Rhizosphere phosphorus depletion by three crops differing in their phosphorus critical levels

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Abstract

It has been reported for many soils that maize (*Zea mays* L.) has a higher soil-P critical level than soybean (*Glycine max* L.) and sunflower (*Helianthus annuus* L). The objective of this work was to compare the rhizosphere P depletion in these three species in order to investigate if they differ in their capacity to acquire soil P. Sequential P fractionation and pH were determined in rhizosphere and nonrhizosphere soil samples from field and greenhouse experiments. Neither sunflower (the species with highest rhizosphere acidification) nor soybean or maize showed a significant relationship between P depletion and rhizosphere pH. The labile P fraction and the NaOH-P_i fraction had lower values in the rhizosphere than in the bulk soil in 38% and 77% of the studied cases, respectively. Sunflower and especially maize presented a more intense P_i depletion than soybean. The comparison between sunflower and maize revealed that neither of them took a clear advantage over the other in terms of P depletion. Rhizosphere P_i depletion was associated with the amount of P acquired by the plants. We conclude that the accessibility to different P pools does not explain the differences in soil-P critical levels among the three species.

Key words: maize / nutritient acquisition / plant roots / soybean / sunflower

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1 Introduction

The application of phosphorus (P) fertilizers is the classical method to overcome soil P deficiency in agricultural fields. Fertilizer recommendations are usually based on the relationship between crop yield and soil P availability determined with specific soil tests (*Dodd* and *Mallarino,* 2005; *Rubio* et al., 2008). The value of soil test P between deficiency and sufficiency is usually termed the P critical level. Crop-yield responses to P fertilization are expected if soil-analysis results in soil test P values below the critical level.

Crops exhibit a great variability in soil-P critical levels (*Dodd* and *Mallarino*, 2005; *Colomb* et al., 2007) and in the ability to cope with P deficiencies through specific mechanisms such as increased associations with mycorrhizae, root morphological traits, or root-induced rhizosphere alterations (*Hinsinger*, 2001; *Vance* et al., 2003; *Lambers* et al., 2006). Whether this ability can explain the interspecific differences in P critical levels is an intriguing question that has not been fully addressed yet.

A greater capacity to access different soil P pools might affect the P critical level. If a plant can acquire the nutrient from a greater number of pools or more P from each of the pools than another plant, the former will be able to take up a higher amount of P, even at a fixed value of soil test P. Therefore, the "meaning" of the soil test P in terms of real P availability will differ between the two plants and this might explain why they eventually differ in their P critical levels.

Soil P is composed of inorganic (P_i) and organic (P_o) forms that differ in their availability for plants. *Hedley* et al. (1982) and *Tiessen* and *Moir* (1993) proposed a method based on the use of a set of chemical solutions with increasing extracting power that remove P from different soil pools. This method has been used to characterize the biogeochemical cycle of P, the effects of land use and management, and to compare the sources of P used by different plant species (*Zoysa* et al., 1999; *Nuruzzaman* et al., 2006; *Li* et al., 2008; *Vu* et al., 2008; *Negassa* and *Leinweber*, 2009).

Soybean, sunflower, and maize are among the most important grain crops in Argentina and in other countries. In Argentina, agriculture is concentrated in the Pampean region, which is mainly covered by highly fertile Mollisols. It has been consistently reported for these Mollisols that soybean and sunflower have lower soil P critical levels (9–12 mg kg^{-1} P-Bray1) than maize (15–18 mg kg^{-1} P-Bray1; *Echeverría* and *García*, 2005). As a consequence, soybean and sunflower are less responsive to P fertilization. These results are consistent with results obtained in the United States. For example, in North Dakota the rank of soil-P critical levels in these crops is soybean < sunflower < maize (*Dahnke* et al., 1992; *Franzen,* 1999; NDSU Extension service: http://www.ag.ndsu.edu/procrop/crn/crnfet05.htm). The same ranking has been found in South Dakota (*Gerwing* and *Gelderman*, 2005). In Iowa, where sunflower is not an agriculturally significant crop, *Dodd* and *Mallarino* (2005) found that the

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soil-P critical level was lower in soybean than in maize. It is largely known that critical P levels may vary among soil types (*Bowman* et al., 1978), which can explain why the ranking of species observed in Mollisols may not be found in other soils (*Colomb* et al., 2007).

