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# ADDED PHOSPHORUS AVAILABILITY AND RE-DISTRIBUTION IN BOTH NATURAL AND CULTIVATED VERTISOLS

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**ABSTRACT**

The fates of both native and added phosphorus were studied in a Leptic Hapludert, by Hedley´s fractionation method. The objectives of this study were to: a) assess the reaction of a natural grassland and a cultivated grassland in a Vertisol with added P by fertilization; b) to study the association between inorganic and organic P labile, moderately labile or non-labile forms with P availability for ryegrass growth; c) to test whether ryegrass growth may reflect the effect of a release of P from the soil with the incubation. Soil samples were taken from: a) Natural soil and, b) Cultivated soil that had been fertilized with 120 kg $ha^{-1}$ $yr^{-1}$ of TSP. In the laboratory, one group of samples was fertilized with P (0-200 mg P $kg^{-1}$) and then incubated 25 days at 28 °C. The other group was also fertilized with P but was not incubated. After P fractionation,, ryegrass was grown in a greenhouse 45 days and the dry yield of shoots was measured. Inorganic labile P forms increased with the level of added P in both soils, and these forms were closely associated with ryegrass growth. The inorganic resistant P forms also increased with the level of added P in both soils, but more P was removed from the incubated samples suggesting that part of the added P was redistributed during the incubation. For both soils, the organic labile P decreased with the level of addition in the incubated samples but increased in the non-incubated samples. The organic more resistant P forms did not differ between the natural and cultivated soil and did not show a consistent distribution with added P or incubation. This suggests that these P forms had little influence on P availability in short or medium periods of reaction between soil and P.

**Key words.** Phosphorus destination, phosphorus fractions, ryegrass.

## DISPONIBILIDAD Y REDISTRIBUCIÓN DE FÓSFORO AGREGADO EN VERTISOLES NATURAL Y CULTIVADO

**RESUMEN**

Se estudiaron los cambios en las formas de fósforo nativo y agregado en un Hapludert Léptico mediante el fraccionamiento de Hedley. Los objetivos fueron: a) evaluar la reacción de un Vertisol en campo natural y pastura cultivada, con el P nativo o ya presente en el suelo y con el añadido por fertilización, b) estudiar la asociación entre P orgánico e inorgánico lábil, moderadamente lábil y resistente respecto a su disponibilidad para el crecimiento del ryegrass, c) probar si el crecimiento del ryegrass refleja el efecto de la liberación de P del suelo con la incubación. Se tomaron muestras de: a) suelo natural y b) suelo cultivado, que había sido fertilizado con 120 kg P $ha^{-1}$ $año^{-1}$ como superfosfato triple. En laboratorio se agregó P (0-200 mg P $kg^{-1}$) a un grupo de muestras de cada suelo y se incubaron 25 días a 28 °C. Otro grupo recibió los mismos agregados de P pero sin incubación. Las muestras incubadas y no incubadas de ambos suelos se analizaron por el método de Hedley, luego se sembró ryegrass en macetas y se cultivaron en invernadero 45 días hasta cosecha. Hubo un aumento del P inorgánico lábil con el P agregado en ambos suelos, que correlacionó con el crecimiento del ryegrass. Las formas inorgánicas resistentes aumentaron con el P agregado, pero se extrajo más P de muestras incubadas que de no incubadas. Para los dos suelos, el P orgánico lábil disminuyó con el P agregado en muestras incubadas, aumentando en no incubadas. No hubo diferencias entre las formas orgánicas resistentes entre los dos suelos, ni se asociaron con el P agregado y la posterior incubación, sugiriendo que estas formas tienen poca influencia en la disponibilidad de P en períodos de corto y mediano plazo. La disminución del P inorgánico lábil estuvo asociada con el aumento del P inorgánico lábil, que a su vez incrementó la disponibilidad de P para el crecimiento de las plantas.

**Palabras clave.** Cambios de formas de fósforo, fracciones de fósforo, ryegrass.

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## INTRODUCTION

In agricultural ecosystems, phosphorus (P) constraints are much more critical than other nutrients because P is removed from the system with the harvested products, and only very limited quantities return to the system in crop residues and animal manure. Therefore, severe P-deficiencies are quite common when no supplementary P-sources are applied to soils. Long-term and continuous application of P fertilizers cause P to accumulate in soil due to low crop P use efficiency (< 25% in the year of application) (Ciampitti *et al.*, 2011; Balemi & Negisho, 2012). Increased amounts of soil P enhance P runoff to surface waters thereby increasing the risk of severe eutrophication, which can be avoided by modifying management practices. Effective management practices that ensure environmental quality can only be developed on the basis of a thorough knowledge of the P status in long-term fertilized soils.

