



# Assessment of phosphorus availability in soil cultivated with ruzigrass



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## ARTICLE INFO

Handling Editor: Junhong Bai

Keywords:

*Urochloa ruziziensis*

Isothermal titration calorimetry

Phosphorus pools

Organic phosphorus

## ABSTRACT

Growing ruzigrass (*Urochloa ruziziensis*) in crop rotation systems has been suggested as a strategy to increase soil phosphorus (P) cycling and P availability. However, despite increased P lability shown in routine soil analysis, decreased grain yields of crops grown after ruzigrass have been observed. The objective of this study was to evaluate soil P availability to maize (*Zea mays*) in low or high-P soil cropped to ruzigrass. Soil P lability was evaluated using Hedley fractionation and pearl resin extractions, and P desorption/adsorption was assessed by isothermal titration calorimetry (ITC). Phosphorus changes in soil-P fractions in the maize rhizosphere were studied in a greenhouse experiment. Growing ruzigrass resulted in higher resin-extractable P and soil organic matter (SOM) contents than fallow. However, in soil cropped with ruzigrass, maize P uptake and P desorption were lower, and P adsorption to soil was higher than soil under fallow. In general, organic P bound to Fe and Al was non-available. Phosphorus sorption as assessed with ITC was a better indicator of P bioavailability to maize than pearl resin and Hedley fractionation, and suggested that P was less bioavailable after ruzigrass due to increased SOM, which resulted in the formation of metal phytate and more effective organo-metal sites for ligand exchange. Greater P solubility and availability in fallowed soil appeared to be partly due to the dissolution of Ca-related P, greater P desorption, and less potential for P adsorption. Isothermal titration calorimetry is a useful semi-quantitative tool for understanding P sorption behavior.

## 1. Introduction

Species of the genus *Urochloa* have been largely adopted in cropping system rotations in Brazil. Ruzigrass [*Urochloa ruziziensis* (R. Germ. and C.M. Evrard) Morrone and Zuloaga] is the most used due to its characteristics as both livestock feed and cover crop in integrated crop-livestock systems. According to Boddey et al. (1996), ruzigrass is well adapted to low soil fertility, low pH, and Al toxicity in soil, and has high yield, palatability and nutritional quality for animal feed. Ruzigrass is also tolerant to low P availability due to a high P uptake efficiency, and has been studied in crop system rotations intended to increase soil P availability through P cycling (Merlin et al., 2013; Almeida, 2014; Almeida and Rosolem, 2016).

The high P uptake efficiency by ruzigrass may be related to an extensive root system (Boddey et al., 1996) and the ability of roots to exude low-molecular weight organic acids (LMWOAs), such as citrate (Wenzl et al., 2001), that give access to Fe- and Al-bound P (Merlin et al., 2015). Almeida and Rosolem (2016) observed an increase in water-extractable P, labile P, and moderately labile P forms in upper soil layers, and a decrease in recalcitrant P in subsurface soil layers as a result of ruzigrass cultivation during the soybean [*Glycine max* (L.)

Merrill] off-season. Hence, P cycling may be increased in systems where ruzigrass is introduced (Merlin et al., 2013; Almeida, 2014; Almeida and Rosolem, 2016). However, Almeida (2014) observed lower leaf P concentration and lower soybean grain yield after growing ruzigrass than in soil kept fallow. Since soybean leaf P concentration was lower after ruzigrass (Almeida, 2014), it was hypothesized that growing ruzigrass reduces soil P availability to subsequent crops partly due to a decrease in P desorption from the soil cultivated with ruzigrass. There is also the hypothesis that P extracted by resin and soil P fractionation are not able to truly estimate P availability to plants grown in rotation with ruzigrass.

Phosphorus is predominantly retained in soil through ligand exchange by 1:1 clay minerals, Fe and Al oxides and hydroxides, and precipitated as Fe, Al and Ca phosphates (Sparks, 2003). By understanding the sorption mechanisms, P management to improve P use efficiency can be better planned (Sharpley, 1995). Sorption isotherms and P sorption index are commonly used in soil P sorption studies (Anghinoni et al., 1996; Campos et al., 2016). However, isotherms and P sorption index are not suited for the determination of precise sorption reactions (Veith and Sposito, 1977). Isothermal titration calorimetry (ITC) has been used as a complementary technique for establishing P

