Soil phosphorus dynamics as affected by Congo grass and P fertilizer

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ABSTRACT: Some plant species can change soil phosphorus (P) availability and this may be an important tool in managing tropical high fixing phosphorus soils. An experiment was conducted to evaluate phosphorus transformations in the soil and phosphatase activity during periods of Congo grass (*Brachiaria ruziziensis,* Germain et Evrard) growth in two tropical soils receiving 20, 40, 80, 160 mg dm^{-3} of inorganic P. Plants were grown for 84 days in 8-L pots. Acid phosphatase activity, P in the microbial mass, soil organic and inorganic P and P accumulation by Congo grass were evaluated. Phosphorus fertilization increased soil P availability, Congo grass yields and P accumulation in the plant. On average, less labile P forms in the soil were not changed by Congo grass; however, the P in the soil extracted with HCl (P-Ca - non labil form) decreased. This decrease may have resulted from the combination of the presence of grass and phosphatase capacity to dissolve less available P in the soil. Thus, soil exploration by Congo grass roots and the subsequent extraction of calcium phosphate may have increased the P concentration in the plant tissue. Despite the decrease in the P extracted from the soil with HCl resulting in increased labile P forms in the soil, the effect of Congo grass on the availability of P depends on the soil type.

Keywords: phosphatase, soil fertility, crop rotation, brachiaria

Introduction

Inorganic phosphorus (P) in the soil occurs mainly as $H_2PO_4^-$ and HPO_4^{2-} adsorbed to the surface of oxides and hydroxides and to organic matter or bound to Ca (Olibone and Rosolem, 2010), depending on acidity and minerals in the clay (Duputel et al., 2013). Hence, soils can be considered a source or sink for phosphorus depending on their characteristics (Pavinato et al., 2009). Organic phosphorus can be a potential P source for plants (Cross and Schlesinger, 1995) after microbial decomposition of organic matter. No-till systems allow the accumulation of organic material on the soil surface, which supplies energy, carbon skeletons and electrons for microorganism growth and development, increasing biomass activity and eventually microbial P (Pavinato et al., 2009). Accumulated P_o in the rhizosphere may result in high microbial activity and a buildup of more stable organic P fractions or a low P_o use-efficiency by plants (Kamh et al., 1999). Microorganisms and plant roots transform P_o into P_i by the synthesis and exudation of enzymes known as phosphatases.

Acid phosphomonoesterases are active mainly at low pH whereas alkaline phosphomonoesterases are active predominantly at higher pH (Eivazi and Tabatabai, 1977). Acid phosphatases activity decreases with P_i availability in the soil due to either an increase in P mineralization or pH (Rengel and Marschner, 2005). The decrease of P_o in cropped pasture soil is closely related to the P concentration of the microorganisms' biomass (Brookes et al., 1984).

The contribution of P_o to the total P available to the plant can be important for plant maintenance primarily in areas with pastures and forests, but this contribution can also be significant in areas cropped under no tillage, with adequate management of cover crops. There are a huge number of microorganisms that can mineralize P_o, such as fungi and bacteria. The phosphatase activity is sensitive to soil management and climate seasonality, and depending on its activity, organic P may or may not be available in the cropping systems. Therefore, these enzymes could be useful in evaluating soil quality, broadening the study and the meaning of soil fertility with the inclusion of a measureable biological component.

Root induced processes markedly affect P availability (Devau et al., 2011), and Congo grass may enhance soil P availability through a decrease in maximum soil P fixation capacity (Janegitz et al, 2013) and an increase in microorganism and acid phophatase activity in the rhizosphere. This more labile P could be available for the next crop. Congo grass has been used in Brazil as forage or cover crop in rotation with cash crops under no-till because it has vigorous shoot and root growth, which benefits nutrient cycling (Calonego and Rosolem, 2013) and soil properties (Garcia et al., 2013). This study aimed to evaluate the activity of acid phosphatases and P transformations during periods of Congo grass growth, as well as to assess a potential increase in organic P as affected by P availability.