Fritz *et al.* (2004) suggest that soil organic P mineralization plays an important role in soil P cycling for plant growth. Quantitative information on the release of available inorganic P ($P_i$) by mineralization from organic P (Po) forms is difficult to obtain because any mineralized $P_i$ is rapidly adsorbed on and then retained by the soil with time, especially in soils with high capacities to adsorb P on their solid phase (Barrow, 1985). However, physicochemical processes underlying the adsorption of P are very important, because the basal mineralization is essential to release available-$P_i$ to the soil solution. These processes are reversible, and $P_i$ in the soil solution can increase or decrease according to the environmental conditions and characteristics of the reaction between soil colloids and P (Barrow, 1983; Mendoza & Barrow; 1987 Picone *et al.*, 2003).

A sequential extraction procedure is used when conventional chemical analyses fail to characterize different P forms (Hedley *et al.*, 1982). Sequential fractionation schemes have been used to assess P transformations in grassland soils (Hedley *et al.*, 1982; Stewart & Tiessen, 1987). The fluctuation or turnover in Po is likely to be reduced in natural grassland soils compared with cultivated soils (Hedley *et al.*, 1982) Picone *et al.* (2007) showed that when the amount of P applied exceeded that removed by products, the excess P was converted to different fractions, but with no increases in Bray-P compared to the initial value. This indicates that the residual P present in the soil may not be always available for plant growth, at least in the short term. Hence, acquiring knowledge on the dynamics and P redistribution after fertilization and crop removal would enable to predict P availability for succeeding crops.

Vertisols are a group of heavy-textured soils which occur extensively in warm temperate regions, as the Province of Corrientes (Argentina). These soils are characterized by high clay contents (montmorillonite), organic matter, water-holding capacity and cation exchange capacity (Fernández López *et al.,* 2006). In Corrientes, these soils are usually devoted to native or cultivated pastures for livestock production. Vertisols from Corrientes and Entre Ríos are high in both total P and organic P (Fernández López *et al.,* 2006), and have shown to release native P under incubation, presumably coming from organic P (Mendoza & Barrow, 1987). The effect of the soil reaction with native or added P was studied on the labile, moderately labile and resistant, inorganic and organic P forms in a Vertisol from the Province of Corrientes. The association between increases in labile P forms coming from organic Po and P availability for ryegrass was also studied. The objectives of this study were to: a) assess the reaction of a natural grassland and a cultivated grassland Vertisol with both native P and added P by fertilization, and the fate of P under these two soil conditions; b) study the association between inorganic and organic P labile, moderately labile or non-labile forms with P availability for ryegrass growth; c) test whether ryegrass growth is associated with the release of P from the incubated soil.

## MATERIALS AND METHODS

### Soils

The experimental site was located in the Province of Corrientes at 29°13'37" S and 58°01'31" W. The selected soil was a heavy Vertisol (Leptic Hapludert) that showed severe limitations to cultivation because of its susceptibility to water erosion together with a moderately to limited water infiltration. Sustainable cultivation of these soils need specific management practices (Escobar *et al.*, 1996).

Sampled sites represented: a) a natural grassland (natural soil) composed of *Andropogon lateralis, Paspalum notatum,* and *Baccharis coridifolia*, and b) a sown *Setaria sphacelata* grassland (cultivated soil), which had been fertilized with a yearly application of 120 kg triple super phosphate (TSP) $ha^{-1}$. Three aboveground-biomass cuts were made after TSP applications. In the cultivated soil, the annual amount of dry biomass removed was 11,000 kg $ha^{-1}$; since plant tissue P concentration averaged 0.172%, then 18.92 kg P $ha^{-1}$ $year^{-1}$ were removed from the cultivated soil. Since the total amount of P added was 24 kg $ha^{-1}$, then 5.08 kg P $ha^{-1}$ remained present in different P forms or were lost from the soil.

### Laboratory incubation experiment

Three sets of 5 top-soil samples (0-15-cm depth) were collected from each field site representing the natural and the cultivated soil condition. Samples in each set were bulked into one 30-kg sample; the resulting three large samples from each field were used in both laboratory and greenhouse experiments. Soil chemical analysesprior to the experiment are shown in Table 1. For the laboratory experiment, one large soil sample from each soil condition (natural and cultivated) was fertilized with $KH_2PO_4$ solutions applied at rates ranging from 0-200 µg P $g^{-1}$ soil. Two replicated moistened 600g subsamples were incubated in plastic bags at 28 °C during 25 days. The incubation technique was used to stimulate the reaction between soil and P and to simulate the reaction that would occur in the field at relatively longer periods (Mendoza & Barrow, 1987). Another group of samples was similarly prepared for each soil condition (natural and cultivated) and fertilized with the same levels of added Pexcept that it was not incubated. These non-incubated samples were fertilized, mixed with P and homogenized, and then analyzed for P-fractionation. Approximately 100 g of soil from each plastic bag was sampled and used for analyzing the P fraction forms of both incubated and non-incubated soil. The P-fractions were separated with the Hedley *et al.* (1982) method as modified by Sattell & Morris (1992). Reagents used and the inorganic and organic P fraction forms extracted by this method are indicated in Table 2. Soil samples were stored at 4 °C after sampling until processed (Rice *et al.*, 1996). At this temperature, the reaction between soil and P is too slow to be detected. The concentration of P in neutralized extracts from each of those P fraction was measured with the molybdate-ascorbic acid blue method (Kuo, 1996).

