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"Luiz de Queiroz" College of Agriculture

Influence of filter cake associated with mineral phosphate sources on soil phosphorus dynamics in sugarcane

Thaís de Marchi Soares

Thesis presented to obtain the degree of Doctor in Science. Area: Soil and Plant Nutrition

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versão revisada de acordo com a Resolução CoPGr 6018 de 2011

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RESUMO

Influência da torta de filtro associada a fontes fosfatadas na dinâmica do fósforo no solo em cana-de-açúcar

Aumentar a eficiência do uso do fósforo (P) é um desafio em solos tropicais devido à alta capacidade de adsorção de P, exigindo altos aportes de P via fertilizantes solúveis para alcançar produtividade aceitável. O uso da torta de filtro (TF), subproduto da indústria sucroenergética, tem apresentado bons resultados no aumento da eficiência do uso de P em curto prazo. O objetivo foi avaliar o efeito a longo prazo da associação de TF com fontes minerais de P - superfosfato triplo (SFT) e fosfato de rocha (FR) - na disponibilidade e dinâmica de P no solo. Para acompanhar a absorção do P liberado na solução do solo, no Capítulo 2 foi desenvolvido um método para quantificar P em folhas de cana-de-açúcar *in vivo* usando espectroscopia de fluorescência de raios-X portátil (pXRF). No Capítulo 3, o efeito da associação da TF com SFT e FR na liberação e dinâmica de P foi avaliado em casa de vegetação. Os tratamentos foram: TF, SFT, FR, SFT+TF (35:65), FR+TF (35:65). O teor de P nas folhas (medido por pXRF), a concentração de P na solução e os compartimentos de P no solo mostraram que o uso da TF afetou positivamente o P lábil e moderadamente lábil, aumentou a solubilização do FR e o teor de P nas folhas, possibilitando reduções do uso de SFT. No Capítulo 4, a cinética de liberação de P pelos tratamentos foi acessada por 10 dias usando um sistema de perfusão em coluna, e a mobilidade e labilidade do P em solo ácido (com e sem calagem) e alcalino, foram avaliadas em solo incubado por 5 semanas. A TF apresentou rápida taxa de liberação de P e a mineralização/dissolução foi facilitada no solo ácido. No solo com calagem, a redução do P aplicado via SFT em SFT+TF não afetou a difusão e disponibilidade de P. Nesta condição de solo, a TF afetou positivamente a difusão do P em FR+TF. Em geral, não foi observado efeito da TF no solo alcalino. Nos Capítulos 5 e 6, avaliou-se o efeito a longo prazo da TF (10 Mg ha^{-1}) associada à SFT e FR (dose de 90 e 180 kg ha^{-1} P_2O_5) na dinâmica do P no solo e no desempenho da cana-de-açúcar, em Agudos (solo arenoso) e em Macatuba (solo argiloso). Em ambos os solos, a TF associada às fontes de P reduziu sua conversão em formas pouco disponíveis no solo e aumentou a aquisição de P pelas plantas. Em Agudos, FR+TF (180 kg ha^{-1} P_2O_5) foi tão eficiente quanto SFT (+ e - TF), em aumentar o P no compartimento lábil e a produtividade, mostrando que o FR pode substituir o TSP. Por outro lado, em Macatuba, apenas SFT+TF (180 kg ha^{-1} P_2O_5) aumentou o P disponível e na última safra apenas TSP, em ambas as doses testadas, teve desempenho melhor que a testemunha. A associação da TF com SFT e FN apresentou diferentes efeitos na liberação e dinâmica de P no solo e no desempenho da cana-de-açúcar dependendo da condição do solo.

Palavras-chave: Subproduto, Superfosfato triplo, Fosfato reativo, *Saccharum spp*

ABSTRACT

Influence of filter cake associated with mineral phosphate sources on soil phosphorus dynamics in sugarcane

Increasing the phosphorus (P) use efficiency is a challenge in tropical soils because of its high P adsorption capacity, leading to excessive P inputs via water-soluble fertilizers to achieve acceptable yields. The use of filter cake (FC), a byproduct of the sugarcane industry, has shown good results in increasing the P use efficiency in the short term. We aimed to evaluate the long-term effect of FC associated with mineral P sources - triple superphosphate (TSP) and rock phosphate (RP) - on the P availability and dynamics in the soil. To track the uptake of P released in the soil solution, in Chapter 2 a method was developed to quantify P in sugarcane leaves *in vivo* using portable X-ray fluorescence (pXRF) spectroscopy. In Chapter 3, the release and dynamics of P from the association of FC with TSP, and RP, were evaluated in a greenhouse experiment. The treatments were: FC, TSP, RP, TSP+FC (35:65), and RP+FC (35:65). The P content in the leaves (measured by pXRF), the P concentration in the solution, and the P pools in the soil showed that the use of FC positively affected the labile and moderately labile P, and increased the RP solubility and the P content in the leaves, enabling reductions in the amount of TSP applied. In Chapter 4 the kinetics of P release by these treatments was accessed for 10 days using a column perfusion system, and the mobility, and lability of P in acidic (limed and non-limed) and alkaline soils, were evaluated in a 5-week soil incubation experiment. The FC showed a rapid P release rate and its mineralization/dissolution was facilitated in the acidic soil. In the acidic-limed soil, the reduction in the amount of P applied as TSP in TSP+FC did not negatively affect the P diffusion and availability. In this soil condition, the FC positively affects the P diffusion from RP+FC. Overall, the FC effect was not observed in the alkaline soil. In Chapters 5 and 6, was evaluated the long-term effect of FC (10 ton ha^{-1}) associated with TSP and RP (rates of 90 and 180 kg ha^{-1} P_2O_5) on the P dynamics in the soil and on the agronomic performance of sugarcane, at Agudos (sandy soil) and Macatuba (clayey soil). In both soils, FC associated with mineral P sources reduced the P conversion into low available P forms in the soil and increased the P uptake by plants. In Agudos, RP+FC (180 kg ha^{-1} of P_2O_5) was as efficient as TSP (+ and - FC), in increasing the labile pool of P and the yield, showing that RP can replace TSP. However, in Macatuba, only TSP+FC (180 kg ha^{-1} of P_2O_5) increased the available P, and in the last harvest only TSP, both doses tested, performed better than the control. Thus, the association of FC with TSP and RP had different effects on the P release and dynamics in the soil and on the sugarcane performance, depending on the soil condition.

