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Can tropical grasses grown as cover crops improve soil phosphorus availability?

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Running Title: Tropical grasses and soil phosphorus availability

Summary

Tropical grasses grown as cover crops can mobilize phosphorus (P) in soil and have been suggested as a tool to increase soil P cycling and bioavailability. The objective of this study was to evaluate the effect of tropical grasses on soil P dynamics, lability, desorption kinetics and bioavailability to soybean, specifically to test the hypothesis that introducing grass species in the cropping system may affect soil P availability and soybean development according to soil P concentration. Three grass species: ruzi grass (*Urochloa ruziziensis*),

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palisade grass (*Urochloa brizantha*), and Guinea grass (*Megathyrsus maximus*) were grown in soils with contrasting P status. Soybean was grown after grasses to assess soil P bioavailability. Hedley P fractionation, microbial biomass P, phytase-labile P, and diffusive gradient in thin films were determined, before and after cultivation. It was found that grasses re-mobilized soil P, reducing the *concentration* of recalcitrant P forms. The effect of grasses on changing the P desorption kinetics parameters did not directly explain the observed variation on P bioavailability to soybean. Grasses and microorganisms solubilize recalcitrant organic P (P_o) forms and tropical grasses grown as cover crops increased P bioavailability to soybean mainly due to the supply of P by decomposition of grass residues in low-P soil. However, no clear advantages in soybean P nutrition were observed when in rotation with these grasses in high-P soil. This study indicates that further advantages in soybean P nutrition after tropical grasses may be impeded by phytate, which is not readily available to plants.

Keywords: *Urochloa ruziziensis*, *Urochloa brizantha*, *Megathyrsus maximus*, Cover crops, Phosphorus pools, Organic phosphorus.

Introduction

Phosphorus (P) uptake by cash crops has been observed to increase after growing cover crops, such as *Lablab purpureus* and *Lupinus albus,* due to release of P by the straw decomposition (Horst *et al.*, 2001) or due to an increase in soil P bioavailable pools (Calegari *et al.*, 2013). Recently, it has been shown that ruzi grass (*Urochloa ruziziensis*) and palisade grass (*Urochloa brizantha*) can mobilize and take up soil recalcitrant P bound to iron (Fe) and aluminum (Al) (Merlin *et al.*, 2015). Under no-till, ruzi grass grown in the soybean (*Glycine max*) off-season enhances P cycling decreasing residual–P concentration in deeper

soil layers (Almeida & Rosolem, 2016). Cover crops may increase soil P availability to subsequent cash crops through an increase in the labile P, decrease of soil organic P (P_o), increase of P desorption kinetics, or by a simple release of readily available P during its decomposition and mineralization.

Organic acids exuded by roots may compete with P for adsorption sites and complex metals, inducing P desorption and solubilization in soil, and may also act as energy source to microorganisms (Hinsinger, 2001). Soil microbial biomass plays two main roles on soil P dynamics, not only as a main driver in mineralization of recalcitrant P_o, but also in the inorganic P (P_i) immobilization (Richardson *et al.*, 2001). The P_o is mainly composed of orthophosphate monoesters (Stutter *et al.*, 2015), which can be classified as labile monoesters such as the breakdown products of DNA, and non-labile monoesters such as inositol phosphates (Shears & Turner, 2007). Phytate is an inositol phosphate that can account for more than 70% of total P_o (Canellas *et al.*, 2004), being the least bioavailable P_o form due to its strong affinity to soil particles and fast precipitation as insoluble forms (Berg & Joern, 2006).

According to Syers *et al.* (2008), the factors controlling soil P availability to plants are the soil solution P concentration and soil P buffer capacity. The diffusion of P into roots is governed by the P concentration gradient between the bulk soil solution and the concentration next to the root surface (Roose & Kirk, 2009). The strong soil P adsorption capacity by Fe and Al oxides in highly weathered soils results in lower soil P solution concentration, and reduce the P diffusion flux (Raghothama & Karthikeyan, 2005). However, growing adapted species in low-P soils may affect the P resupply by the soil solid phase due to changes in the soil P pools (Almeida *et al.*, 2018).

A closer look into P dynamics in the rhizosphere of tropical grasses is needed to achieve a better understanding of the potential of these grasses in inducing P cycling. The objective of this study was to evaluate the effect of tropical grasses on soil P dynamics and availability to soybean as a subsequent crop, to test the hypothesis that introducing grass species in the cropping system may affect soil P availability to soybean according to soil P concentration. Namely, this work aims to unravel how different cover crop grasses will affect: a) soil P pool distribution; b) P_i lability and desorption kinetics; and c) P_o dynamics, especially those involving phytate.