### Greenhouse incubation experiment

Plant pots were filled with 500 g of the experimental soils (natural or cultivated) either incubated or not incubated and sown with 30 pre-germinated ryegrass (*Lolium perenne*) plants per pot. Plants were grown at an air temperature of 27 ± 5 °C during 45 days, when plants were cut 5mm above the soil surface, oven-dried and weighed. All pots were watered with a solution containing 12.5 mg N as $NH_4NO_3$, 4.48 mg N and 12.5 mg K as $KNO_3$, to prevent eventual N deficiencies in the plants. In addition, all pots were given 7.5 mg N as $NH_4NO_3$ every 15 days (twice during the experiment).

The flexible equation used by Barrow & Mendoza (1990) was used to describe the response curve of ryegrass shoot dry biomass to added P in all experimental soils:

$$Y = A - [B/ (1 + M C X^{N})^{1/M}], \quad (1)$$

where Y is aboveground dry matter yield per pot; X is the amount of added P, A is the maximum yield when P does not limit growth, A-B is the yield without added P, and M, C and N are coefficients. For a given soil and non-limiting P concentrations, shoot growth in either incubated or non-incubated soils approached the same maximum dry matter yield (A). In soils with moderate to high status of either native P or P from previous additions, the value of B may be different for incubated and non-incubated soils; consequently the response curve for each soil condition may also differ as to the values of M, C and/or N. Specific meaning of these coefficients can be found in Mendoza and Barrow (1990). Two fitted curves were considered to be the same when no statistical differences were found (p>0.05) between the residual sum of squares of the observed values. The simplex method of Nelder & Mead (1965) was used to single-out the coefficient values that gave the smallest residual sum of squares.

### Multivariate analysis

Principal component analysis (PCA) was used to separate P fractions of the non-incubated from the incubated samples of each soil condition in an attempt to differentiate the categories of the P extracted from the soil. The analysis was performed with the statistical software Infostat (Di Rienzo *et al.*, 2012). Association between variables was estimated with Pearson´s correlation coefficient.

## RESULTS AND DISCUSSION

### Soils

The soil used in this study showed an acid reaction in the top layer, low available P (Pa) for pasture growth but high levels of total P extracted (Pte) and was previously used by Fernández López *et al.* (2006). With the exception of soil organic carbon (CO), which was higher in the natural soil than in the cultivated soil, there were no statistical significant differences (p>0.05) in any of the chemical properties studied between both soils (Table 1). These observations confirmed previous results that found that the extracted P from different fractions did not show significant differences, except for the fraction of P extracted by ultrasonication (Fernández López *et al.*, 2006). This allowed us to confirm that the differences in P forms between the natural and the cultivated soils can be attributed to differences in the reaction between soils and P (native or added) during the period of incubation.

**Laboratory incubation experiment**

The plots in Figure 1 show the relationships between the extracted P forms (Table 2) from the incubated versus the non-incubated soils at each level of P addition for the natural and the cultivated soil condition. For each level of P addition, any point located above the 1:1 relationship line, indicates that more P was extracted from the incubated soil with respect to the non-incubated soil whereas any point below the 1:1 line indicates that more P was extracted from the non-incubated soil.

The inorganic labile P ($NaHCO_3$-Pi) increased with the level of added P in both the natural and cultivated soils (Fig. 1a). The 1:1 line separates the fitted line of the natural soil located above the 1:1 line from the fitted line of the cultivated soil located below the 1:1 line. This indicates that for the natural soil, more P was extracted with incubation than from the non-incubated samples for each level of added P. However, for the cultivated soil, more P was extracted from the non-incubated than from the incubated samples

Table 1. Initial physical and chemical properties of the natural and cultivated soil used in the experiment (from Fernández López *et al.,* 2006).
Tabla 1. Propiedades físicas y químicas al comienzo del experimento para el suelo en su condición natural y cultivado (datos Fernández López *et al.,* 2006).

| Sites | Sand | Silt | Clay | pH | OC | Pa | Pte | Ca | Mg | K | Na | $CO_3^{2-}$ | Fe | Al |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | | | | $g\ kg^{-1}$ | $mg\ kg^{-1}$ | | $cmol\ kg^{-1}$ | | | | $g\ kg^{-1}$ | | |
| Natural | 55.7a | 21.0a | 23.3a | 5.3a | 21b | 6.6a | 142.3a | 14.84a | 1.04a | 0.20a | 0.34a | 0.003a | 0.53a | nd |
| Cultivated | 57.6a | 19.8a | 22.6a | 5.2a | 18a | 8.5a | 147.3a | 14.06a | 1.93a | 0.14a | 0.30a | 0.004a | 0.65a | nd |

Column means followed by the same letter are not significantly different ($\alpha = 0.05$) by Tukey's multiple range test. nd: not detected.
OC (organic carbon, Walkley-Black), Pa (available phosphorus, P Brayl), Pte (total extracted P, Acid-Diluted Hydroxide), Ca, Mg, K, Na (Exchanges cations), $CO_3^{2-}$ (water-soluble carbonate), Fe and Al active (Acid Ammonium Oxalate).

