization increases the extractable P content determined by Bray-Kurtz and Olsen-Watanabe methods in soils under pastures and crops (Verna et al. 2005; McKenzie et al. 1992; Beck and Sanchez 1994). An estimate of plant available P is obtained by relating data from soil analysis (Olsen or Bray) to the response of crops to P fertilization. The P value at which yield approaches the asymptote can be considered the critical value required in order to maintain the optimum productivity. These extraction methods do not provide information about the P-replenishing ability of soil. Since the P remaining in the soil solution after plant uptake is replenished by labile fractions of P that are in equilibrium with other mineral or organic P fractions, it is very important to know the magnitude of each P fraction in order to understand the repositioning mechanisms. Research conducted on unfertilized and highly weathered soils showed that the P organic forms are the main sources of available P for plants (Beck and Sanchez 1994, Tiessen et al. 1992) while the Pi-NaOH and Pi-$NaHCO_3$ are the major fractions in the Mollisols (Tiessen et al. 1984). In the southeast of the Buenos Aires province (Argentina) in a long-term fertilization experiment, the addition of high rates of P increased the labile inorganic fraction only during the first year after P application (Zamuner and Culot 1999). The labile organic fraction showed changes according to the amount of P applied.

However, there are a lot of questions concerning the qualitative and quantitative alterations of phosphate ions in the medium-term due to fertilization strategies. From the agronomic point of view, the knowledge of the forms in which P is found in the soil, and the comprehension of the mechanisms that account for the replacement of soluble P extracted by plants would be useful tools for the selection of a fertilization strategy that would provide a better P supply to crops.

The objectives of this work were: 1) to assess the effect of several rates of phosphate fertilizer on the P fractions of different labilitiy, and 2) to evaluate various phosphate fertilization strategies (fractioned and non-fractioned application) on P fractions of different lability.

2. Materials and Methods

Soil samples were used from a long-term experiment with 8 years of wheat monoculture, located at the INTA-Balcarce experimental station in the province of Buenos Aires (37° 45´ S 58° 18´ W).

The long-term experiment was designed to evaluate different strategies of phosphorus fertilization, comparing fertilization applied only at the beginning of the experiment with repeated fertilization over time. Rates were calculated so that they matched after several years.

The region is mesothermal-humid to sub-humid, with 13.7 °C annual average air temperature and 927.5 mm annual average rainfall (1976-95), occurring mostly in the spring-summer period. The dominant soil is a Typic Argiudol, fine, mixed, thermic with a loam surface horizon of medium depth and granular structure. Initial soil organic carbon was 36.6 g kg^{-1} and pH 5.8 (soil:water ratio 1:2). Soil characteristics associated with P at the beginning of the research are shown in **Table 1**.

During the 8 years of the experiment, wheat was tilled following conventional management in the area and different phosphate fertilization strategies were applied. A combination of low and high fertilization rates and two forms of application, a single one and a fractioned one, were used. The low rate (L) corresponded to 88 kg P ha^{-1} and the high rate (H) to 176 kg P ha^{-1}. The lower rate is the recommended one in the area for wheat when the expected yield is 4,000 kg ha^{-1} and available soil P is 9 to 11 mg P ha^{-1} (Echeverria and Garcia 2005). The single (S) application is a complete rate (176 or 88 kg P ha^{-1}) that was applied at the beginning of the experiment. In the fractioned (F) application, an annual rate of 22 or 11 kg P ha^{-1} was applied for 8 years to H or L rate respectively at the end of

Table 1. Soil characteristics and soil phosphorus fractions (P) at the beginning of the experiment

	Concentration
Total organic carbon	36.6 g kg^{-1}
pH (soil:water ratio 1:2.5)	5.8
Fe free	0.75%
Cation exchange capacity	26 meq 100 g^{-1}
Soluble cations	
Ca	11.1 meq 100 g^{-1}
Mg	3.2 meq 100 g^{-1}
K	2.2 meq 100 g^{-1}
Clay	265 g kg^{-1}
Loam	337 g kg^{-1}
Sand	398 g kg^{-1}
Extractable P-Bray 1	10.30 mg P kg^{-1}
Total P	471.11 mg P kg^{-1}
Total inorganic P	84.60 mg P kg^{-1}
Total organic P	386.51 mg P kg^{-1}
Organic P extractable with $NaHCO_3$ 0.5 M	24.48 mg P kg^{-1}
Organic P extractable with NaOH 0.1 N	52.20 mg P kg^{-1}
Inorganic P extractable with $NaHCO_3$ 0.5 M	9.11 mg P kg^{-1}
Inorganic P extractable with NaOH 0.1 N	33.00 mg P kg^{-1}

the experiment. From the combination of rates and application strategies the 5 treatments were as follows: control, low rate-single application (LS), low rate-fractioned application (LF), high rate-single application (HS) and high rate-fractioned application (HF).