The fact that maize has a higher soil-P critical level than soybean and sunflower may indicate that maize is less capable of depleting soil P pools. If this is true, the accessibility to different pools of soil P will be a main factor to determine the soil-P critical level of each crop. The objective of this work was to compare the rhizosphere P depletion in soybean, sunflower, and maize. We followed an integrative approach by conducting both field and greenhouse experiments, allowing us to compare the performance of soybean, sunflower, and maize at the rhizosphere levels under different conditions.

2 Material and methods

Modern cultivars of soybean (*Glycine max* L. cv. Don Mario 4800 RR), sunflower (*Helianthus annus* L. cv. Paraíso 20), and maize (*Zea mays* L. cv. DK628 RR) were evaluated under field and controlled conditions. In all cases, soils were Mollisols involving a wide range of plant-available P (Tab. 1). In these Mollisols, clay content is the key parameter to assess soil P retention (*Gutierrez Boem* et al., 2008; *Rubio* et al., 2008).

2.1 Field experiment

The field was located in the Pampean region (35°02′ S, 60°16′ W), in Alberti, Buenos Aires, Argentina. The soil was a silt loam Typic Argiudoll (Soil I in Tab. 1). The three crops were arranged in a randomized complete block design with five replicates. Each experimental unit had an area of 21 m^2 (6 m × 3.5 m). Row spacing was 70 cm. The crops were sown with 36, 6, and 7 plants m^{-2}, for soybean, sunflower, and maize, respectively. Following the practices performed by local farmers, plots with maize and sunflower received a broadcast presowing application of 70 kg N ha^{-1} as urea. Soybean plots received no N fertilizer but seeds were inoculated with *Bradyrhizobium japonicum*. Total rainfall from sowing to sampling date (80 days after sowing) reached 382 mm.

2.2 Pot experiment A

Seeds of the three species were sterilized by shaking in a 10% (v/v) NaOCl solution for 15 min and rinsing with sterilized water. Then, seeds were germinated in paper towels moistened with a $CaCl_2$ 0.08 M solution in darkness at 25°C. After 4 d, germinated seedlings were transplanted into individual cylindrical pots (30 cm high; 11 cm diameter), containing 3.7 kg of soil. Treatments were arranged in a factorial experiment with two factors (soil and species) and five replicates. The soil factor was composed of three soils (Soils II, III, and IV; Tab. 1) collected from agricultural fields located in the Pampean region. Pots without plants were included in the experiment and considered as bulk soil. The species factor was composed of four levels: soybean, sunflower, maize, and bulk soil. Soybean seeds were inoculated with 3 mL kg^{-1} with a commercial formulation of *Bradyrhizobium japonicum* strains (10^9 bacteria mL^{-1}). Maize and sunflower pots were fertilized with 1 g of N as $Ca(NO_3)_2 \cdot 4\ H_2O$. Each pot was watered daily to 80% of soil water-holding capacity. Pots were randomly distributed on a 20 m^2 area protected by a rain shelter (90% transparence polycarbonate sheets). After 90 d growth, plants were harvested to extract rhizosphere soil samples.

2.3 Pot experiment B

Two different soils (Soils V and VI) differing in P-Bray were selected (Tab. 1). The experimental design of pot experiment B was similar to the one described above for pot experiment A. The difference was that in this case, pots were filled with layers of soil and river sand. The purpose of these layers was to concentrate the roots in the fertile soil layer to exacerbate the rhizosphere effects. The proportion of these layers from top to bottom of the pot was: one part of sand (0.31 kg), four parts of soil (1.25 kg), and seven parts of sand (2.17 kg). Water evaporation was reduced to a minimum through the

Table 1: Main characteristics of the soils (0–20 cm) used in the field (Soil I), and pot experiment A (Soils II, III, and IV) and pot experiment B (Soils V and VI). In experiment A, pots were filled with one layer of homogeneous soil. In experiment B, pots were filled with layers of soil and river sand. Concentration of extractable P in the layer of soil of Soils V and VI were 45 and 72 mg kg^{-1}, respectively. River sand had negligible content of P. Values reported for experiment B result from the P concentration of the whole pot.