Table 2. Reagents used and P fractions extracted by the Hedley's fractionation method.
Tabla 2. Reactivos utilizados y fracciones de P extraídas mediante el método de fraccionamiento de Hedley.

| No. | Reagent | Fraction designation | P forms | Phosphorus compound extracted |
|---|---|---|---|---|
| 1 | 30 mL 0.5 *M* $NaHCO_3$ pH 8.5 | $NaHCO_3$-Pi<br>$NaHCO_3$-Po | Labile Pi and Po | Labile Pi forms available to plants. Labile Po forms and Po held at the internal surfaces of soil aggregates. |
| 2 | 30 mL 0.1 *M* NaOH | NaOH-Pi<br>NaOH-Po | Moderately labile Pi and Po | Pi partially dissolves Fe-Al phosphates, and Pi is desorbed from sesquioxides.<br>Po strongly held by chemisorption to Fe-Al |
| 3 | 20 mL 0.1 *M* NaOH, sonicate, add 10 ml 0.1 *M* NaOH | Sonic-Pi<br>Sonic-Po | Occluded Pi and Po | Pi and Po held at internal soil aggregates. |
| 4 | 30 mL 1.0 *M* HCl | HCl-Pi | Non-labile Pi | Acid soluble Pi dissolved from calcium phosphates. |
| 5 | 5 mL 12.5*M* HCl, heat, add 10 mL 12.5 *M* HCl followed by 5 mL $H_2O$ after 1 h | Res-Pi | Residual Pi | Occluded Pi. |
| 6 | 10 mL 12.5 *M* HCl + 5 mL 30% $H_2O_2$ | Res-Po | Residual Po | The most stable organic phosphates |

Note: Pi = inorganic phosphorus, Po = organic phosphorus.

for each level of P addition. Hence, two straight and significant different lines were fitted to deal separately with extracted Pi labile form from either the natural or the cultivated soil.

The NaOH-Pi (inorganic moderate labile P) extracted by NaOH–which is supposed to release P from Fe-Al phosphates and desorbed P from the surfaces of sesquioxides–increased with the level of added P in both natural and cultivated soils (Figure 1c). In this case, less P was extracted with the incubation from both soil conditions compared to the non-incubated soil (Figure 1c). The points and the two straight fitted lines were located below the 1:1 line. This result differed with the inorganic labile P ($NaHCO_3$-Pi) extracted from the natural soil (Figure 1a), where more P was extracted with the incubation. These two contrasting results for the natural soil with respect to the incubation suggest that the increase of the inorganic labile P-forms extracted are associated with the decrease of inorganic moderately-labile P at a same level of addition. Hedley *et al.* (1982) suggested that the re-distribution of P between different pools is due to both chemical reactions taking place during incubation and the conversion of soil P to microbial P to be later released back to the soil with microbe death.

The more resistant Pi forms (Sonic-Pi, Res-Pi) also increased with the level of added P in both soils, and together with Pi-HCl, the plots show that the points were over the 1:1 line indicating that more P was extracted from the incubated samples (Figure 1e, 1g, 1h). These fractions did not show a consistent relationship as observed for the labile ($NaHCO_3$-Pi) and moderately labile Pi (NaOH-Pi) forms with respect to both soil conditions. In one case (Pi-sonic, Figure 1e), more P was extracted from the cultivated soil, in another other case (Res-Pi, Fig. 1h), more P was extracted