P fertilizer was added as triple superphosphate [$Ca(H_2PO_4)_2.H_2O$) applied to the sowing line up to a rate of 88 kg P ha^{-1}. In the treatments with higher rates, the rest of the fraction was broadcasted. It was introduced to the soil with a broadcast spreader before seeding. Every year, urea was applied to all trials so that N was not a limiting nutrient for crop growth. N rates were uniform for all treatments but varied annually from 80 to 120 kg N ha^{-1} in agreement with the content of available N at sowing between 0 and 120 cm of depth. The experimental design was of complete randomized blocks with 3 repetitions.

The amount of P removed by the crop during eight years was calculated using the yield and P concentration in grain. The P input was calculated as a function of the amount of fertilizer applied, and the output from the crop yield and P concentration in grain (Berardo and Grattone, personal communication). The balance was assessed from the difference between the P input and the output in the system.

To evaluate the P sink applied as fertilizer, composite samples from the superficial 18 cm were taken in each trial. In the laboratory, the plant residues were manually eliminated and the soil samples were air-dried, screened with a 0.5 mm mesh and kept at room temperature until their analysis. The following was determined:

- Total P (TP) was obtained from calcination (Saunders and Williams 1955); total inorganic P (TIP) by H_2SO_4 1N extraction (Saunders and Williams 1955); and total organic P (TOP) from the difference between TP and TIP.

- Inorganic P soluble in $NaHCO_3$ 0.5 M pH 8.5 (Pi-$NaHCO_3$) was quantified according to Bowman and Cole (1978). Total P soluble in $NaHCO_3$ 0.5 M pH 8.5 was obtained from digestion of $NaHCO_3$ extract with HNO_3 and $HOCl_4$ (Shaw 1959). Organic P soluble in $NaHCO_3$ 0.5 M pH 8.5 (Po-$NaHCO_3$) was calculated as the difference between total soluble P and Pi-$NaHCO_3$.

- Inorganic P soluble in NaOH 0.1 N (Pi-NaOH) was determined according to Bowman and Cole (1978). Total P soluble in NaOH 0.1 N was obtained by digestion of the alkaline extract with HNO_3 and $HOCl_4$. Organic P soluble in NaOH 0.1 N (Po-NaOH) was calculated as the difference between total and inorganic P, both soluble in NaOH 0.1N.

- Extractable P-Bray 1 (Bray and Kurtz 1945).

Concentration of inorganic P forms obtained with the different extractants was determined using the Murphy and Riley (1962) colorimetric technique. All the analyses were carried out 3 times for each composite sample. P concentration was expressed in mg P kg^{-1}. The P balances were calculated using the P applied annually and the average annual P offtake in the harvested crops.

Applying Statistical Analysis System procedures (SAS Institute Inc. 1996), variance analyses and comparisons between the selected treatments through orthogonal contrasts were carried out for a complete randomized block design with two repetitions and five treatments. The following contrasts were assessed: control vs. fertilized, single application vs. fractioned application (HS-LS vs. HF-LF), high rate in a single application vs. high rate in fractioned application (HS vs. HF) and low rate in a single application vs. low rate in fractioned application (LS vs. LF). A linear model (SAS Institute Inc. 1996) was used to describe the relationship between Bray 1 and another available P test.

3. Results and Discussion

1. P balance

Accumulated dry matter production with the HS treatment was similar to that obtained by applying the same rate but fractioned during eight years (34.03 and 36.5 Mg ha^{-1}, respectively). When the lower P rate was applied, in a single or fractioned application, dry matter production was also similar (32.03 and 32.15 Mg ha^{-1} respectively, data not shown). These results suggest that the P applied in a single rate at the beginning was as available through time as the P applied at a fractioned rate. Fertilization with P increased grain P concentration, regardless of the rate or strategy employed (0.25 % and 0.30% in control and fertilized treatments respectively). Exported P by gain was 112.30-109.60-92.89-96.40 and 64.09 kg ha^{-1} for HS-HF-LS-LF and control respectively (data not shown).