Soil	Experiment	Extractable P§	pH	OM	Clay	Silt	Sand
		/ mg kg^{-1}		/ %	/ %	/ %	/ %
I	Field	10	6.2	3.65	16.2	56.2	26.6
II	Pot A	12	6.5	3.65	6.2	40.5	53.3
III	Pot A	14	6.4	3.30	6.2	50.8	42.9
IV	Pot A	17	6.2	4.40	20.0	59.6	20.4
V	Pot B	15	6.2	2.55	22.5	71.3	6.2
VI	Pot B	24	6.3	3.80	3.8	18.7	77.4

§ Extractable P determined with the Bray 1 method, organic matter (OM) with Walkley-Black, and texture with the pipette method. pH was measured at a 1:20 soil-to-water ratio.

sand layer. Sunflower and maize pots were added 1 g of N (as urea) per pot. Each pot received 70 cm^3 of a nutrient solution with the following composition: K_2SO_4 (2.5 mM), $CaSO_4$ (1.2 mM), $MgSO_4$ (0.5 mM), Fe EDTA (0.005 mM), H_3BO_3 (0.5 mM), $MnSO_4$ (1.5 mM), Zn SO_4 (1.5 mM), Cu SO_4 (0.5 mM), and MoO_3 (0.143 mM). Each pot was watered daily to 80% of soil water-holding capacity. After 90 d growth, plants were harvested and rhizosphere soil samples were taken.

2.4 Sampling and measurement of rhizosphere soil and P concentration

Samples of the rhizosphere soil from soybean, maize, and sunflower roots were obtained following the method proposed by *Riley* and *Barber* (1971). Rhizosphere soil was collected by carefully separating roots from loosely adhering soil (maximum 2 mm from the root surface) with a paintbrush. In the field experiment, these samples were taken from roots located within the first 10 cm of surface soil. In the pot experiments, the whole root system was sampled. At the same time of the rhizosphere soil collection, samples of bulk soil (*i.e.,* nonrhizospheric) were obtained from each experiment. Rhizosphere- and bulk-soil samples were air-dried and sieved (0.5 mm) before measurements. Rhizosphere- and bulk-soil pH were determined from a 1:20 soil-to-water-ratio suspension. This low relation was due to the low volume of soil obtained from rhizosphere-soil samples. Shoots were cut at ground level and dried at 60°C for 3 d to determine dry weight. Subsamples (70 mg) of aerial plant tissue were ashed at 500°C for 24 h. The ashes were dissolved in 8 mL of 0.1 M HCl, and P concentration was measured colorimetrically.

2.5 Soil-P fractionation

A modified version of the procedure of *Hedley* et al. (1982) was used to sequentially fractionate soil P from rhizosphere- and nonrhizosphere-soil samples (*Tiessen* and *Moir*, 1993). Briefly, six P fractions were determined as follows. Two anionic exchange-membrane (AEM) strips (saturated with $NaHCO_3$) were added to 0.5 g of soil and shaken for 16 h at constant temperature (24°C) with 30 mL deonized water. When the process was completed, the AEM strips were shaken with 20 mL 0.5 M HCl overnight. The soil suspension was centrifuged, and water discarded. The soil was further sequentially extracted with 30 mL of 0.5 M $NaHCO_3$ (pH 8.5), 0.1 M NaOH, and 1 M HCl by shaking for 16 h at constant temperature (24°C). At each step, the soil suspension was centrifuged and P concentration was measured. Total P in the $NaHCO_3$ and NaOH fractions was quantified after digestion of an aliquot (by adding ammonium persulfate and 0.9 M H_2SO_4). The $NaHCO_3$ and NaOH extracts were acidified to precipitate organic matter (pH 1.5, by adding 0.9 M H_2SO_4). The difference between total P and inorganic P (P_i) was assumed to be organic P (P_o). The residual P was not differentiated between P_i and P_o and was determined after H_2SO_4/H_2O_2 digestion. Phosphorus concentration in the extracts was determined colorimetrically with the molybdate-ascorbic acid procedure after pH adjustment using *p*-nitrophenol as an indicator.