Materials and methods

The approach used a greenhouse experiment with soil taken from plots of a long-term experiment in Botucatu, Brazil (22º50′00″ S; 48º25′31″ W; altitude of 806 m), where soybean [*Glycine max* (L.) Merrill] had been cropped since 1998. The soil is a Rhodic Hapludox (Soil Survey Staff, 2014) with 67% sand and 21% clay. For the experiment, soil was collected from the 0–0.20 m depth, and accommodated in 9 L plastic pots.

The experimental design was a 2 × 3 factorial in randomized complete blocks, and two control treatments, with eight replications. The treatments consisted of two soil P levels, three grass species, and non-cultivated controls without grasses. The soil P levels were characterized as low, for the soil that did not have P fertilizer added; and high, which had received a total of 305 kg/ha of P as triple superphosphate (TSP) from 2001 to 2014. The grass species were ruzi grass [*Urochloa ruziziensis* (R. Germ. and C.M. Evrard) Morrone and Zuloaga], palisade grass [*U. brizantha* (A. Rich.) R.D. Webster], and Guinea grass [*Megathyrsus maximus* (Jacq.) B.K. Simon and S.W.L. Jacobs].

Conducting the experiments

Five grass plants were grown in pots for 60 days and then desiccated with glyphosate, simulating the usual desiccation management in field. Pots from four replicates were disassembled to evaluate grass shoots, roots, and rhizosphere soil, while the pots from remaining four replicates were maintained intact after grass desiccation. Rhizosphere soil was considered the soil adhered to the roots, and was gently separated by hand-shaking. Fifteen days after desiccation, grass shoots from the intact pots were chopped into pieces and accommodated on the soil surface, and 6 seeds of soybean were sown. After thinning, two soybean plants were grown per pot up to flowering.

Soil chemical characterization

For all soil analyses, soil samples were air-dried and passed through a 2-mm sieve. The soils were initially analyzed for chemical characterization (Table 1). Soil available P was extracted using pearl resin (Resin–P), as well as calcium (Ca), magnesium (Mg), and potassium (K), according to Raij *et al.* (2001). Soil pH in $CaCl_2$, soil organic matter (SOM), potential acidity (H+Al), and cation exchange capacity (CEC) were determined according to Raij *et al.* (2001).

Phosphorus fractionation

Soil P fractionation was performed according to the method of Hedley *et al.* (1982) with the modifications proposed by Condron & Goh (1989). Briefly, 0.5 g of air-dried soil was subjected to the following sequential extraction: anion exchange resin (AER) strips; sodium bicarbonate ($NaHCO_3$), 0.5 mol/L; sodium hydroxide, 0.1 mol/L (0.1 NaOH); hydrochloric acid, 1 mol/L (HCl); and sodium hydroxide, 0.5 mol/L (0.5 NaOH). After extraction, 0.1 g of the soil was subjected to digestion (HNO_3 + $HClO_4$) for the extraction of residual P. In acid extracts obtained with AER, HCl, and nitroperchloric digestion, the following P fractions

were analyzed: AER–P, HCl–P, and Residual–P, respectively. The alkaline extracts were divided into two aliquots. In the first aliquot of each alkaline extract, the following inorganic P (P_i) fractions were obtained: $NaHCO_3$–P_i, 0.1 NaOH–P_i, and 0.5 NaOH–P_i. The second aliquot was subjected to digestion with ammonium persulfate and sulfuric acid in an autoclave to determine the total P (P_t) content. The molybdate unreactive P was calculated as the difference between P_t and P_i and was here termed as organic P (P_o). Thus, the following extracted P fractions were obtained: $NaHCO_3$–P_t, $NaHCO_3$–P_o, 0.1 NaOH–P_t, 0.1 NaOH–P_o, 0.5 NaOH–P_t, and 0.5 NaOH–P_o. An analytical triplicate was used throughout the fractionation analysis. The sum of all extracted P fractions was labeled Total–P. The data was expressed as the change (Δ) for each P fraction between the samples collected before (time 0) and after grass growth.

Phytase labile phosphorus

Phytase labile P was assayed in soil extracts with 0.25 mol/L NaOH plus 0.05 mol/L ethylenediaminetetraacetic acid (NaOH-EDTA). Samples of 2 g of air dried soil were extracted with 20 mL of extractant on a reciprocal shaker for 16 h. Phytase labile P ($P_{Phy\text{-}lab}$) was determined using a commercially available phytase (Natuphos, EC 3.1.3.8; BASF SE, Ludwigshafen, Germany). Briefly, soil extracts (100 µL) were combined with 100 µL of phytase (100 nKat/mL) diluted in a buffer (50 mmol/L acetate, pH 5.5) and incubated at 37ºC for 16 h. Organic P hydrolysable by phytases was inferred by the difference of P_i content measured after the incubation of samples with phytase and samples with denatured phytase.