Materials and Methods

An experiment was conducted in a greenhouse, in 8-L pots filled with 9.2 kg of two soils (Table 1): a clayey Rhodic Hapludox (RH) and a Typic Haplustult (TH) (Soil Survey Staff, 2010). These soils were chosen because they are representative of Brazilian soils, RH was taken in Botucatu, state of São Paulo, Brazil (22°49′ S, 48°25′ W, altitude 800 m); while TH was taken in Luís Eduardo Magalhães, in the state of Bahia, Brazil (12° 19′31″S, 46°13′26″W, altitude 820m). Lime was applied (CaO: 39

%, MgO: 13 %) in order to increase base saturation to 70 %, and the soil was moistened to soil water retention capacity and kept moist for 60 days. This was done because it is closer to the soil environment where the forage is grown in rotation with cash crops. Next, 100 mg dm^{-3} of N and 100 mg dm^{-3} of K were added as urea and potassium chloride, respectively. Phosphorus was added as triple superphosphate at 20, 40, 80, 160 mg dm^{-3}, and Congo grass (*Brachiaria ruziziensis,* Germain et Evrard, sin. *Urocloa ruziziensis*) was grown or not in the pots, with four replicates.

Congo grass seeds were pre-germinated in a germination chamber for 48 h at 25 °C after dormancy-breaking by immersion in concentrated sulfuric acid for 10 min and washing in distilled water, and transferred to the pots. Eight days after planting, they were thinned to four seedlings per pot. Soil moisture was maintained between 70 and 100 % of the soil retention capacity, by weighing and replacing water loss daily. Congo grass was harvested 84 d after planting and dried in a forced air oven at 65 °C for 4 d. Dry matter yields were then determined. Subsequently, the material was ground and P concentration in the tissue was determined using nitric-perchloric wet acid digestion. Part of the soil was dried under ambient temperature conditions and part was saved for microbial P analysis.

Soil pH (0.01 M CaCl$_2$) was determined after harvest. Acid phosphatase activity was estimated colorimetrically by determining the amount of *p*-nitrophenol released by the enzyme when the soil was incubated with a solution of *p*-nitrophenyl sodium phosphate and toluene at 37 °C for 1 h (Tabatabai and Bremner, 1969). Organic phosphorus was calculated as the difference between results from unburned and burned (muffle furnace at 550 °C for 2 h) samples as in Olsen and Sommers (1982).

Soil samples were extracted using Hedley et al.'s (1982) P fractionation method, with modifications as proposed by Condron and Goh (1989). Specifically, 1.5 g of soil samples were quickly subjected to extraction conditions in sequential order. Shortly after, labile inorganic P was extracted with anion exchange resin. Next, labile inorganic and organic P were extracted with 0.5 M NaHCO$_3$, moderately labile organic P were extracted with 0.1 M NaOH, P was extracted sequentially with 1

M HCl. Then, occluded inorganic and organic P from soil micro aggregates were extracted with 0.5 M NaOH. Residual P was extracted with H$_2$SO$_4$, and total organic P was calculated as the difference between results obtained from unburned and burned samples. All of the samples were analyzed in triplicate. After these extractions the samples were digested with H$_2$SO$_4$ + H$_2$O$_2$. P$_i$ was analyzed as in Dick and Tabatabai (1977) in the NaHCO$_3$ and NaOH extracts. In addition, total P was analyzed after acid digestion.

The amount of P in the microbial mass was obtained by calculating the difference between the amount of P$_i$ extracted using 0.5 M NaHCO$_3$ (pH 8.5) from fresh soil fumigated with CHCl$_3$ and the amount extracted from non-fumigated soil. Fresh soil was used because the amount of P that CHCl$_3$ releases in dry soil is very low (Brookes et al., 1982).

The results of each soil were subjected to ANOVA as a 2 x 5 factorial (with and without plants and 5 P rates) design in completely randomized blocks with four replications. The parameters of P in the soil were correlated with P uptake by Congo grass. Means were compared using the t-test (LSD, $p < 0.05$). When appropriate, equations were fit to the results.

Results

Congo grass dry matter yields had a response to P rates regardless of soil type, up to 80 mg kg^{-1} of P (Figure 1). However, yields were higher in RH than in TH. In addition, plant P concentrations increased linearly with fertilizer rates. Some plants can modify the soil chemical properties such as pH and the cation exchange

Table 1 – Chemical characteristics from two tropical soils (Rhodic Hapludox - RH and Typic Haplustult - TH) used in the experiment.