According to *Tiessen* and *Moir* (1993), the P fractions separated with this procedure corresponded to the following P pools, (1) labile P_i (resin P + $NaHCO_3$-P_i): this fraction represents the plant-available P, associated with solution and weakly sorbed inorganic P; (2) NaOH-P_i: inorganic P with lower plant availability and is considered to be associated with amorphous and some crystalline Al and Fe phosphates; (3) HCl-P_i: inorganic P associated with calcium; (4) $NaHCO_3$-P_o: easily mineralizable organic P; (5) NaOH-P_o: more stable forms of organic P; and (6) residual P: inorganic and organic P in resistant or occluded pools.

2.6 Statistical analysis

Experimental units for each experiment were arranged in a randomized complete block design. Analysis of variance and regression analyses of plant and soil properties were conducted. Homogeneity of variances was tested using Bartlett's procedure. In case of rejection, data were transformed. Significant differences among means were determined by LSD at the 5% probability level.

3 Results

3.1 Biomass and P accumulation and rhizosphere acidification

As expected, maize showed the highest biomass accumulation in both the field and pot experiments (Tab. 2). However, this generalized higher growth of maize was translated into higher P accumulation in only two of the six soils evaluated in this study. Significant differences between species in P accumulation were observed only in Soils V and VI from pot experiment B. In these two soils, soybean had the lowest P accumulation, maize the highest, and sunflower showed values intermediate between them.

Rhizosphere acidification was consistently greater in the soils with sunflower than in soils with soybean and maize (Fig. 1), whereas no clear differences were observed between the soils with these last two species. Sunflower acidified the rhi-

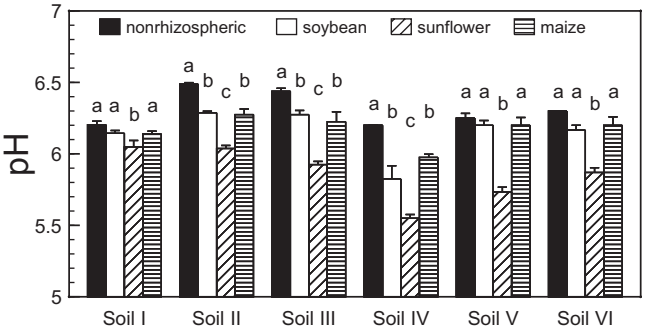

Figure 1: pH values from rhizosphere and nonrhizosphere soil of soybean, sunflower, and maize in the field experiment (Soil I), pot experiment A (Soils II–IV), and pot experiment B (Soils V–VI). Bars indicate standard error. Columns in the same soil with different letters are significantly different ($P < 5\%$).

Table 2: Aboveground biomass accumulation for soybean, sunflower, and maize in the field experiment (Soil I), pot experiment A (Soils II–IV), and pot experiment B (Soils V–VI). Shoot P content was measured only in the pot experiments. Different letters denote significant differences between species in each experiment (LSD test, $P < 5\%$).

Soil	Field experiment	Pot experiment. A			Pot experiment. B	
	I	II	III	IV	V	VI
	aboveground biomass / kg m^{-2}	/ g plant^{-1}				
Soybean	1.50 b§	16.7 b	16.3 a	14.0 a	13.4	9.6 a
Sunflower	1.38 b	13.6 a	15.3 a	15.5 a	16.7	26.3 b
Maize	2.19 a	30.0 c	34.6 b	27.3 b	24.6	61.3 c
	aboveground P content / g m^{-2}	/ mg P plant^{-1}				
Soybean	2.01	17.6	24.7	20.2	18.7 a	14.2 a
Sunflower	1.73	17.3	17.1	24.3	20.8 ab	35.3 b
Maize	1.96	20.6	21.0	16.5	27.6 b	68.1 c

§ Different letters denote significant differences between species (LSD test, $P < 5\%$).

zosphere in all the studied soils, while soybean and maize in only three of them (Fig. 1). Average differences between rhizosphere and bulk soil were 0.4, 0.1, and 0.1 pH units for sunflower, soybean, and maize, respectively. Rhizosphere pH ranged from 5.5 to 6.0 in sunflower, 5.8 to 6.2 in soybean, and 5.9 to 6.2 in maize. The linear regression between rhizosphere acidification and P depletion (measured as the difference between the bulk soil and the rhizosphere) of P fractions (including HCl-P_i) was not significant in any case (not shown).