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sorption reactions, providing a sensitive and direct quantitative measure of heat in a reaction (Penn and Warren, 2009; Penn and Zhang, 2010; Lyngsie et al., 2014). Penn and Warren (2009) studied P adsorption by kaolinite using ITC, and observed that reactions of P adsorption by ligand change resulted in exothermic reactions, whereas phosphate precipitation with Al resulted in endothermic reactions. The same authors observed that  $\text{NaH}_2\text{PO}_4$  titration in  $\text{AlCl}_3$  and  $\text{FeCl}_3$  solutions resulted in an endothermic reaction, because of P precipitation with Al and Fe. Titration of HCl to kaolinite was exothermic due to mineral dissolution. Another exothermic reaction was the adsorption of fluoride to kaolinite. These observations were confirmed with soils of mixed mineralogy, displaying exothermic reactions with ligand exchange to variable charged minerals (Penn and Zhang, 2010; Penn et al., 2014). Moreover, it was found that precipitation of Ca phosphate was exothermic (Penn and Zhang, 2010).

The objectives of this study were to directly measure the amounts and forms of soil P available to maize plants in soil previously cultivated with ruzigrass at two different soil P levels, and evaluate P sorption-desorption using ITC in the context of relative P uptake and soil P availability.

## 2. Materials and methods

Soil samples were collected from a long-term experiment in Botucatu, Brazil ( $22^\circ 50' 00''$  S;  $48^\circ 25' 31''$  W; at altitude 806 m), cropped under no-till since 1998. The area selected for the long-term experiment had not been cropped for several years before 1998. From 1998 to 2005, no-till soybean was grown in the summer in rotation with black oat (*Avena strigosa* Schreb.) or triticale (x *Triticosecale* Witt.) in the fall-winter, and pearl millet [*Pennisetum glaucum* (L.) R. Br.] in the spring. Since 2006, soybean has been grown in rotation with ruzigrass or fallow. The soil is a well-drained Rhodic Hapludox (Soil Survey Staff, 2014) with 67% of sand and 21% of clay. Selected chemical characteristics of the soil are presented in Table 1.

Soil samples were taken from plots with different P fertilization and crop rotation for soil analysis and for a greenhouse experiment. Treatments comprised the combination of the following long-term fertilizer applications and cropping systems: low-P soil samples taken from plots not fertilized with P since 1998, and high-P soil that has received a total of  $427 \text{ kg ha}^{-1}$  of P applied as triple superphosphate (TSP) from 1998 to 2012; and presence or absence of ruzigrass grown as a cover crop during the off-season. Soil samples were taken from depths of 0–5 and 0–20 cm in November 2012, after ruzigrass desiccation. Six soil subsamples were randomly taken from the 0–5 cm depth of each plot using a 50 mm diameter core sampler and composited. Three soil subsamples were collected from the 0–20 cm depth of each plot using a shovel to obtain a sufficient amount to perform a greenhouse experiment.

Subsamples from both depths were air-dried and passed through a 2-mm sieve for subsequent chemical analysis of available P (Resin-P)

**Table 1**  
Selected chemical characteristics of the soil at different depths before the long-term experiment was planted in 1998.

Depth	pH <sup>a</sup>	SOM <sup>b</sup>	Resin-P <sup>c</sup>	H + Al <sup>d</sup>	K	Ca	Mg	CEC <sup>e</sup>
cm		$\text{g kg}^{-1}$	$\text{mg kg}^{-1}$		$\text{mmol}_c \text{ kg}^{-1}$			
0–5	5.2	21	18	27	2.7	21	13	64
5–10	5.1	18	16	28	0.7	17	11	57
10–20	4.6	19	5	34	0.6	8	6	48

<sup>a</sup> Soil pH measured in calcium chloride solution.

<sup>b</sup> Soil organic matter.

<sup>c</sup> Phosphorus extracted with pearl resin. According to Raji et al. (1986).

<sup>d</sup> Potential acidity.

<sup>e</sup> Cation exchange capacity.