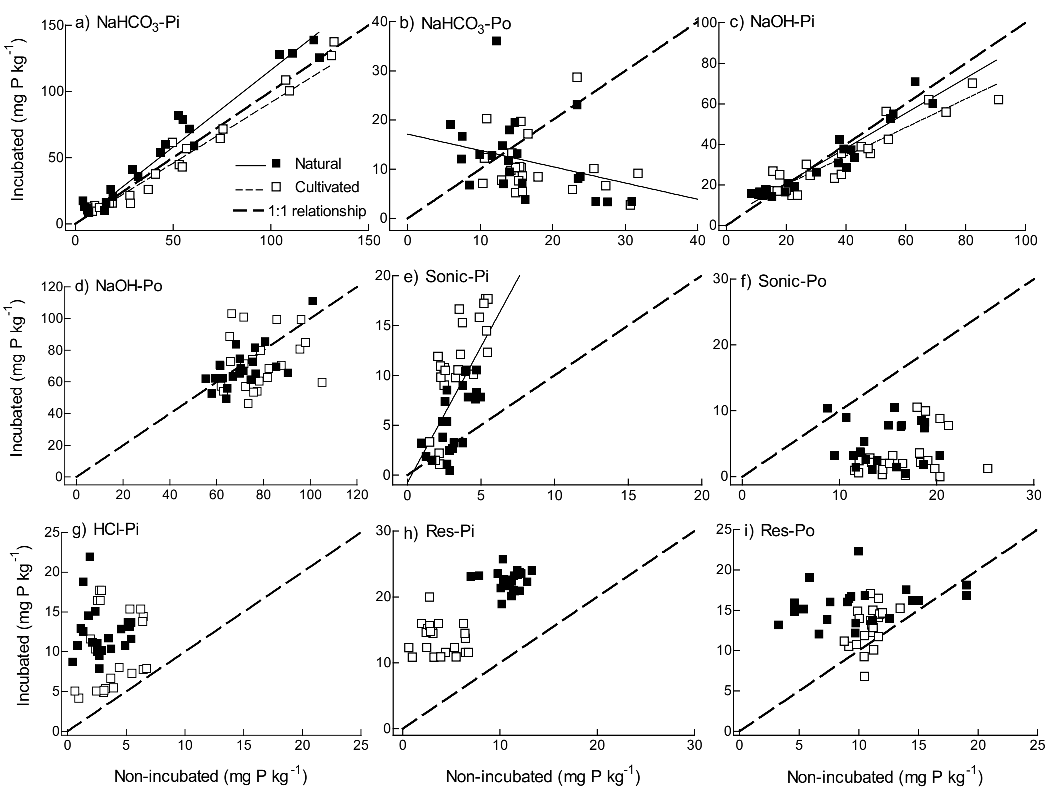


Figure 1. Relation between P fractions extracted from non-incubated and incubated samples from natural (filled symbols) and cultivated soils (empty symbols) that had been fertilized with a range of added P (0-200 mg P $kg^{-1}$). The P-fractions are: a) $NaHCO_3$-Pi, b) $NaHCO_3$-Po, c) NaOH-Pi, d) NaOH-Po, e) Sonic-Pi, f) Sonic-Po, g) HCl-Pi, h) Res-Pi, i) Res-Po.

Figura 1. Relación entre las fracciones de P extraídas de las muestras no incubadas e incubadas para el suelo natural (símbolos llenos) y cultivado (símbolos vacíos) que habían sido fertilizadas con distintos niveles de P (0-200 mgP $kg^{-1}$). Las fracciones extraídas son: a) $NaHCO_3$-Pi, b) $NaHCO_3$-Po, c) NaOH-Pi, d) NaOH-Po, e) Sonic-Pi, f) Sonic-Po, g) HCl-Pi, h) Res-Pi, i) Res-Po.

from the natural soil, and for HCl-Pi (Figure 1g), the points of the two soils were mixed together forming a cloud. These three P fractions are associated with either non-labile or more retained P-forms, which are less likely to be released from the soil in a short or intermediate period of reaction to the soil solution. The results suggest that these fractions could be inferred to be directly associated with the added P. Zhang *et al.* (2004) concluded that the moderately labile P (NaOH-Pi) acted like a large reserve of fertilizer-P. The Res-Pi fraction would be a secondary reserve of fertilizer P, as found in the highland plains soils of Ethiopia (Duffera & Robarge, 1996) and in corn soils of Québec (Zhang & Mackenzie, 1997).

The pattern of the organic P (Po) extracted from the soils was different from the pattern of the extracted Pi. The fitted line of the organic labile P-forms ($NaHCO_3$-Po), for both the natural and cultivated soil decreased significantly with the level of P addition when the soil was incubated, but the organic labile P increased with the level of P addition for the non-incubated soils (Figure 1b). This inverse relationship between the non-incubated and the incubated samples suggests that transference of these labile organic P forms to inorganic P forms may occur during the incubation, and this is consistent with the increase of inorganic labile P with the level of addition of P observed in Figure 1a. Previous studies have addressed the biological transformations associated with the release of P from residues of organic matter after cropping, and the subsequent accumulation and turnover of organic P during decomposition of organic matter (Fritz *et al.*, 2004). Inorganic P-fractions from a natural soil ($NaHCO_3$-Pi, NaOH-Pi and HCl-Pi) have been found stable with time, presumably because these fractions were supplied by P released from organic P fractions through mineralization (Zhang *et al.*, 2004). Mendoza & Barrow (1987) reported the release of P already present in two Argentinean Vertisols after long periods of reaction between soil and P. The authors suggested that part of this release was the result of biological activity causing the decomposition of organic P which was transformed to labile inorganic P.