Soil	pH	O.M.	P[1]	H+Al	K	Ca	Mg
	(0.01M CaCl$_2$)	g dm^{-3}	mg dm^{-3}	———— mmol$_c$ dm^{-3} ————			
RH	4.1	12	3	60	0.2	2	0
TH	5.2	8	6	45	1.1	8	4
	Clay		Silt		Sand		
	———————— g kg^{-1} ————————						
RH	750		200		50		
TH	670		210		20		

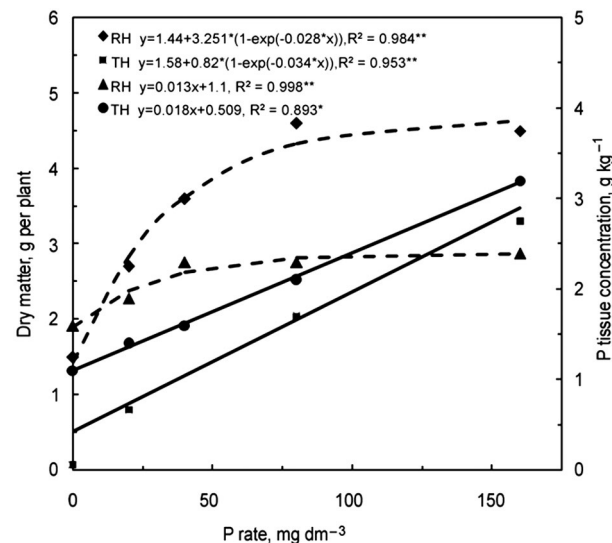[1]P – soil phosphorus extracted with pearl resin. O.M. – Organic matter

Figure 1 – Congo grass dry matter (dashed lines) and P concentrations (solid lines) in the tissue as affected by P rates in two tropical soils; RH: Rhodic Hapludox; TH: Typic Haplustult. **R^2 Significant ($p > 0.01$); *R^2 Significant ($p > 0.05$).

capacity by exuding organic acids in the rhizosphere (Devau et al., 2011). However, in the present experiment the average pH was 5.2 in RH and 5.4 in TH and it was not affected by phosphate fertilization or Congo grass (results not shown).

The microbial biomass P increased with P rates, with a larger increase in RH than in TH (Figure 2). The values of P in the microbial biomass were larger in the presence of grass ($p < 0.05$), with no interaction with P rates. For example, at the lowest rates of P the values observed in the absence and in the presence of grass were 11.9 and 14.6 µg g^{-1}, respectively, reaching 43.3 µg g^{-1} for the largest rate of P added, in the presence of Congo grass in RH (Figure 2). In TH differences between the values observed in the presence and absence of grass were also significant as in RH ($p < 0.05$), but in this case there was an interaction with P rates.

Acid phosphatase activity decreased with phosphate fertilization and increased in the presence of Congo grass (Figure 3, $p < 0.05$). This activity was, in general, higher in RH, where the lowest rate of P resulted in the average production of 669 µg of p-nitrophenol g^{-1} h^{-1} while the largest rate resulted in an average value of 541 µg of p-nitrophenol g^{-1} h^{-1}. The differences in acid phosphatase activity in TH samples were lower, with values of 568 and 481 µg of p-nitrophenol g^{-1} h^{-1} at the lowest and largest rate of P, respectively (Figure 3).

Phosphate fertilization up to 80 mg kg^{-1} increased soil labile P irrespective of grass cultivation (Figure 4), but the presence of Congo grass resulted in higher P availability in RH. However, there was no effect on the inorganic labile fraction (NaHCO$_3$) of P (Table 2). For the P inorganic fraction extracted with 0.1 M NaOH there was a response to the added P and again there was no Congo grass effect (Table 2). A response to P fertilizer was also observed for HCl extracted-P, which was reduced after Congo grass cultivation.

Soil P$_i$ increased with P rates (Table 2). An increase in the fraction of non-labile P (0.5 M NaOH) as affected by P fertilization was observed independently of grass cultivation (Table 2). The values of this non-labile P fraction were greater for RH than for TH. There was no effect of P rates or the use of Congo grass on the P residual fraction (Table 2). Labile P$_o$ was affected by P application in both soils, but there was no effect of grass or interactions (Table 3). There was no effect ($p < 0.05$) of treatments on other forms of P$_o$.

As expected, P fertilization had no effect on the organic P complexed with iron and aluminum. Grass cultivation produced no effect ($p < 0.05$) on this fraction and there were no significant interactions between the treatments (Table 3).