Similarly, to the fractionation analysis, P_t, P_i and P_o extracted by NaOH-EDTA were also assayed. The difference between NaOH-EDTA–P_t and NaOH-EDTA–P_i corresponds to NaOH-EDTA–P_o. The change (Δ) in these parameters to the initial concentration and the changes in percentage of $P_{phy\text{-}lab}$ in relation to P_o were calculated.

Microbial biomass phosphorus

The microbial biomass P (MBP) was determined according to Stutter *et al.* (2015) and references cited therein. Samples of 80 g of soil were placed in a container and wetted with ultra-pure Milli-Q (MQ) water to approximately 50% water holding capacity to re-establish microbial activity, and incubated for 72 h. After incubation, the soil slurry was prepared by adding MQ water and mixing the soil until maximum retention was reached. Quadruplicates of soil slurry (1 g of dry weight equivalent) were extracted for 16 h in 10 mL of MQ water with AER strips either with or without addition of 0.4 mL hexanol. After 16 h, the resins were eluted with 0.5 mol/L HCl and the concentration of P was measured. The MBP was estimated as the difference between samples extracted with and without hexanol. A correction factor to account for sorption of P to soil solid phase was determined from soil samples spiked with 20 mg/g of P.

Diffusive gradient and equilibrium in thin films

Soil labile P was measured using diffusive gradient in thin films (DGT) and soil solution P was assessed using diffusive equilibrium in thin films (DET) as in Menezes-Blackburn *et al.* (2016b) and references cited therein. A binding layer containing ferrihydrite was used for the DGT test. More information about the preparation of the diffusive and binding layer are published in Menezes-Blackburn *et al.* (2016b).

The DET devices were deployed in soil slurry prepared as in MBP analysis. On the next day, the DGT devices were deployed for 48 h. The DGT and DET devices were deployed in duplicates for each experimental replicate. The diffusive and binding layers were eluted in 0.5 mL of H_2SO_4 solution, 0.25 mol/L. The concentration of P in the diffusive layer of DET devices (P_{DET}) is expressed as the equilibrium concentration to soil solution P. The

concentration of labile P (P_{DGT}) at the surface of the DGT devices was calculated using eq. 1 (Zhang & Davison, 1995).

$$P_{DGT} = \frac{M\Delta g}{DAt} \quad (1)$$

where M is the accumulated P mass in the binding layer, A is the surface area of the DGT sampling window, t is the deployment time, Δg is the total thickness of the diffusive gel layer and the filter membrane, and D is the diffusion coefficient of P in the diffusive gel. The P_{DGT} could be converted to an effective concentration (P_E) using eq. 2 (Zhang *et al.*, 2001).

$$P_E = \frac{P_{DGT}}{R_{diff}} \quad (2)$$

The diffusive only ratio (R_{diff}) between P_{DGT} and soil solution P (P_{DET}) was calculated using a dynamic numerical model (DIFS) (Harper *et al.*, 2000). The ratio (R) of measured P_{DGT} concentration to the P_{DET} was calculated as in eq. 3.

$$R = \frac{P_{DGT}}{P_{DET}} \quad (3)$$

The relative resupply from solid phase ($R-R_{diff}$) was calculated subtracting the R_{diff} from the R ratio. Using the DIFS model, the T_c was also obtained.

Plant analysis

The grass biomass was harvested, and roots were separated from shoots. Soybean was harvested at flowering (after 53 days of emergence). The remaining grass straw on the soil surface was also collected. All plant material was dried at 65ºC to determine dry mass. Concentration of P in plant materials was determined according to Jackson (1973). The P released from grass straw was measured by the difference on P accumulated in the grass shoot right after desiccation and 53 days after. The decomposition of grass straw was based on the difference of shoot dry weight of grass after desiccation and after 53 days.

Statistical analysis

Data were subjected to ANOVA considering a 2 × 3 factorial in randomized complete blocks, with four replications, and means were compared using Tukey's test ($p < 0.05$). To allow a better comparison of the grass effect, the control treatment was not included in ANOVA and Tukey's test. Instead, a second ANOVA was performed, considering a 2 × 4 factorial in randomized complete blocks, with four replications, and Dunnett's test ($p < 0.05$) was used to compare the significance of the difference between different treatments and the respective controls. All the statistical analyses were performed using SAS software (SAS Inst., North Carolina, U.S.).

Results and discussion

Soil phosphorus pools: distribution and availability

The calculated change (Δ) in soil P concentrations in each pool before and after grass cultivation can be interpreted as a decrease in the P pool (negative Δ values). This indicates that P was transferred to other pools or taken up by the grasses, while an increase in the P pool (positive Δ values) indicates that P was accumulated into this pool (Table 2). Great changes in P pools were observed, since the Δ was obtained from the rhizosphere soil, a particular region where the effect of roots and microorganisms is much higher than in the bulk soil.