3.2 Comparison of soil-P fractions between rhizosphere and nonrhizosphere soil

The six evaluated soils varied in their distribution of P among the different fractions (Fig. 2). Soils with originally lower P-Bray content (Soils I to IV; Tab. 1) had proportionally less P in the more available fractions (labile Pi, NaOH-P_i). Complementarily, Soils I to IV had proportionally more P in the resistant fractions (NaOH-P_o and residual P), whereas the P-rich soils (Soils V and VI) showed a more even distribution of P among the six fractions. When the labile P_i of the rhizosphere was compared to that of the bulk soil, depletion was observed only in one soil (out of the six evaluated) in soybean, two soils in sunflower, and four soils in maize (Fig. 2). In contrast, the NaOH-P_i fraction showed the greatest differences between rhizosphere and nonrhizosphere soil: depletion was observed in four soils with sunflower and in five soils with soybean and in maize (Fig. 2).

Depletion of the fractions HCl-P_i, $NaHCO_3$-P_o, NaOH-P_o, and residual P was infrequent: it was not significant in 83% of the cases. Rhizosphere P accumulation, as revealed by higher P contents in the rhizosphere compared to the bulk soil, was only observed in few cases without a clear identification of a specific fraction more prone to concentrate P in the vicinity of the roots (Fig. 2).

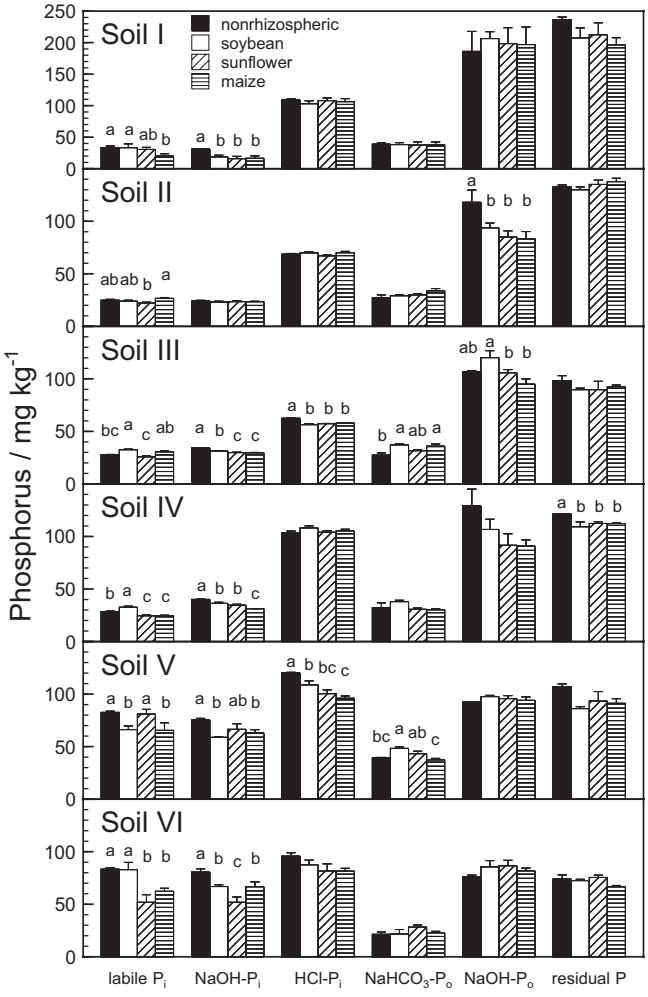

Figure 2: Concentration of P in various P fractions in rhizosphere and nonrhizosphere soil of soybean, sunflower, and maize in the field experiment (soil I), pot experiment A (soils II–IV), and pot experiment B (soils V–VI). Bars indicate standard error. Columns in the same soil and P fraction with different letters are significantly different ($P < 0.05$). Fractions without letters denote no treatment effects.

3.3 Comparison of soil P depletion among species

Interspecific differences in the depletion of P fractions in the rhizosphere were detected in most of our experiments (Fig. 2). These differences varied among soils and were mainly detected in the labile P_i and NaOH-P_i fractions. The HCl-P_i, $NaHCO_3$-P_o, and NaOH-P_o fractions showed only one significant difference among crops (Fig. 2).