Discussion

In general, P tissue concentrations in Congo grass were above the sufficiency range reported for tropical grasses (Malavolta et al., 1997), from 0.8 to 1.2 g kg^{-1}.
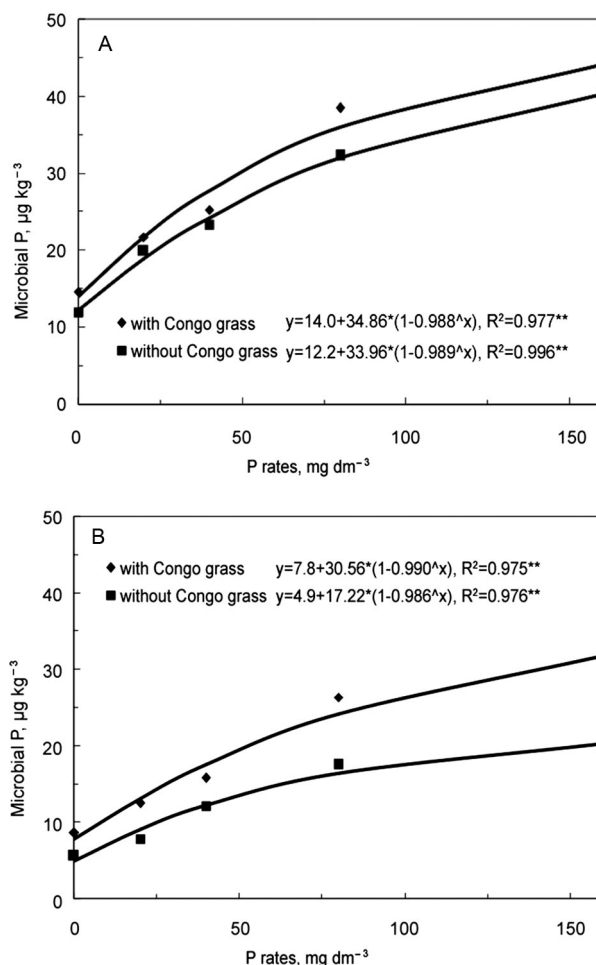

Figure 2 – Microbial P as affected by P rates (with and without Congo grass) in two tropical soils. A: Rhodic Hapludox; B: Typic Haplustult. **R^2 Significant ($p > 0.01$); *R^2 Significant ($p > 0.05$).