Phosphate ions have little mobility because of their tendencies to be adsorbed on colloid surfaces and to form insoluble compounds with bi and trivalent cations (Stevenson and Cole 1999), and therefore the main soil P removal is by crops. The P balance was negative (-64 kg P ha^{-1}) in the control, close to zero with low fertilizer rates (-5 and -8 kg ha^{-1} for LS and LF, respectively), and positive in the treatments with high fertilizer rates (64 and 66 kg P ha^{-1} for HS and HF, respectively). Theses results show the importance of fertilization in the maintenance of P availability for crops. Moreover, when the P balance is positive, the P from the fertilizer that is not extracted by the grain should accumulate in the soil. As P is not retained in a single fraction of the soil but in several, it is expected that the positive or negative P balance is associated with increases or decreases in the P content in different soil fractions.

2. Effect of fertilization treatments on total phosphorous

The concentrations of TP, TOP and TIP from soil samples under different phosphate fertilization strategies are shown in **Figure 1**.

No significant change in the TP concentration (p = 0.52) was produced by applying different fertilization strategies (an average of 412 mg P kg^{-1}). This concentration is similar to the TP concentration values from 400 to 800 mg P kg^{-1} reported in previous investigations carried out on soils of the same area by Echeverria and Navarro

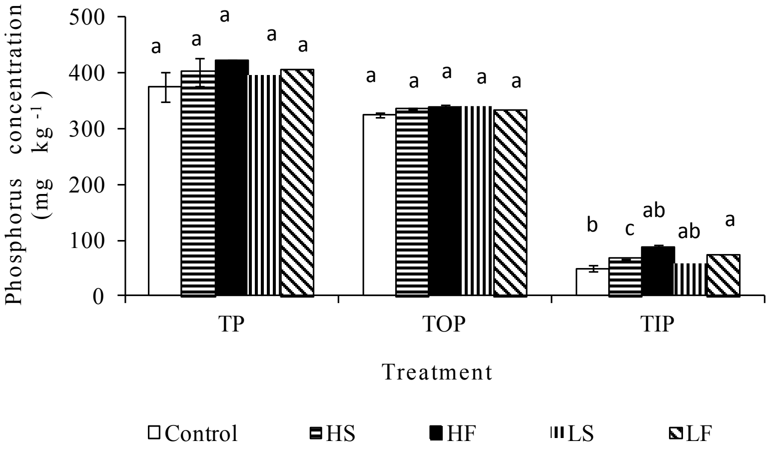

Figure 1. Soil total phosphorus (TP), total inorganic P (TIP) and total organic P (TOP) for high rate (H), low rate (L), single (S) or fractioned (F) application of phosphate fertilizer in wheat monoculture. Bars show standard deviations.

(1984) and Zamuner et al. (2008). Although the differences were not statistically significant, in the soil where high rates of P were applied, 5% more TP was quantified than in the non-fertilized soil in agreement with positive P balance. Verna et al. (2005) reported that when samples from a soil cultivated with corn-wheat sequences during 29 years were fertilized annually with P and N, the TP content increased compared with the original soil. Blake et al. (2003) also reported changes in the total P content associated with a positive or negative balance after 35 years under crops. This shows that TP changes require long periods under cultivation and therefore a period of 8 years was not sufficient to detect significant differences in this variable.

Organic P forms (TOP) were dominant regardless of the mineral fertilization that was applied. No significant differences due to fertilization were found ($p = 0.56$) averaging 344 mg P kg^{-1} (**Figure 1**). Studies of soils of this area agree that 80% of TP in the arable layer is in the organic form (Picone et al. 2003). This result is linked to the high content of organic matter in the soils of the region.

The fraction with the lower value but the most sensible to fertilization effects was TIP. Fertilized soils had a significantly ($p = 0.0074$) higher inorganic P concentration than the control or LS treatment. Similarly Takahashi and Anwar (2007), investigating the wheat yield response to long-term fertilization and evaluating soil P fractions after 23-year fertilization on an Andosol, concluded that TIP was greater in the treatments with P applications than the treatment without P applications, with no significant difference in TOP among the treatments.