Maize presented a more intense labile-P_i depletion than soybean in four out of the six soils (Fig. 2). The other P fractions presented fewer differences between these two species but in no case, soybean depleted more P than maize. More depletion in maize than in soybean was observed in two soils in the NaOH-P_i fraction, in one soil in the HCl-P_i fraction, and in one soil in the $NaHCO_3$-P_o fraction. Comparisons between sunflower and soybean revealed that soybean had a tendency to deplete less P than sunflower. The significant differences indicating more depletion by sunflower were observed in labile P_i (three soils) and NaOH-P_i (two soils). Finally, the comparison between sunflower and maize revealed that neither of them took clear advantage over the other in terms of P depletion. More P depletion in sunflower than in maize was observed in two soils in the labile P_i fraction and in one soil in the NaOH-P_i fraction. Maize depleted more labile P_i than sunflower in one soil (Fig. 2).

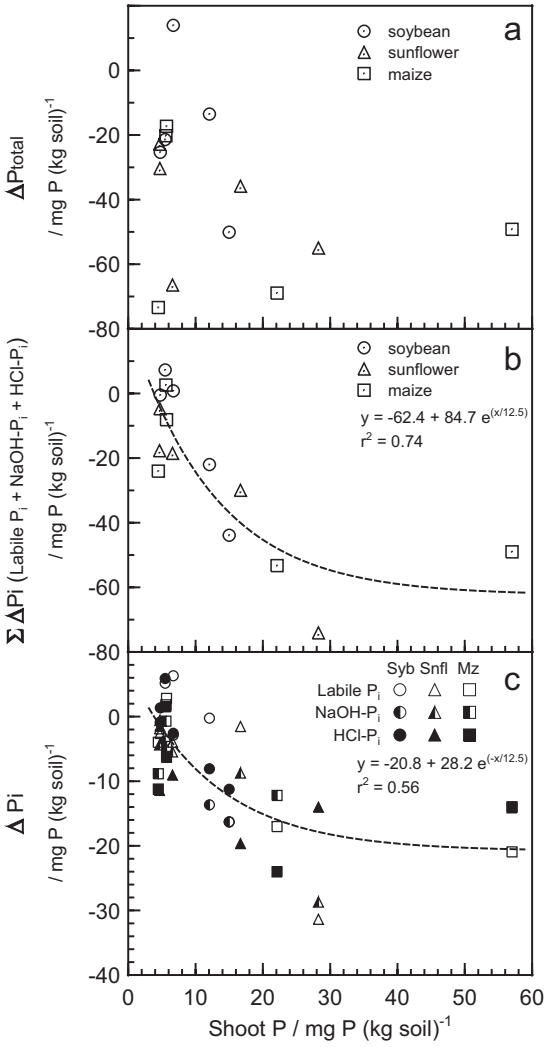

Figure 3: Difference between P from rhizosphere and nonrhizosphere soils as a function of the aerial P content in plants of soybean, sunflower, and maize per unit of soil mass in pot experiments A and B. Please note that the total soil mass differed between both experiments, as explained in section 2 (3.7 and 1.25 kg in experiments I–IV and V–VI, respectively). Rhizosphere depletion of total P (a), the sum of the three inorganic P fractions (b), and each inorganic P fraction (c) are shown for the three species. Functions were fitted to all data as there was no significant difference between functions fitted separately to each species (in b and c) or P fraction (in c) (F test, $P > 5\%$).

3.4 Rhizosphere P depletion and plant P uptake

A strong relationship between the depletion of the labile P_i, NaOH-P_i, and HCl-P_i fractions and the amount of shoot P uptake per unit of soil weight was observed in the pot experiments (Fig 3b, 3c). Those pots that presented a reduced plant P uptake (points on the left side of Fig. 3) showed very little variation between the P_i in the rhizosphere and the P in the bulk soil. The cases in which depletion of the P_i fractions was clearly noticeable appeared only in those pots in which P uptake was higher than a value around 10 mg P (kg soil)$^{-1}$. Functions fitted for each species, and each P_i fraction was not significantly different to each other (F test, $P > 5\%$). Therefore, the relationship between P_i depletion and P uptake was represented by one function fitted to all data (Fig. 3b, 3c). These results indicate that: (1) the three P_i fractions were prone to rhizosphere depletion; (2) P depletion was driven by plant P uptake; (3) the relationship between P_i depletion and P uptake was similar for the three P_i fractions and the three species. On the contrary, no significant relationship between total P depletion and plant P uptake was observed (Fig. 3a).