Even so, dry matter yields and P tissue concentrations increased with P application (Figure 1). P was immobilized in the soil with increased fertilization (Figures 2 A and B). This phenomenon is observed in soils where the management system allows for organic material accumulation on the surface, and conditions are favorable for microbial development. Similar data were obtained by Rheinheimer et al. (2002), who observed an increase in P stored in the microbial biomass after addition of P. Immobilized P in the microbial biomass can be released into the soil by rupture of the microbial cells, and also by the interactions with the microfauna that feed on microorganisms and subsequently release these stored nutrients into the soil (Buchanan and King, 1992). The P contained in the biomass acts, therefore, as a protection for this nutrient, diminishing its fixation by soil colloids (Paul and Clark, 1996) and increasing the efficiency of phosphate fertilization in the long range by immobilizing part of the P from the fertilizer into the biomass.
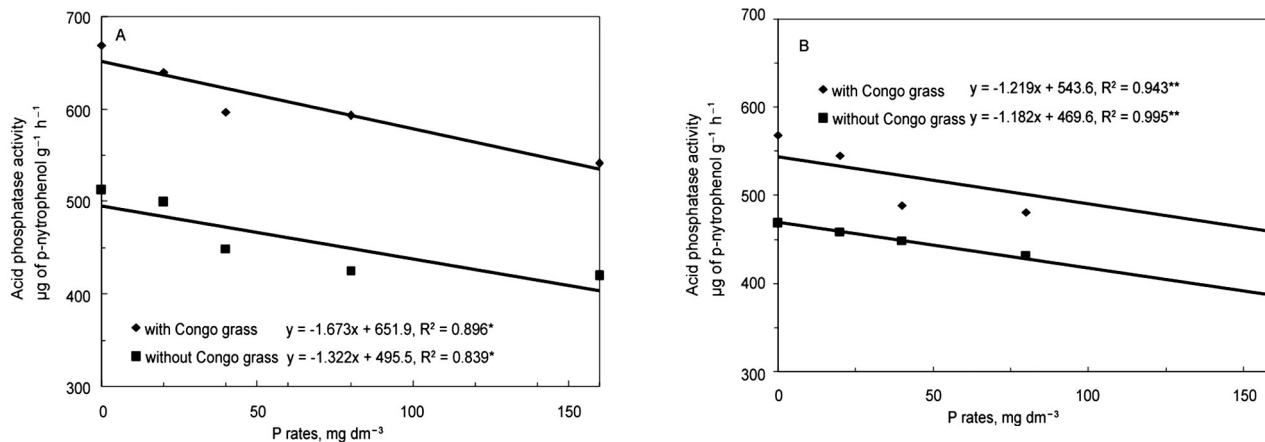
Figure 3 – Acid phosphatase activity (µg de p-nitrophenol g^{-1} h^{-1}) as affected by P rates (with and without Congo grass) in two tropical soils. A: Rhodic Hapludox; B: Typic Haplustult. **R^2 Significant ($p > 0.01$); *R^2 Significant ($p > 0.05$).
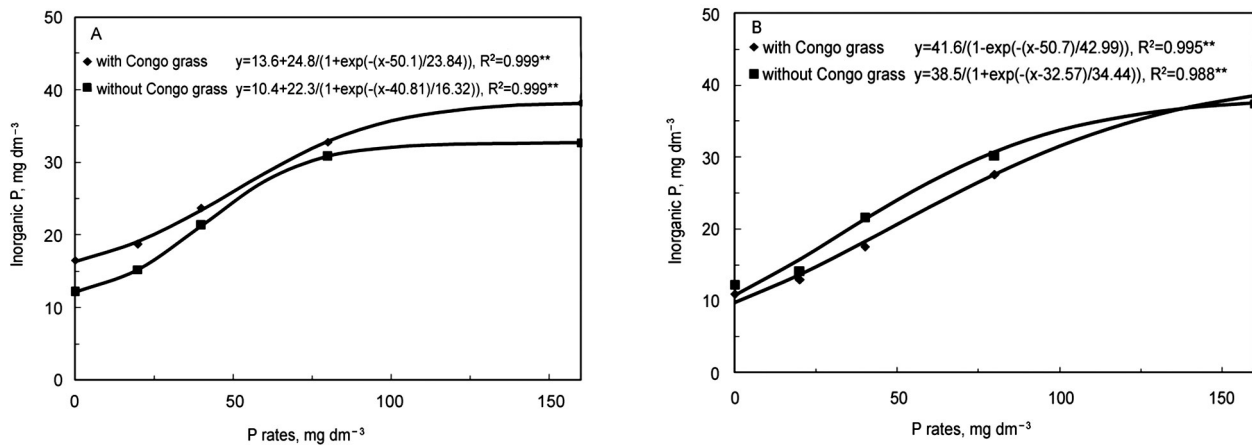

Figure 4 – Inorganic available P extracted with pearl resin as affected by P rates (with and without Congo grass) in two tropical soils A: Rhodic Hapludox; B: Typic Haplustult. **R^2 Significant ($p > 0.01$); *R^2 Significant ($p > 0.05$).

In addition to the P microbial biomass, the action of phosphate solubilizing microorganisms, the mycorrhizal fungi, and acid and alkaline phosphatases production by microorganisms are responsible for the mineralization of P_o (Dick and Tabatabai, 1993). In some species, the activity of enzymes such as acid and alkaline phosphatases in dissolving less soluble phosphates has been demonstrated (Rengel and Marschner, 2005), including ruzigrass - *Brachiaria ruziziensis,* Germain et Evrard (Louw-Gaume et al., 2010). In the experiment reported herein, addition of P decreased acid phosphatase activity in both soils (Figure 3 A and B), as expected (Bünemann et al., 2012). According to Dick and Tabatabai (1993), microorganisms would be the main sources of phosphatases in the soil. Thus, their large biomass, high metabolic activity and short life span, with several generations per year, would result in the production and release of large amounts of extracellular enzymes.

In sequence, anion exchange resin (AER) and bicarbonate extracted the amount of labile P_i. A high availability of the bicarbonate fraction has been observed by most of the authors who have worked with soil P fractionation, indicating that the amounts extracted by the AER do not represent all of the available soil P (Hedley et al., 1982; Guo and Yost, 1998). In the present study, soil labile P ($NaHCO_3$-P) fractions P were increased by P fertilization. However, Congo grass did not alter the labile inorganic fraction of P (Table 2), but increased AER P in one of the soils, the Rodhic Hapludox (Figure 4), regardless of P rates. The increase in acid phosphatase was higher in RH, which may have affected P availability. Reports of phosphate fertilization promoting alterations in the labile P fraction exist, mainly when soluble sources of P are used (Olibone and Rosolem, 2010). An increase in the labile organic fraction with rates of P has also been observed (Table 3). The observed effects with

Table 2 – Soil inorganic P fractions: labile, bound to Iron and Aluminum (P-Fe/Al), bound to Calcium (P-Ca), non Labile, residual and total inorganic as affected by P rates, and with or without Congo grass in two tropical soils (Rhodic Hapludox - RH and Typic Haplustult - TH).