The moderately labile and non-labile organic P forms did not show a clear causal relationship with the level of added P in the natural and the cultivated soils (Figure 1d, f, and i). The amounts of extracted P from these organic forms did not show any definite trend in response to the levels of added P. The particular effect of soil incubation on the extraction of organic P-forms vis-$\alpha$-vis inorganic P-forms in both natural and cultivated soils (Figure 1a, 1i) could be attributed to the tendency of the former forms to be converted into the latter forms (Xu *et al.*, 2013).

**Greenhouse incubation experiment**

The fitted response curves of the ryegrass shoot dry matter to added P with respect to the incubated and the non-incubated samples were different between the natural and the cultivated soil (Figure 2a, b). In the natural soil, the shoot yield was highest in incubated soils and largely independent of the amount of added P, as shown by the flat response curve (Figure 2a). The difference between the incubated and non-incubated soil was more marked at low levels of added P, and then both fitting curves approached a common value of the shoot yield at high levels of added P (Table 3). In the cultivated soil, the response curve of shoot dry matter to added P in non-incubated soils increased at low levels of added P, to smoothly flatten out at the highest P additions (Figure 2b). The incubation of the samples in the cultivated soil decreased shoot dry matter compared to non-incubated samples, particularly at medium additions of P. In fact, at these medium levels of added P the response curves for incubated and non-incubated soils were quite different with lower dry matter values for incubated soils. Subsequently, both response curves tended to reach the same maximum yield at the largest P additions (Figure 2b).

Equation (1) described adequately both differences between the natural and the cultivated soil and the differences within a soil between the non-incubated and incubated treatment (Table 3). These observations could be revealing two different processes, the different displacements of the reaction equilibrium between the soil and P during the period of incubation between the two soil conditions (natural and cultivated soil); and the effect of P already present in the soil which reacted differently with the soil during the period of incubation. These two processes varied the concentration of P in the soil solution from which plants take up P to grow. In the natural soil (Figure 2a), soil incubation stimulated the release of the existing P and thus increased the level of P in the soil solution over and above the level of P applied to the soil. However, in the cultivated soil, the incubation promoted the soil retention of added P and the net balance resulted in a decrease of P in the soil solution, compared with the non-incubated soil which received additional P just before sowing. In this case, we interpret that part of the inorganic labile P or moderately labile P were transferred to non-labile forms during incubation. When P is not limiting for plant growth, dry matter yield tended to a common value with

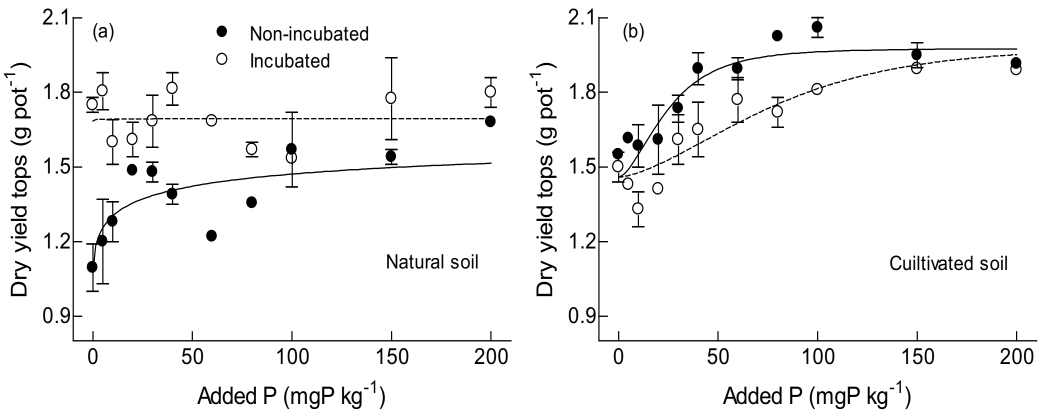


Figure 2. Relationship between aboveground dry ryegrass biomass and added P (0-200 mg P kg$^{-1}$) as fitted by equation (1) to data from incubated and non-incubated natural (a) and cultivated (b) soil conditions. Bars show the mean standard errors.

Figura 2. Relación entre la materia seca de aérea de ryegrass y el P agregado (0-200 mg P kg$^{-1}$) ajustados por la ecuación (1) en los tratamientos no incubados e incubados para el suelo natural (a) y cultivado (b). Las barras indican el error estándar de la media.

increasing added P in both incubated and non-incubated soils for the two soil conditions (Figure 2b). This explanation is consistent with the reversibility of the phosphate sorption process proposed by Barrow (1983).