4 Discussion

4.1 Rhizosphere pH

The three plant species differed in their capacity to modify the rhizosphere pH. Whereas rhizosphere acidification was systematically greater in sunflower than in maize and soybean, no significant differences were found between the last two species (Fig. 1). *Zaccheo* et al. (2006) observed that sunflower plants lowered the initial soil pH but only when ammonium fertilizers were applied. In soybean and maize, only half of the soils showed statistically significant rhizosphere acidification. In legumes such as soybean, a greater rhizosphere acidification is expected when biological fixation is the predominant nitrogen source (*Aguilar* and *Van Diest,* 1981; *Hinsinger* et al., 2003), but when nitrogen comes simultaneously from the soil and from the atmosphere, rhizosphere pH is less predictable (*Nye,* 1981). Although active nodules were visible in all soybean plants, we do not know the proportion of nitrogen from the soil and from the air taken up by soybean plants. In the case of maize, the lack of generalized rhizosphere acidification observed is in line with that found by *Li* et al. (2007; 2010) and *George* et al. (2002).

The decrease in rhizosphere pH may promote P acquisition in those soils in which calcium phosphates are a major component of soil P (*Lindsay* and *Moreno*, 1960; *Hinsinger,* 2001; *Devau* et al., 2009), as is the case of Pampean Mollisols (*Ciampitti* et al., 2011). In our case, neither sunflower (the species with highest rhizospheric acidification) nor soybean or maize showed a significant relationship between HCl-P_i depletion and rhizosphere pH. This suggests that the acidification observed may have not been enough to activate the release of calcium phosphates. An alternative explanation is that calcium phosphates more reactive to pH are extracted to a large extent early in the sequential extraction procedure and are present in the labile P_i fractions. The HCl-P_i fraction would contain mainly the less soluble and pH-reactive calcium phosphates. Relative large changes in pH would be necessary (especially at a relatively neutral pH) in order to markedly change P solubility in this fraction (*Li* et al., 2008).

4.2 Phosphorus fractionation

To assess the effect of root activity on soil P fractions, we used a methodology based on the comparison between rhizosphere and nonrhizosphere soil. Differences between both soil zones can obviously be linked to root activity but it is erroneous to attribute the rhizosphere effects only to plant P acquisition. The quantity of each P fraction in the rhizosphere is the result of a series of simultaneous processes. For example: (1) plant P uptake and efflux; (2) secretion of root compounds that can mobilize soil P_i; (3) transfer of P to the rhizosphere from the bulk soil *via* diffusion (although it is a slow process); (4) P-related processes driven by the enhanced biological activity such as pH alterations, microbial-related P immobilization or release, and release of enzymes or chelating compounds; (5) mycorrhizal activity; and (6) changes in soil P dynamics driven by the depletion of P fractions, which may prompt a replenishment from other fractions (*Lambers* et al., 2006; *Vance* et al., 2003). The assumption of all P-fractionation methods is that the different fractions can be classified in terms of their bioavailability and sensitivity to undergo the abovementioned processes (*Bowman* and *Cole,* 1978; *Hedley* et al., 1982). However, it should be taken into account that the comparison between rhizosphere and nonrhizosphere P fractions only reveals the net balance of the counteracting processes mentioned above and does not allow the discrimination of the individual effect of any of them, including plant P uptake. In this paper, we refer to this net balance as P accumulation or P depletion when it indicates more or less P in the rhizosphere than in the nonrhizosphere soil, respectively.