Growing grasses depleted P_i in the firsts extracts of the sequential fractionation (AER–P, $NaHCO_3$–P_i) (Table 2), which are considered the most available soil P fractions (Cross & Schlesinger, 1995). Depletion of these P fractions had also been observed in the rhizosphere of *Zea mays*, *Lablab purpureus,* and *Mucuna pruriens* (Horst *et al.*, 2001). The decrease in Resin–P in the control treatments with high P level results from the equilibrium between soil solution P and P in the solid phase. Soil P_i equilibrium is a dynamic combination

of sorption and desorption processes, which transfer P_i between the solid and solution phases. The control treatment with low P level had a small increase in AER–P; possibly, this was a consequence of the MBP mineralization (Macklon *et al.*, 1997).

The sum of P_o fractions extracted with $NaHCO_3$, 0.1 and 0.5 NaOH accounted for 46% to 56% of total soil P fractions when Residual–P was not considered. Organic P accounted for approximately 60% and 54% of total soil P extracted with NAOH-EDTA, for soils with low and high P levels, respectively. The high proportions of P_o in the soil suggest the importance of the mobilization of this fraction to improve plant P nutrition (Rodrigues *et al.*, 2016).

The simple incubation of moist uncultivated soil of the control treatments at greenhouse conditions caused a significant change in P_o pools, indicating an active microbial role in redistributing these fractions. These uncultivated control samples behaved remarkably different at different P levels: while in the low-P soils there was a movement from residual P towards more labile pools, the opposite was observed at high P levels, where P was continuously fixed into this less labile pool (Residual-P). Conversely, the presence of plants changed these trends, causing a depletion of Residual–P and an increase of alkali soluble P_o fractions in the rhizosphere (Table 2). The HCl-extractable P was decreased in the low-P soil and it was increased in the high-P soil compared with the initial concentrations, regardless of the effect of grass cultivation.

Despite the fact that Residual–P may be considered as a combination of inorganic and organic stable P forms strongly associated with the mineral fraction, P re-mobilization was mainly observed in Residual–P due to grass cultivation. Several studies have shown that Residual–P can be depleted by plant uptake (Almeida *et al.*, 2018).

When grasses were grown, there was an increase in P_o pools extracted with $NaHCO_3$, and 0.5 mol/L NaOH (Table 2), which is in accordance with the observations in field studies with crop rotations under no-till (Almeida & Rosolem, 2016; Rodrigues *et al.*, 2016), and in pot studies (Horst *et al.*, 2001). However, for soil with high P level, the P_o extracted with 0.1 mol/L NaOH was decreased by the grasses. According to Beck & Sanchez (1994), NaOH-extractable P_o is an important source of P in weathered soils that have not had P fertilizer added, which may have contributed to the decrease of this fraction observed in the soil with high P level in the present study. Studies have shown that phytate content in soils is frequently found to be major fraction of P_o and may account for the most part of total P_o (Canellas *et al.*, 2004), however here the $P_{Phy\text{-}lab}$ was approximately 23% and 13% of P_o extracted with NAOH-EDTA in the present study, for the low- and high-P soils, respectively. Chapuis-Lardy *et al.* (2001) reported that in Cerrado oxisols P_o appears to be mainly in form of stable monoesters in the $NaHCO_3$ and 0.5 NaOH extracts. Therefore, the increase in P_o observed in $NaHCO_3$ and 0.5 NaOH extracted from P fractionation in this experiment may be related to the increase in $P_{Phy\text{-}lab}$, which is poorly labile.

Since the $P_{Phy\text{-}lab}$ increased in all the treatments, soil microorganisms could have desorbed the recalcitrant P_o including phytate, increasing $P_{Phy\text{-}lab}$, where no changes in phytate content were expected, since no plants were grown. The decrease in the Residual–P and the increase in P_o pools is an indication that recalcitrant P_o forms from Residual–P changed to less recalcitrant P forms in the P_o pools. These changes could turn phytate or other recalcitrant organic compounds to forms more accessible by phytases, increasing the $P_{Phy\text{-}lab}$. Although the interaction of phytate with inorganic soil compounds has been much studied, less is known about phytate interactions with the soil organic compounds (Nanny & Minear, 1994). The increased $P_{Phy\text{-}lab}$ could indicate an important possible way to further improve P nutrition of subsequent crops. If soybean or grasses had mechanisms to a higher access the

$P_{Phy\text{-}lab}$ pool, possibly even higher increases in soybean P uptake and dry mass could have been observed.

In the soil with high P level, a possible competition of grass roots with microorganisms occurred, since MBP was lower in the soil cultivated with grasses than in the control (Table 4). The MBP was higher in the high P control than in the low P control, indicating that P is a limiting nutrient for the microbes in these soils.