P rates (mg dm⁻³)	NaHCO₃ P		0.1 M NaOH -P		HCl-P		0.5 M NaOH -P		Residual P		Total P	
	With	Without	With	Without	With	Without	With	Without	With	Without	With	Without
					mg dm⁻³							
						RH						
0	22.9	21.8	32.1	34.3	3.8	8.8	18.9	17.2	45.6	44.2	369	356
20	23.7	22.5	33.9	36.5	10.1	14.5	21.9	20.9	48.6	45.1	421	389
40	30.7	29.3	35.1	36.8	21.7	21.4	25.2	24.5	44.3	44.6	456	412
80	34.8	33.8	32.5	33.1	16.5	19.4	26.7	25.8	46.2	46.9	489	436
160	36.1	35.9	33.6	32.9	13.5	17.2	29.1	28.6	47.5	45.3	512	472
Average	29.6	28.6	33.4	34.7	13.1	16.2	24.3	23.4	46.4	45.2	449	413
P rates (D)	2.5*	3.1*	2.2*	2.2*	3.9*	3.9*	3.6*	3.6*	ns	ns	17.2*	17.2*
Congo grass (B)	ns[1]	ns	ns	ns	2.5*	2.5*	ns	ns	ns	ns	ns	ns
D × B	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
						TH						
0	18.2	17.5	28.6	30.1	1.9	3.2	21.5	18.5	38.9	35.6	286	245
20	19.3	18.5	28.1	32.8	9.2	12.4	20.7	21.1	37.2	35.1	302	269
40	21.5	21.9	29.7	34.6	13.7	14.6	22.6	23.6	37.9	34.7	334	318
80	21.9	19.4	31.2	34.9	12.9	13.6	23.5	24.3	38.0	34.3	359	349
160	22.5	21.4	33.2	36.2	14.8	19.6	24.5	23.7	39.5	35.8	383	371
Average	20.6	19.7	30.1	33.7	10.5	12.6	22.5	22.2	38.3	35.1	332	310
P rates (D)	2.1	2.4	2.6*	ns	ns	ns	2.1*	2.1*	ns	ns	12.5*	12.5*
Congo grass (B)	ns	ns	ns	ns	3.1*	3.1*	ns	ns	ns	ns	ns	ns
D × B	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[1]ns – non significant; *LSD ($p < 0.05$).

Table 3 – Organic P fractions: labile (NaHCO₃), P adsorbed to iron and aluminum (0.1 M NaOH), non labile (0.5 M NaOH) and total as affected by P rates (with or without Congo grass) in two tropical soils (Rodhic Hapludox - RH and Typic Haplustult - TH).

P rates (mg dm⁻³)	NaHCO₃ P		0.1 M NaOH -P		0.5 M NaOH -P		Total P	
	With	Without	With	Without	With	Without	With	Without
			mg dm⁻³					
				RH				
0	9.8	12.8	17.8	16.8	30.8	30.9	96.2	89.6
20	10.7	14.9	16.5	17.8	31.5	29.8	98.8	91.2
40	12.5	16.3	18.9	17.1	32.5	30.1	97.3	93.8
80	17.9	17.9	19.2	18.3	31.6	29.2	101.2	94.1
160	20.5	19.2	19.7	18.7	32.0	31.2	102.4	94.5
Average	14.2	16.2	18.4	17.7	31.6	29.5	99.1	92.6
P rates (D)	4.3*	4.3*	ns[1]	ns	ns	ns	ns	ns
Congo grass (B)	ns	ns	ns	ns	ns	ns	ns	ns
D × B	ns	ns	ns	ns	ns	ns	ns	ns
				TH				
0	7.8	15.3	15.4	13.2	26.9	27.2	64.5	65.0
20	8.9	14.6	14.1	13.8	25.8	26.5	67.2	64.2
40	10.5	17.9	14.8	14.0	25.1	26.8	66.8	64.8
80	12.7	16.9	15.6	14.3	26.4	26.4	65.8	65.4
160	13.5	21.5	16.8	15.9	27.1	27.3	66.2	66.2
Average	10.6	17.2	15.3	14.2	26.2	26.0	66.1	65.1
P rates (D)	5.9*	5.9*	ns	ns	ns	ns	ns	ns
Congo grass (B)	ns	ns	ns	ns	ns	ns	ns	ns
D × B	ns	ns	ns	ns	ns	ns	ns	ns