The distribution of P fractions followed a particular pattern in each of the six soils, associated with the level of initial soil test P (Bray 1). Soils with originally low or medium levels of P (less than 17 mg kg^{-1} Bray-1 P—Soils I to IV; see Tab. 1) had proportionally less P in the more available fractions (labile P_i, NaOH-P_i). In these soils, the sum of NaOH-P_o and residual P fractions constituted around 70% of total soil P. Conversely, the experiments that included a layer of P-rich soil (45 and 72 mg kg^{-1} Bray-1 P—Soils V and VI), exhibited a rather even distribution of P pools without a clear dominance of any fraction. All the tested soils were homogeneous in terms of soil taxonomy (Mollisols), current use (all were sampled from agricultural fields), and history of fertilization (they had received P-fertilizer doses just to compensate annual exports of P, or even less, along their history of agriculture). The main contrast among them was the length of the period after they had been introduced to agriculture, which means that the soils with currently low P values were exhausted over a longer time and exported more P through harvests. In such sense, observed divergences in P fractionation between soils poor and rich in P would indicate that crop P removals generated a redistribution of P among fractions.

Overall, our results indicate that the depletion of rhizosphere P did not closely follow the range of bioavailability of P fractions predicted by the methodology (*Hedley* et al., 1982; *Tiessen* and *Moir*, 1993), especially in the range of the more active fractions. This confirms previous reports where the distribution of P fractions was used to assess the depletion of rhizosphere P (*George* et al., 2002; *Li* et al., 2008; *Hassan* et al., 2012; *Wang* et al., 2012). We observed that the labile P fraction had lower levels in the rhizosphere than in the bulk soil in only 38% of the studied cases. Conversely, the NaOH-P_i fraction was the most sensitive to rhizosphere depletion, showing lower values in the rhizosphere than in the bulk soil in 77% of the cases. Other studies have also found a fast depletion of the NaOH-P_i fraction (*Vu* et al., 2008; *Nuruzzaman* et al., 2006; *Wang* et al., 2012). A possible explanation is that the mobilization of P from the NaOH-P_i fraction preserved the pool of labile P, despite plant P uptake (*Ciampitti* et al., 2011; *Wang* et al., 2012). With regard to the organic P fractions, no significant differences were observed between the rhizosphere and the bulk soil in most cases. This correlates favorably with results obtained by *Rose* et al. (2010), although other reports have found that the organic fractions were fairly active (*Nuruzzaman* et al., 2006; *Li* et al., 2008; *Steffens* et al., 2010).

Our findings offer evidence that the interspecific differences in rhizosphere P depletion were not aligned with the ranking of soil P critical levels. Therefore, the lower P critical levels and lower responsiveness to P fertilization of soybean and sunflower compared to maize found in previous studies would not be generated by a greater accessibility to soil P sources. In fact, soybean was less effective at depleting P from the rhizosphere than sunflower and maize, whereas no clear differences were found between the latter two species. Interspecific differences were mainly observed in the labile-P_i and NaOH-P_i fractions, but none of the three species showed a specific preference for utilizing a particular fraction. Maximum rhizosphere P depletion and interspecific differences were observed when soil P exhaustion was exacerbated by growing plants in pots filled with sand free of P and only a layer of soil, where P was confined (pot experiment B). In such sense, P depletion was observed in those cases where greater plant P demand per unit of soil occurred and it was less likely to be detected when P uptake was low (Fig. 3). The fact that soil P depletion was associated to the amount of P taken up by the plants is in good agreement with the classic concept that uptake rates are driven by nutrient demand (*White,* 1972; *Jungk* et al., 1990).

5 Conclusion

Our studies, specifically designed for the comparison at the rhizosphere level of crops that differed in their P critical levels in agricultural fields of the United States and Argentina (sunflower = soybean < maize), revealed significant variations in the root-mediated soil P depletion. However, these variations in rhizosphere P depletion followed a different ranking (maize = sunflower > soybean), which means that the accessibility to soil pools does not appear to be a relevant factor determining soil-P critical levels. Instead, the rhizosphere P depletion was associated with the amount of P acquired by the plants. Further studies are needed to elucidate which other mechanisms may be responsible for the divergent soil-P critical levels of crops. Among them are root morphological characteristics, mycorrhizal responsiveness, internal P requirements, and other traits related to P acquisition or utilization efficiency (*Lambers* et al., 2006; *Fernandez* et al., 2009, 2011; *Simpson* et al., 2011).

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