Inorganic phosphorus lability and desorption kinetics

The low Total–P content observed in the present study is in agreement with the range of Total–P observed in similar weathered soils from Cerrado by Chapuis-Lardy *et al.* (2001), from 301 to 456 mg/kg in natural and pasture soils, respectively. Because of low Total–P content and high adsorption P capacity, the most labile P pools are low, and the P_{DET} in the low-P soil was below the limit of detection by the malachite green method (1 µg/L). Since the P_{DET} was not measured, it was not possible to calculate a series of other parameters, such as K_d, R, R-R_{diff}, and T_c, for the soil with low P level. Therefore, Table 5 shows only results of these parameters for soil with high P level.

The contrasting differences observed in Resin–P, P_{DGT}, and P_E is not a surprise (Mason *et al.*, 2010). In the present study, P concentration extracted with resin was lower than the control treatment in the high-P soil; however, no differences were related to soybean plants grown on soil previously cultivated or not with grass, in the soil with high P level (Table 5). In soil with a lower P level, Resin–P was also not related with soybean response to P availability. In the high P soil, the results obtained with DGT corresponded with the response of soybean to P, which indicates a higher accuracy of this method to predict P availability than resin (Mason *et al.*, 2010). However, in the low-P soil, P_E was negatively affected by grasses, while soybean P uptake was higher after grasses than in the control. This

contrasting response of soybean plants and P_E may be a consequence of the different mechanisms to induce P mobilization by soybean plants, as root exudation and microbial growth stimulation (Hinsinger, 2001; Richardson *et al.*, 2001), and also due to P release from cover crop residues (Horst *et al.*, 2001).

The calculated ratio (R) between P_{DGT} and P_{DET} resulted in higher values than the most part of soils analyzed by Menezes-Blackburn *et al.* (2016b), meaning that in this highly weathered soil, the contribution of P_i diffusion is small compared with the replenishment of pore water P_i, due to its desorption from the solid phase. The R_{diff} is the hypothetical ratio of the P_{DGT} to the concentration in the soil solution if no resupply occurs (only pore water P diffusion). Since R_{diff} was low, $R\text{-}R_{diff}$ was the dominant component on plant P bioavailability. Surprisingly, T_c values were low, and also different from those of the temperate soil samples analyzed by Menezes-Blackburn *et al.* (2016b), underlying the importance soil-P buffering capacity in this system. The soil used in this study has a very interesting P desorption behavior, because while soil solution P is low, P resupply to soil solution is fast, evidenced by the high K_d. This suggests that these soils can adsorb a high amount of P with low energy, despite oxisols being known to have a very high P sorption capacity. Nevertheless, it is worth noticing that the experimental soil has more than 60% of sand particles, which may have contributed to the low P adsorption energy.

As observed by Nunes *et al.* (2008), Guinea grass has a high P demand. Growing Guinea grass resulted in a lower content of P_{DET} than the other species in the high P soil, and consequently, P availability was even more dependent from the resupply from the solid phase, and the sorption rate constant was 10 times higher in the soil cultivated with Guinea grass than with the other species. However, the decrease in P_{DET} resulted in an increased gradient between the soil P in solution and the solid phase (K_d), leading to a much smaller T_c.

Plant growth and phosphorus uptake

Soil P was a limiting factor only to Guinea grass, which makes sense because ruzi grass and palisade grass are better adapted to P-poor soils (Rao *et al.*, 1996). In the high P soil, the smaller dry mass of Guinea grass compared with ruzi grass and palisade grass was probably due to the lower P uptake (Table 6). The higher demand for P by Guinea grass than the Urochloa species is possibly due to its low capacity to mobilize less labile P forms.

Several plant species have been reported to exude compounds that increase soil P bioavailability into the rhizosphere, due to solubilization and mineralization of P_o, such organic acids and phosphatases (Hinsinger, 2001). It has been reported that ruzi grass and palisade grass are able to exude high amounts of organic acids (Ishikawa *et al.*, 2000) that may stimulate rhizosphere microorganisms (Menezes-Blackburn *et al.*, 2016a). Urochloa species are highly adapted to low soil P fertility, however, the mechanisms responsible need further research. Several soil microorganisms can produce phosphatases, and the relationship between plants and these microorganisms is important in the soil P cycle, in order for plants to acquire P from soil recalcitrant P sources (Richardson *et al.*, 2001). According to Hayes *et al.* (2000), extractable P_o increases with citric acid concentration, which could enhance P availability in the rhizosphere. Guinea grass seems to not be able to feed rhizosphere microorganisms as the other species, which also accounts for a lower shoot P accumulation in this species. The DIFS derived parameters also reflect the higher P demand by Guinea grass, depleting P in the soil solution, impairing plant P acquisition and P resupply, and eventually limiting the proliferation of microorganisms due to P competition.