and Ca extracted with pearl resin (Raji et al., 1986), pH in  $0.01 \text{ mol L}^{-1}$   $\text{CaCl}_2$ , SOM, and potential acidity (H + Al), as described in Raji et al. (2001). Soil P fractionation was conducted based on a modification of the Hedley fractionation (Hedley et al., 1982) as proposed by Condron and Goh (1989). Briefly, 0.5 g soil samples were placed in centrifuge tubes and subjected to the following extraction sequence: anion exchange resin (AER) type AR-204SZRA (GE Water & Process Technologies, Pennsylvania, U.S.);  $0.5 \text{ mol L}^{-1}$  sodium bicarbonate ( $\text{NaHCO}_3$ ) pH 8.5;  $0.1 \text{ mol L}^{-1}$  sodium hydroxide (0.1-NaOH);  $0.1 \text{ mol L}^{-1}$  hydrochloric acid (HCl); and  $0.5 \text{ mol L}^{-1}$  NaOH sodium hydroxide (0.5-NaOH). After extraction, the soil was dried in an oven at  $50^\circ \text{C}$ , ground in an agate mortar, and 0.1 g of the soil was subjected to nitric-perchloric digestion for the extraction of residual P (Residual-P). A standard spike ( $500 \text{ mg kg}^{-1}$  of P) was added to few soil samples to assess recovery efficiency of the nitric-perchloric digestion, which was in average 92%, approximately. The acid extracts obtained with AER, HCl, and nitric-perchloric digestion were used to determine inorganic P ( $\text{P}_i$ ) concentrations using the molybdate blue and ascorbic acid method (Murphy and Riley, 1962). The alkaline extracts obtained with  $\text{NaHCO}_3$  and NaOH were divided into two aliquots. In the first aliquot of each extract, the concentration of  $\text{P}_i$  was determined immediately after extraction to prevent the hydrolysis of organic P ( $\text{P}_o$ ) present in the extract (Dick and Tabatabai, 1977). Accordingly, the following  $\text{P}_i$  fractions were obtained:  $\text{NaHCO}_3\text{-P}_i$ ,  $0.1\text{-NaOH-P}_i$ , and  $0.5\text{-NaOH-P}_i$ . The second aliquot was subjected to digestion with ammonium persulfate [ $(\text{NH}_4)_2\text{S}_2\text{O}_8$ ] and sulfuric acid ( $\text{H}_2\text{SO}_4$ ) in an autoclave to determine the total P ( $\text{P}_t$ ) content of each extract. The difference between the  $\text{P}_t$  and  $\text{P}_i$  was assumed to equal  $\text{P}_o$  for each extract. Thus, the following extracted P fractions were obtained:  $\text{NaHCO}_3\text{-P}_o$ ,  $\text{NaHCO}_3\text{-P}_t$ ,  $0.1\text{-NaOH-P}_o$ ,  $0.1\text{-NaOH-P}_t$ ,  $0.5\text{-NaOH-P}_o$ , and  $0.5\text{-NaOH-P}_t$ . All fractionation analyses were done in triplicate. The P concentration was determined using a spectrophotometer (Model 600S, FEMTO, Brazil). The sum of all extracted P fractions from fractionation analysis was labeled as Total-P, including Residual-P. Comparison of total P measured directly with summed P fractions revealed that the fractionation recovered an average 80% of total P.

### 2.1. Isothermal titration calorimetry

Samples collected from the 0–5 cm depth were subjected to tests of soil P desorption and adsorption by isotherm titration calorimetry (ITC), model CSC 4200 (CSC Inc., Utah, U.S.) at  $25^\circ \text{C}$ . Since no-till results in more pronounced effects on P availability in the uppermost soil layers, the 0–5 cm layer was chosen. Soils were titrated with P to obtain a semi-quantitative indicator of the ability to sorb P along with some indicator of P sorption mechanisms. In addition, titration and extraction with citric acid was chosen to investigate potential P desorption because citric acid is excreted by plant roots to increase P bioavailability in the rhizosphere (Jones and Darrah, 1994; Hinsinger, 2001).

The ITC has a sensitivity of  $0.418 \mu\text{J}$  detectable heat effect and a “noise level” of  $\pm 0.0418 \mu\text{J}$  (deconvoluted signal). All samples were analyzed with 25 titrations of 0.01 mL of titrant in 300 s intervals. For each experiment, a blank was determined by titration of the respective solution into deionized (DI) water, and subtracted from the measurements of the samples. For the ITC analysis, 50 mg of soil sample was placed in a 1.3 mL ampule filled with 0.75 mL of distilled water and titrated with the appropriate solution. Quality and Assurance (Q & A) procedures involved applying a precise sweep of electrical inputs into the ITC prior to measuring samples, for the purpose of instrument calibration via measurement of ampule heat coefficients. In addition, two different types of check samples were utilized as part of Q & A. Due to the fact that temperature is highly controlled to prevent heating or cooling, drift is  $< 100 \mu^\circ\text{C}$  over 24 h, making the analysis highly repeatable (Penn and Warren, 2009). For P sorption, a solution of  $0.01 \text{ mol L}^{-1}$   $\text{NaH}_2\text{PO}_4$  was used as the titrant, according to Penn and

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