When a high rate of phosphate fertilizer was fractioned and applied annually, soil TIP concentration increased significantly compared to when the whole application was applied at the beginning of the investigation (88.95 and 68.37 mg P kg^{-1}, respectively). These results were not the same for a low P rate of fertilization where no significant differences due to fertilization strategies were found, with an average of 64.91 mg P kg^{-1} (**Figure 1**).

3. Effects of fertilization treatments on phosphorous fractions

The concentrations of organic P fractions in the soil after 8 years of wheat monoculture with different rates and strategies of phosphate fertilization application are presented in **Table 2**.

The Po extracted with $NaHCO_3$ has been considered to be an active fraction of soil Po because it represents compounds such as glycerophosphate and ribonucleic acid that are readily exchanged with the soil (Bowman and Cole 1978). Po-NaOH is associated with humic compounds (Schoenau et al. 1989). The organic fractions were not affected by the fertilization rate or strategy. This is in agreement with the TOP findings. The Po-$NaHCO_3$ concentration represented 5% of the TP (21.69 mg P kg^{-1}) and the NaOH extractable Po averaged 21% of the TP (85.31 mg P kg^{-1}). Similarly, McKenzie et al. (1992) concluded that inorganic fertilization increases inorganic P reservoirs but that it has no effect on labile and moderately labile organic forms on the long term. Ciampitti et al. (2011), analyzing the effect of continuous P fertilization on soils of the Pampas Argentinas, reported that P fertilization increased the Po-$NaHCO_3$ form at the site with a high sand content (566 g kg^{-1}), which could generate a low soil P sorption capacity. However, there was no difference in Po-$NaHCO_3$ in soils with similar properties. Ciampitti et al. (2011) reported that fertilization exerted a large influence on the Po-NaOH fraction. Our experiment was carried out under conventional tillage in contrast to that of Ciampitti et al. (2011), which was under no tillage. Cabria et al. (2005) reported that continuous agriculture under conventional tillage caused a reduction in macroaggregate mass and a decrease in Fe concentration associated with organic compounds. According to Schoenau et al. (1989), Po-NaOH is associated with humic compounds and adsorbed to Al and Fe. Therefore, as both the soil organic matter and Fe concentration are reduced with conventional tillage, the capacity of the soil to retain P in the form of Po-NaOH may also be reduced.

Inorganic P concentrations obtained with the different extractants is presented in **Table 2**. The Pi-$NaHCO_3$ represented a small proportion of soil TP (about 2-3%), while Pi-NaOH represented 10% of TP. Both inorganic fractions were affected by fertilization in different ways, and so the results obtained from the contrasting treatments and strategies are presented separately.

Table 2. Effect of rate and form of phosphorus application on different forms of phosphorus (P) concentration: organic, inorganic and extractable-Bray 1

TREATMENT	Organic P		Inorganic P		P-Bray 1
	Po-$NaHCO_3$	Po-NaOH	Pi-$NaHCO_3$	Pi-NaOH	
		----------------	mg kg^{-1}	----------------	
HS	23.49	84.16	11.53	48.10	19.73
HF	23.97	83.38	14.80	59.55	29.47
LS	21.94	101.77	9.46	40.92	15.75
LF	20.08	72.17	10.18	39.94	17.99
Control	20.90	84.52	7.62	28.37	11.27
CONTRASTS					
Control vs. fertilized	0.74	0.96	0.03	0.009	0.003
HS-HF vs. LS-LF	0.20	0.86	0.08	0.018	0.003
HS vs. HF	0.89	0.98	0.11	0.084	0.006
LS vs. LF	0.33	0.31	0.27	0.940	0.29

H = high rate. L = low rate. S = single application. F = fractioned application.