There was no difference in the total P released from the straw of palisade grass and the other grasses (Table 6). The P re-mobilization by grasses, which resulted in the decrease of the Residual–P and supply of P during the grass straw decomposition, seems to be the factor responsible for the higher soybean dry matter yield and P uptake than in the soil kept

fallow, as observed by Horst *et al.* (2001) growing *Lablab purpureus*. According to Canellas *et al.* (2004), the addition of crop residues on the soil surface results in increased soil diester P, which is considered a labile P form. This effect was observed only in the low-P soil, indicating that P fertilizer application created a P sufficiency condition that overcame the effect of grasses in increasing P bioavailability to soybean. In the soil with high P level, even with the P re-mobilization due to grass growth, and the large increase of the Residual–P in the control treatment, soybean P uptake was not affected. The large differences in DIFS derived parameters observed between grass species in the soil with high P level were not reflected in differences in soybean P uptake, since this soil shows a high capacity to resupply P to soil solution.

Almeida & Rosolem (2016) have shown that ruzigrass increases labile soil P forms in the long-term; however, the authors were not able to determine if the increase resulted either from changes in the less labile P pools or by deposition of P from ruzigrass residues. In the present study, analyzing the grasses effects in the short-term, the contribution of P deposition from grass residues seems to be the main factor improving P availability to soybean in low-P soil. A closer look into P dynamics in the rhizosphere of tropical grasses revealed a depletion of labile P forms, which may result in higher P adsorption capacity and lower soil P desorption, as observed by Almeida *et al.* (2018) and also here through a lower P_E concentration in low-P soil. According to Almeida *et al.* (2018), ruzigrass should result in the accumulation of recalcitrant P_o forms in soil. In the present study, it was observed that tropical grasses and microorganisms may expose recalcitrant P_o forms, resulting in increased concentration of these P_o forms, and showing that a great improvement in P availability may depend not only on the release of P from grass residues, but also on the mineralization of recalcitrant P_o forms such as phytate.

Conclusions

The soil P pool distribution is highly affected by tropical grasses grown as cover crops. Ruzi grass, palisade grass or Guinea grass increased P cycling, decreasing the less available P forms, regardless of soil initial P level. Grasses seems to solubilize recalcitrant P_o forms, exposing phytates, and consequently increasing non-labile P concentration. The soil used in this study showed a capacity to resupply P to soil solution quickly, even with a very high P sorption. Nevertheless, changes in P desorption kinetics did not seem to explain the observed differences in P uptake by soybean.

The supply of P by decomposition of grass residues is the key factor to improve soybean P nutrition, and consequently increase soybean yield. When the soil P concentration is higher due to P fertilizer application, no clear advantages in soybean P nutrition were observed when in rotation with these grasses, rejecting the hypothesis that grass species improve the subsequent soybean P uptake in high-P soil.

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Table 1 Selected chemical characteristics of initial soil samples collected before grasses grown, as a function of soil phosphorus (P) level.

	Soil P level	
	Low P level	High P level
Chemical soil characterization		
pH[a]	5.5	5.7
	------- mg/kg -------	
Resin–P[b]	8	19
	--------- g/kg --------	
SOM[c]	19	18
	----- $mmol_c/kg$ -----	
H+Al[d]	12.5	13.2
K	2.0	1.1
Ca	24	22
Mg	0.16	0.14
CEC[e]	39	36

[a]Soil pH measured in calcium chloride solution.
[b]Phosphorus extracted with pearl resin.
[c]Soil organic matter.
[d]Potential acidity.
[e]Cation exchange capacity.

Table 2 Changes (Δ) in soil phosphorus (P) fractions content between soils sampled before (Time 0) and after grasses growth, as a function of soil P level and grass species, and a control treatment with soil kept fallow.