**Relationship between laboratory and greenhouse experiments**

The responses of shoot dry matter yield observed in the natural and cultivated soil (Figure 2a, 2b) are consistent with the inorganic labile P extracted by $NaHCO_3$ from both the non-incubated and incubated samples observed in the laboratory experiment (Figure 1a). For the natural soil, in both experiments (laboratory and greenhouse), the results show that more P was displaced from the incubated soil samples than for the non-incubated samples. The P extraction from the soil by the chemical solution of $NaHCO_3$ and the shoot yield of ryegrass reflected a similar result. That is, more P extracted from the soil is associated with more shoot dry ryegrass yield. Plant roots were more sensitive to the nature of P-sources or P-categories than soil tests to detect shifts in available-P, as observed earlier (Mendoza *et al.*, 2009). Theoretically, any plant can be more sensitive in detecting the differences in P availability and reflect this in plant growth comparing with any chemical solution, especially when plants grow shorter than longer periods of time as in the present study. Mendoza *et al.* (2009) suggested that part of this release was the result of biological activity causing the decomposition of organic P that was transformed to labile inorganic P.

In this case, we cannot exclude a release of P from organic P forms during the incubation which increased the P availability for plant growth in the soil solution. Consistent results between both experiments (laboratory and greenhouse) were also observed for the cultivated soil. In

Table 3. Values for the coefficients of the equation (equation 1)* and determination coefficient describing the relationship between aboveground ryegrass dry biomass and added P in either incubated or non-incubated natural and cultivated soils.

Tabla 3. Valores de los coeficientes (Ecuación 1) y coeficiente de determinación que describe la relación entre la materia seca aérea del ryegrass y el P agregado en muestras incubadas y no incubadas de los suelos natural y cultivado.

| Soil | Trat. | A | B | M | C | N | $R^2$ |
|---|---|---|---|---|---|---|---|
| Natural | Non - Inc. | 1.695 | 0.6207 | 1.2709 | 0.1886 | 0.5228 | 0.679 |
| | Incubated | | 0.0089 | 1.0299 | 0.7752 | 0.6143 | |
| Cultivated | Non - Inc. | 1.976 | 0.5166 | 0.3875 | 0.0051 | 1.6003 | 0.770 |
| | Incubated | | | 0.1138 | 0.0010 | 1.5471 | |

*Equation (1) $Y = A - [B/(1 + M\,C\,X^{N})^{1/M}]$.

the laboratory, less P was extracted by $NaHCO_3$ with the incubation which was reflected by a lower ryegrass yield. This indicates that the reaction between soil and P is complex, dynamic and bi-directional with time, and that the available P for plant growth may increase, decrease or remain unchanged with time.

The inorganic moderately labile P forms (NaOH-Pi) extracted from the soil were consistent with the ryegrass yield response observed for the cultivated soil condition but did not reflect the same consistent result for the natural soil condition. The NaOH-Pi solution extracted more P from the non-incubated samples than from the incubated ones (Figure 1c), and this is consistently reflected by the ryegrass growth, which grew better in non-incubated than in incubated soils (Figure 2b). However, this was not the case for the natural soil, where ryegrass grew better in the incubated soils (Figure 2a).

The ryegrass shoot dry matter did not show consistency with the non labile inorganic P (Sonic-P; HCl-Pi; Res-Pi) and with the moderately and non-labile organic P forms extracted from the soils, nor with cultivation or with incubation, suggesting that these non-labile P forms had little influence on the availability of P for plants growth in short and/or medium periods of time.

The P fractionation techniqueis a suitable approach to detect changes in P caused by chemical and/or biological reactions. Mendoza & Barrow (1987) reported the release of P already present in some Argentinean soils (Vertisols included) after long periods of reaction between soil and P. Part of this release was the result of biological activity causing the decomposition of Po from organic matter. In addition, it is known that a solution of $NaHCO_3$ removes both Pi and Po from the soil and that this is important because it represents an active soil Po fraction for plant growth (Rubio *et al.*, 1998; Guo *et al.*, 2000).

### Multivariate analysis

The PCA analysis of the different P forms in the experimental non-incubated and incubated soils samples accounted for 77% of the total variance, 49% represented by axis 1 and 28 % by axis 2. This analysis clearly separated the incubated from the non-incubated samples from both the natural and cultivated soil conditions (Figure 3). The positive part of axis 1 was associated with the incubated samples and the negative part with the non-incubated