• *Control vs. fertilized treatments*

Fertilization significantly increased the concentration of Pi labile and moderately labile forms in the soil. Inorganic P extracted with $NaHCO_3$ 0.5 M was 51% higher in fertilized than in control treatments (11.49 and 7.62 mg P kg^{-1}, respectively, **Table 2**). Similarly, the Pi-NaOH concentration was also increased with fertilization, with an average concentration of 47.13 mg P kg^{-1} in fertilized soils significantly higher than the 28.37 mg P kg^{-1} from the unfertilized soil (**Table 2**). The results obtained agree with the values registered by other authors from a wide range of soils showing an increase in the Pi-$NaHCO_3$ and Pi-NaOH fractions with P applications (Wang et al. 2007; Picone et al. 2007; Verna et al. 2005; Guo et al. 2000; O´Halloran 1993; Selles et al. 1995). The increases in Pi-$NaHCO_3$ and Pi-NaOH, indicate that these fractions are an important sink of added fertilizer according to Picone et al (2007) and Ciampitti (2011), who studied the transformations of P in Mollisols. Equilibrium reactions between labile Pi (Pi-$NaHCO_3$) and soil solutions are responsible for re-establishing the phosphate ion concentration in the soil solution when it diminishes due to radical absorption and/or microbial immobilization. Chemisorption of Pi (Pi-NaOH) to amorphous or some crystalline Fe and Al oxides restore labile Pi in the medium term. In the medium term and in the absence of phosphate fertilization, P extraction by crops should be expressed by the lowering of some reservoirs responsible for its replacement, if crop productivity is sustained.

• High rate vs. low rate

When the high P rate was applied, the soil concentration of all inorganic fractions was higher than the one obtained in the plots fertilized with the low rate, regardless of the fertilization strategy used (**Table 2**). Concentration of Pi-$NaHCO_3$ was 34% higher for the high rate than for the low one, 13.16 and 9.82 mg P kg^{-1} for HS-FH and SL-FL, respectively. In a similar way, the soil Pi-NaOH concentration that received the high P rate was significantly higher than that reached with treatments with a low rate of fertilizer (**Table 2**). The average values obtained were 53.82 and 40.43 mg P kg^{-1} for SH-FH and SL-FL, respectively. This increase was more marked in more strongly retained P, and available in the medium term (Pi-NaOH). These results show that if a high fertilizer rate is applied, an increase of P forms available for plants in a short and medium term occurs, a situation that is in agreement with the positive P balance. It can therefore be inferred that this is the main sink of excess added P. Zhang and McKenzie (1997) evaluated the changes in P fractions after the addition of inorganic P fertilizer at normal rate and a high rates in a four-year corn monoculture. They similarly concluded that Pi-NaOH was the sink of added P, while most Pi-$NaHCO_3$ was indirectly contributed to by the formation of Pi-NaOH. Pi-NaOH was the sink for fertilizer P, probably due to the formation of amorphous or crystaline Fe and Al phosphates favored by low pH. Although no soil pH values during the experiment were registered, it is possible that a small decrease occurred. This could have been due in part to the application of a high rate of triple superphosphate that diminishes pH when solubilized, and also due in part to the higher carbon availability through residues returning to the soil that releases acids when degraded, since there was a higher dry-matter production from the fertilizing treatments with high P rates.

• Single application vs fractioned application

The effect of the fertilization strategy (single or fractioned application) was analyzed for each rate in particular. When a low rate of fertilizer was applied no significant differences between single and fractioned application were found in any of the inorganic fractions evaluated (**Table 2**). This is consistent with the soil P balance near to zero. It can therefore be inferred that, when fertilizing with a low P rate, equilibrium between inorganic and organic P remained unchanged (Zhang and Mc Kenzie 1997).

However, when a high rate was added, a greater amount of P was retained under moderately labile inorganic forms (Pi-NaOH) than when the same amount was added in one application (**Table 2**). A study carried out at the beginning of this investigation showed that sorption processes were more important in soils fertilized at high rates than in the control during the first year following fertilization (Zamuner and Culot 1999). From this it could be inferred that frequent application at a high P rate in excess of crop demands would enhance the Pi chemical adsorption process to Fe and Al oxides that are NaOH extractable.

4. Relationship between available P and phosphorus fractions

After 8 years of wheat monoculture there was a concentration of 11.3 mg kg^{-1} of available P-Bray 1 in an unfertilized soil. This is considered as "low" P availability for a wheat crop, with a high probability of response to phosphate fertilization. The critical soil P level in the Pampas Region is 17 mg P-Bray 1 kg^{-1} (Echeverría and García 2005; Zamuner et al. 2006b). According to Zamuner et al. (2006a), this value corresponds to 18 mg P-Mehlich kg^{-1}, 11 mg P-Olsen kg^{-1} and 23 mg P-Anion Exchange Resin kg^{-1}. Therefore, the fertilized soil P-Bray 1 values between 18 and 29 mg kg^{-1} are near to maximum yield and the fertilizer response is likely to be null.