	Grass species					
Soil P level	Ruzi grass	Palisade grass	Guinea grass	Average	Control	Time 0
	---------- AER–P (mg/kg) ----------					
Low P level	-0.30 Ab[a,]*	-0.55 Ac*	-0.13 Aa*	-0.32	1.31	5.3
High P level	-1.85 Bab	-2.46 Bb	-1.31 Ba*	-1.87	-2.26	11.9
Average	-1.08	-1.50	-0.72			
	---------- $NaHCO_3$–P_i (mg/kg) ----------					
Low P level	-3.05	-1.73	-2.21	-2.33	-1.56	8.6
High P level	-2.57	-2.29	-2.91	-2.59	-2.79	9.3
Average	-2.81	-2.01	-2.56			
	---------- $NaHCO_3$–P_o (mg/kg) ----------					
Low P level	5.04	3.53	3.30	3.96 A	2.60	6.5
High P level	2.02*	1.63*	1.48*	1.71 B	-1.57	8.7
Average	3.53	2.58	2.39			
	---------- 0.1 NaOH–P_i (mg/kg) ----------					
Low P level	-15.65 Bb*	-13.63 Bab*	-8.88 Ba	-12.72	-8.62	37.4
High P level	0.73 Aab	5.83 Aa	-0.59 Ab	1.99	-8.81	34.3
Average	-7.46	-3.90	-4.73			
	---------- 0.1 NaOH–P_o (mg/kg) ----------					
Low P level	17.98	14.62	13.20	15.27 A	14.48	55.5
High P level	-12.06*	-10.69*	-9.82*	-10.86 B	-33.16	93.4
Average	2.96	1.96	1.69			
	---------- HCl–P (mg/kg) ----------					
Low P level	-2.05	-2.50	-2.60	-2.38 B	-1.89	13.8
High P level	1.76	0.54	0.90	1.07 A	1.38	12.6
Average	-0.14	-0.98	-0.85			
	---------- 0.5 NaOH–P_i (mg/kg) ----------					
Low P level	-4.27	-1.12	-3.86	-3.08	-2.57	37.2
High P level	0.64	-2.84	-1.65	-1.28	0.83	35.9
Average	-1.82	-1.98	-2.75			
	----------0.5 NaOH–P_o (mg/kg) ----------					
Low P level	11.03	10.50	9.14	10.23 A	7.97	26.2
High P level	2.29*	1.22*	3.84*	2.45 B	-17.74	32.2
Average	6.66	5.86	6.49			
	---------- Residual–P (mg/kg) ----------					
Low P level	-34.66*	-35.95*	-23.95*	-31.62 B	-14.26	162.9
High P level	-21.75*	-19.93*	-18.64*	-20.21 A	58.49	158.1
Average	-28.21	-27.94	-21.30			

[a]Average followed by the same lowercase letter in the line and uppercase in the column were not significantly different, as a function of soil P level and grass species (Tukey, $p < 0.05$); *Indicates a significant difference between each treatment and the control treatment (Dunnett, $p < 0.05$).

Table 3 Changes (Δ) in soil phosphorus (P) concentration extracted with sodium hydroxide and Ethylenediaminetetraacetic acid (NaOH-EDTA), and soil phytase labile P ($P_{phy\text{-}lab}$) concentration and changes in percentage of $P_{phy\text{-}lab}$ according to organic P extracted with NaOH-EDTA (NaOH-EDTA–P_o). Changes calculated between soils sampled before (Time 0) and after grasses growth, as affected by soil P level and grass species, and a control treatment with soil kept fallow.

	Grass species					
Soil P level	Ruzi grass	Palisade grass	Guinea grass	Average	Control	Time 0
	---------- NaOH-EDTA–P_i (mg/kg) ----------					
Low P level	-3.36	-1.70	-1.42	-2.16 A[a]	-2.61	25.9
High P level	-14.08	-13.08	-13.11	-13.42 B	-14.00	50.9
Average	-8.72	-7.39	-7.26			
	---------- NaOH-EDTA–P_o (mg/kg) ----------					
Low P level	1.07	1.66	1.25	1.33 A	2.28	39.6
High P level	-2.11	-3.71	-5.47	-3.76 B	-2.70	58.6
Average	-0.52	-1.03	-2.11			
	---------- Phytase labile P (mg/kg) ----------					
Low P level	5.46 Aa	3.72 Aab	2.48 Bb	3.73	5.32	5.24
High P level	3.17 Aa	4.77 Aa	5.30 Aa	4.41	3.31	3.44
Average	4.31	4.24	3.66			
	---------- Phytase labile P (%) ----------					
Low P level	9.87 Aa	7.21 Aab	4.47 Ab*	7.19	9.82	13.23
High P level	7.45 Ab	10.97 Aab	12.52 Ba*	10.31	7.21	5.87
Average	8.66	9.09	8.50			

[a]Average followed by the same lowercase letter in the line and uppercase in the column were not significantly different, as a function of soil P level and grass species (Tukey, $p < 0.05$); *Indicates a significant difference between each treatment and the control treatment (Dunnett, $p < 0.05$).

Table 4 Microbial biomass phosphorus (P), as affected by soil P level and grass species, and a control treatment with soil kept fallow.

Soil P level	Grass species			Average	Control
	Ruzi grass	Palisade grass	Guinea grass		
	---------- Microbial biomass P (mg/kg) ----------				
Low P level	0.63	0.54	0.51	0.57	0.60
High P level	0.70*	0.66*	0.42*	0.72	1.15
Average	0.67 a[a]	0.60 ab	0.44 b		

[a]Different letters in rows indicate significant differences (Tukey, $p < 0.05$);
*Indicates a significant difference between each treatment and the control treatment (Dunnett, $p < 0.05$).