[1]ns – non significant; *LSD ($p < 0.05$).

grass cultivation are most likely due to the increased availability of P_i from the fertilizer. Plants use organic sources of P almost as efficiently as mineral sources, given the hydrolysis of P_o, liberating P_i (Tarafdar and Claassen, 1988). The organic soil P can act as a source or sink for the available P, depending on soil management and fertilization techniques used. The increase in P availability on the soil surface layers with the adoption of no-till can result in a significant transformation of P_i into P_o.

The fraction of P extracted with 0.1 M NaOH generally increased with P application, although no significant effects of Congo grass cultivation or interactions were observed (Table 2). Schoninger et al. (2012) observed that *brachiaria* grass reduced the most available P forms only in the 0-1 mm layer from the rhizosphere. Under field conditions, a large amount of P added as fertilizer is adsorbed in soil and remains in moderately labile fractions (Pavinato et al., 2009). As expected, the rates of P did not have an effect on the organic P forms extracted with 0.1 M NaOH (Table 3).

The P extracted with HCl represents the calcium phosphate that can be provided from primary minerals in the soil, or in other words, calcium phosphates formed "in situ" (Magid et al., 1996), and also from the addition of phosphate fertilizers. This fraction is considered unavailable for the majority of plant species (Tiessen et al., 1984). Alterations in P-Ca levels were observed in the present experiment and the largest values were obtained with 17.5 mg dm^{-3} of P (Table 2). The data presented confirm the ability of Congo grass to use soil P forms of lower lability. The most common mechanism of P solubilization by plants and microorganisms is the exudation of organic acids, mainly citrate, malate and oxalate (Richardson et al., 2011), and it has been shown that Congo grass can exude organic acids in the rhizosphere (Wenzl et al., 2001).

The increases observed in non-labile P fraction (0.5 M NaOH) are attributed to the addition of the phosphate fertilizer (Table 2). The absence of an effect in the non-labile organic forms can be attributed to the low lability of this fraction in the soil due to high ionic strength exerted by the extraction with 0.5 M NaOH. This fraction is considered to complement the inorganic and organic forms obtained by the extraction with 0.1 M NaOH (Hedley et al., 1982).

The increases in the total P_i fraction are attributed to the rate of P applied as treatments (Table 2). In no-till systems, the P-total values become larger over time, favoring the cycling of the nutrient into the soil, increasing the activity of the microorganisms and, consequently, the mineralization of P_o. Pavinato et al. (2009) observed that phosphate fertilizer promoted P accumulation in less available fractions in the soil and, eventually this phosphorus could migrate to more labile fraction and be available to plants. Thus, the weathering factors and the presence of plants seem to be very important in the dynamics of P, besides the actual application of phosphate fertilization.

The P_o fraction was not affected by the grass. The effect of cover crops, mainly legumes, enhancing P availability has been observed before (Kamh et al., 1999; Richadson et al. 2011; Hassan et al., 2013), but results on the effect of no-till on available P are inconclusive and depend on the soil type (Zibilske et al., 2002; Pavinato et al., 2009; Devau et al., 2011). However, these results were obtained in a pot experiment where roots were very close together and the proportion of rhizosphere soil is higher than it would be under field conditions. Hence, the magnitude of these changes may not be sufficient to foster an improvement in the nutrition of subsequent crops in a rotation system.

Conclusions

Congo grass dry matter yields, as well as plant P accumulation increases with the addition of P fertilizer, which lead to an increase in P accumulation in the soil microbial biomass. Phosphorus fertilization decreases soil acid phosphatase activity, which is increased by Congo grass. Congo grass promotes alterations in some P fractions, and is able to take up P from less labile pools. It may also increase resin extracted P, but it depends on the soil. The introduction of Congo grass in the rotation may enhance P use efficiency of the system over time. The effects can be high P uptake efficiency including the exploration of less labile forms along with vigorous growth and P accumulation in the plant.

Acknowledgements

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