P-balancing shows that fertilized soils with a high rate of P have a P net gain and, in accordance with that, the initial extractable P-Bray 1 increased with time. The control that had a net P loss showed no decrease on the initial level of available P, which remained relatively constant. This indicates that the P forms extracted by Bray 1 removed by the crop are restored by other P forms. Similar results were reported by Kuo et al. (2005).

The source of P absorbed by the plants is the soluble fraction of soil P, which is replenished by a labile fraction (organic and inorganic). This last fraction is in equilibrium with low and moderately labile fractions. The extractants used in the soil analysis mainly evaluate available P without identifying the corresponding fraction. In order to investigate the relationship between the available P and the different soil P fractions of the soils, available P-Bray 1 was related to each soil P fraction. The addition of labile and moderately labile inorganic reservoirs was positively related to P-Bray 1 values (**Figure 2**), which suggests that these fractions could be suppliers of available P. The Pi-$NaHCO_3$ is considered readily available in all soils (Guo and Yost 1998). The Pi-NaOH fractions can be considered as low or moderately labile according to the soil studied. The results agree with those of Tiessen et al. (1984) who reported that in Mollisols a great amount of labile P was derived from inorganic $NaHCO_3$ and NaOH extractable forms, whereas in more weathered soils the mineralization of organic P may be a major determinant of fertility. Highly weathered soils contain lower amounts of organic matter, an acid pH and more active Al and Fe sesquioxides, and so the solubilities of secondary Pi forms are much lower (Havlin et al. 2005) than in slightly weathered soils. In Mollisols, the high organic matter content and neutral pH increase P availability.

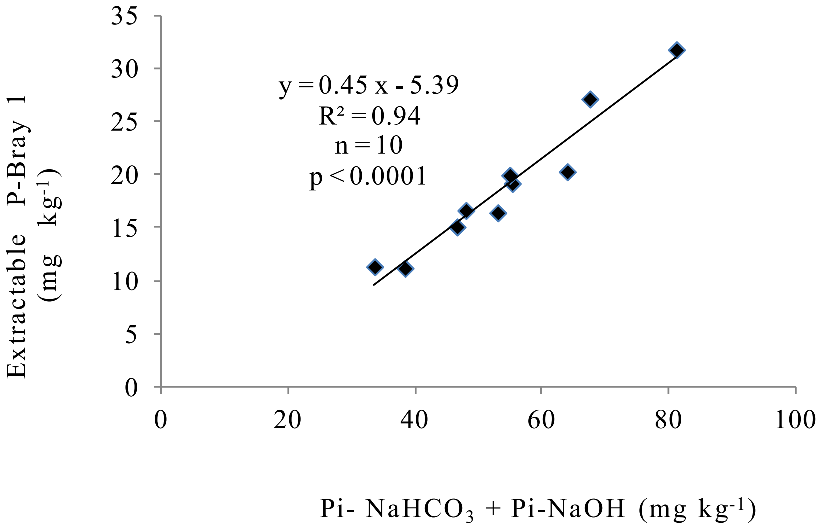

Figure 2. Relationship between extractable P-Bray 1 and the sum of inorganic P extractable with $NaHCO_3$ (Pi-$NaHCO_3$) plus inorganic P extractable with NaOH (Pi-NaOH).

4. Conclusions

The rate and the strategy of applied P affected soil P fractions and their relationship with P availability for wheat. When fertilization exceeded the requirements of the wheat crop (high P rate) there was an accumulation of P in the soil, evidenced by the increase of available P that was closely associated with the increment in labile (Pi-$NaHCO_3$) and moderately labile (Pi-NaOH) inorganic forms. When fertilization only compensates the P extracted by the crop (low rate), the soil P balance was near to zero and the P availability in the soil did not change. With regard to the strategy of fertilizer application when the rate was low, there was no difference between a single application or it being split over time. At the high P rate, the split application increased the availability of P, associated with a differential enrichment of soil inorganic P extractable with NaOH.

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