Table 5 Soil available phosphorus (P) extracted with pearl resin (Resin–P), labile P (P_{DGT}) and effective phosphorus concentration (P_E), as affected by soil P level and grass species, and a control treatment with soil kept fallow. Soil solution P (P_{DET}), resupply potential (K_d), and response time of the system (T_c) as a function of grass species, and a control treatment with soil kept fallow, in soil with high P level.

Soil P level	Grass species			Average	Control
	Ruzi grass	Palisade grass	Guinea grass		
	---------- Resin–P (mg/kg) ----------				
Low P level	6.9	6.8	6.8	6.8 B[a]	7.8
High P level	10.2*	9.3*	10.2*	9.9 A	13.0
Average	8.6	8.0	8.5		
	---------- P_{DGT} (µg/L) ----------				
Low P level	2.2	1.9	2.2	2.1 B	3.7
High P level	8.8	8.7	8.9	8.8 A	8.6
Average	5.5	5.3	5.6		
	---------- P_E (mg/L)----------				
Low P level	0.10*	0.07*	0.10*	0.11 B	0.17
High P level	0.41	0.40	0.41	0.41 A	0.40
Average	0.25	0.24	0.26		
	---------- P_{DET} (µg/L) ----------				
High P level	30.7 a	31.7 a	15.3 b*	27.8	33.5
	---------- K_d (cm^3/g) ----------				
High P level	340 b	299 b	682 a*	421	362
	---------- R ----------				
High P level	0.30 b	0.28 b	0.60 a*	0.37	0.30
	---------- R-R_{diff} ----------				
High P level	0.27 b	0.26 b	0.58 a*	0.35	0.28
	---------- T_c (s^{-1}) ----------				
High P level	485 a	384 a	47 b*	391	648

[a]Average followed by the same lowercase letter in the line and uppercase in the column were not significantly different, as a function of soil P level and grass species (Tukey, $p < 0.05$);
*Indicates a significant difference between each treatment and the control treatment (Dunnett, $p < 0.05$).

Table 6 Grass shoot, root, and total dry matter, grass shoot phosphorus (P) uptake before desiccation, P release from grass straw 60 days after desiccation, and decomposition of grasses straw, as affected by soil P level and grass species. Soybean shoot dry matter and shoot P uptake, as affected by soil P level and grass species, and a control treatment with soil kept fallow.

	Grass species				
Soil P level	Ruzi grass	Palisade grass	Guinea grass	Average	Control
	---------- Grasses shoot dry matter (g/pot) ----------				
Low P level	22.8	22.3	19.3	21.5	-
High P level	22.2	21.9	21.0	21.7	-
Average	22.5 a[a]	22.1 a	20.2 b		
	---------- Grasses root dry matter (g/pot) ----------				
Low P level	8.4	9.1	7.9	8.5 B	-
High P level	11.6	10.7	10.1	10.8 A	-
Average	10.0	9.9	9.0		
	---------- Grasses total dry matter (g/pot) ----------				
Low P level	31.2 Aa	31.4 Aa	27.1 Bb	29.9	-
High P level	33.7 Aa	32.6 Aa	31.13 Ab	32.5	-
Average	32.5	32.0	29.2		
	---------- Grasses shoot P uptake (mg/pot) ----------				
Low P level	23.0 Ba	23.3 Ba	15.0 Bb	20.4	-
High P level	30.6 Aa	33.4 Aa	29.4 Aa	31.1	-
Average	26.8	28.3	22.2		
	---------- P released from grass straw (mg/pot) ----------				
Low P level	5.12	4.98	2.06	4.05 B	-
High P level	5.78	6.26	6.24	6.09 A	-
Average	5.45	5.62	4.15		
	---------- Grasses decomposition (%) ----------				
Low P level	32.3	23.9	31.4	29.2	-
High P level	30.1	19.9	29.3	26.4	-
Average	31.2 a	21.9 b	30.4 a		
	---------- Soybean shoot dry matter (g/pot) ----------				
Low P level	10.2 Bb*	13.7 Ba*	11.2 Bab*	11.7	7.0
High P level	25.9 Aa	23.9 Aa	26.7 Aa	25.5	25.8
Average	18.1	18.8	18.9		
	---------- Soybean shoot P uptake (mg/pot) ----------				
Low P level	16.9*	18.6*	16.3*	17.3 B	12.7
High P level	39.0	37.3	39.1	38.5 A	39.3
Average	27.5	28.0	27.6		

[a]Average followed by the same lowercase letter in the line and uppercase in the column were not significantly different, as a function of soil P level and grass species (Tukey, $p < 0.05$); *Indicates a significant difference between each treatment and the control treatment (Dunnett, $p < 0.05$).