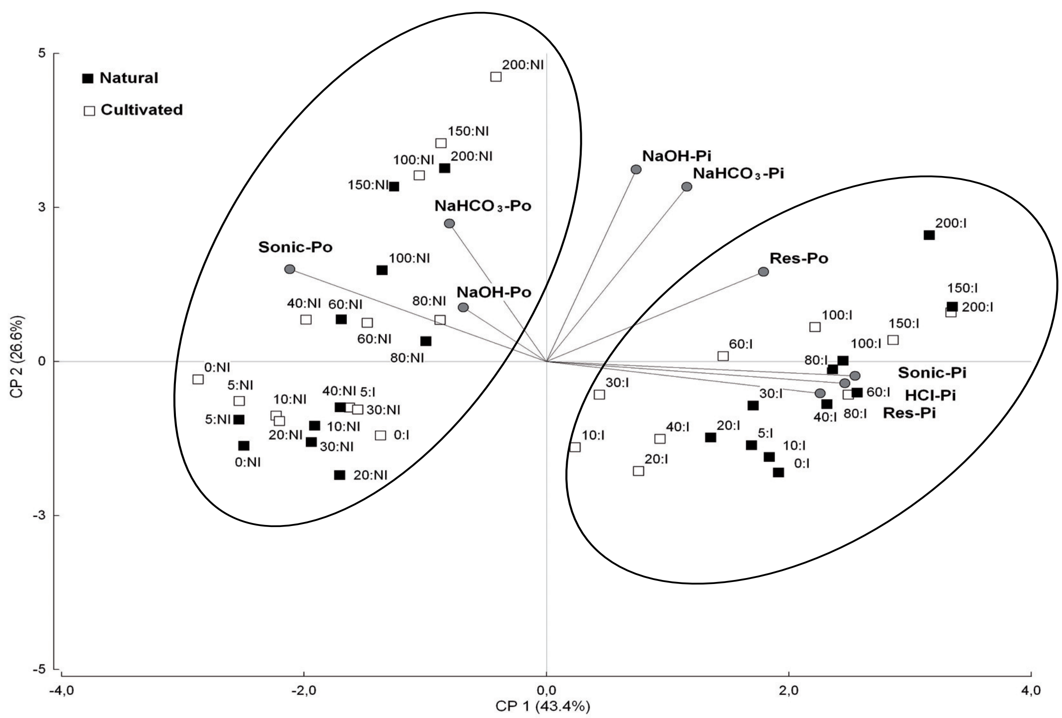


Figure 3. Biplot for P fractions in natural (filled symbols) and cultivated soils (empty symbols) which were either incubated (I) or non-incubated (NI) with a range of added P (0-200 mgP $kg^{-1}$). Incubated (I) and non-incubated (NI) samples are enclosed in separate ellipses.

Figura 3. Biplots para fracciones de fósforo en el suelo natural (símbolo lleno) y cultivado (símbolo vacío) que fueron incubados (I) o no-incubados (NI) con distintas dosis de fósforo agregado (0-200 mgP $kg^{-1}$). Las muestras incubadas (I) y no-incubadas (NI) están agrupadas en elipses separadas.

samples. The axis 2 was associated with the levels of added P, which increased from the negative to the positive scale of axis 2 (Figure 3). The PCA separated both, the inorganic from the organic P forms and the labile from the less labile P forms of both soil conditions. This suggests that the P extracted from the soil samples come from different pools of soil P.

With the exception of Res-Po, axis 1 was positively correlated with the non-labile inorganic P forms: HCl-Pi ($r=0.94$, $\rho<0.001$), Res-Pi ($r=0.97$, $\rho<0.001$) and Sonic-Pi ($r=0.98$, $\rho<0.001$); but negatively correlated with non-labile organic P forms: Sonic-Po ($r=-0.88$, $\rho<0.001$). As showed in Figure 1, this suggests that a fraction of the Po form was transferred to Pi-forms during the incubation period; similar results were found by Zhang & Mackenzie (1997). This explanation is consistent with results from other studies on the relation between Po and added Pi (McGill & Cole, 1981; Guo *et al.*, 2000). In contrast, axis 2 was associated positively with both the inorganic and organic labile P forms $NaHCO_3$-Pi ($r=0.86$, $\rho<0.001$) and $NaHCO_3$-Po ($r=0.70$, $\rho<0.001$), and with the moderately inorganic P form extracted by NaOH-Pi ($r=0.92$, $\rho<0.001$). These forms are expected to be more correlated with the P availability for plant growth.

## CONCLUSIONS

Phosphorus already present in the soil and/or added P reacts differently with the natural and the cultivated Vertisol. In the natural condition, available P increases under incubation as a consequence of the release of native P or P already present from the soil. However, in the cultivated condition, available P decreased with the incubation as a consequence of the retention of P by the soil, which was transferred to both inorganic and/or organic labile P forms at first, and later, the added P became stabilized under more resistant forms with time. This is probably a consequence of the reaction between soil and the different categories or soil P status. The reaction is complex, dynamic and bi-directional, and the available P for plant growth may increase, decrease or remain unchanged with time.

The labile organic P-form were mineralized under incubation conditions, and organic non-labile P forms did not change with added P in time. This suggests that recalcitrant organic P forms are stabilized in soil, and they may have little influence on plant growth in the short or medium term.

The residual P present in the soil may not be always available for plant growth. Knowledge on P redistribution to different P fractions after fertilization and crop removal could be a useful tool to predict the P availability for succeeding crops.


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