Keywords: Byproduct, Triple superphosphate, Reactive phosphate, *Saccharum spp*

1. GENERAL INTRODUCTION

Phosphorus (P) is a relatively scarce macronutrient in terrestrial ecosystems, limiting crop yield in around 40% of the world's arable soils (Balemi and Negisho, 2012). Because of its complex dynamics and diversity of species in the soil (Kruse et al., 2015) even when the total P content is high, only a small portion (<1%) of the inorganic P (Pi) and organic P (Po) is available in the soil solution (0.05 to 0.30 μg P mL^{-1}) (Bolan, 1991; Bünemann, 2015).

Both, Pi and Po, can be strongly bound (or fixed) to the soil´s solid phase and thus, are found in a varied range of compounds with different solubility, reactivity, and bioavailability in the soil (Liu et al., 2014). The Pi compounds usually represent the greatest part of total P in the soil varying between 35 to 70% of the total P and are found predominantly in the soil minerals, and in small portions, as plant-available Pi in the soil solution (Frossard et al., 1995; Devau et al., 2010). The Po may constitute 30 to 35% of the total P in most soils (Sharpley, 1985), but it can be higher in grassland and forests (Achat et al., 2010). The Po, such as orthophosphate monoesters (inositol phosphates) and diesters (organic polyphosphates and phosphonates), is mineralized into forms available to plants by microorganisms (Zhu et al., 2018).

Two theories can explain the nature of phosphate in the soil: the precipitate-particulate, and the adsorption-penetration. Although the former has been questioned (Barrow et al., 2022), both theories are still well-accepted (Penn and Camberato, 2019). Thus, P is found in soil precipitated with iron (Fe), aluminum (Al), or calcium (Ca), inner and out-sphere adsorbed to the surface of Fe and Al oxides and the edges of clay minerals, in solution (HPO_4^{2-} or $H_2PO_4^{-}$), in organic components, or as part of insoluble compounds (including rock minerals from the parent material and organic matter) (Dollard and Billard, 2003; Khan et al., 2014; Barrow et al., 2022).

The pH is one of the soil parameters which determines its ability to retain P (Kana et al., 2011). The maximum P availability for plants occurs at pH between 5.5 and 7.0. Increasing the pH leads to P precipitation as Ca-phosphates, while its reduction induces the formation of Fe and Al phosphates (Wright, 2009). In addition to pH, the type and amount of minerals in the clay fraction also determines the P species (Kana et al., 2011; Shigaki and Sharpley, 2011). In tropical soils, the P availability is mainly guided by adsorption and/or precipitation reactions of Pi and Po forms to (oxy) hydroxides of Fe and Al, and by the adsorption to 1:1 clay minerals, and organic matter (OM) (Wang et al., 2014; Nascimento et al., 2018; Withers et al., 2018).

Because of the high P sorption capacity of tropical soils, supplementation with P has been done by annually applying large amounts of soluble inorganic fertilizers to optimize crop growth and development (Chowdhury et al., 2014; Roy et al., 2016; Van Dijk et al., 2016). However, this practice has been proven inefficient, because the maximum P availability in the soil from soluble fertilizers comes shortly after the application, and a great part of the nutrient turns into low soluble forms (Beauchemin et al. 2003; Hedley and McLaughlin 2005; Khatiwada et al. 2012; Wang et al. 2017). For example, in the sugarcane (*Saccharum spp*.) production in Brazil, only 10 to 20% of the P added at the crop establishment is absorbed by the plants in the first season (Raij, 2004).

The low P use efficiency by sugarcane is a matter of concern because 20% of Brazil's total consumption of soluble inorganic P fertilizers (FAOSTAT, 2017) is destined for the production of this crop. Adding more complexity to the matter, the country is highly dependent on these fertilizer imports (Withers et al., 2018) which has the market dominated by China, Marocco, the United States of America, and Russia (USGS, 2022). Moreover, the manufacturing of P sources requires expensive processing of rock phosphates (RP), and although the exact size of the global commercial reserves of RP is not known (Sattari et al., 2012; Van Kauwenbergh et al., 2013), it is a finite natural resource that must be preserved. Thus, the security of sugarcane production in Brazil is susceptible to a crisis in the fertilizers industry, RP scarcity, and geopolitical issues.

Brazil is the largest worldwide sugarcane producer (FAOSTAT, 2019), and to supply the increasing global demand for clean and renewable energy the sugarcane fields have expanded mainly to areas previously occupied by pasturelands (Taniwaki et al., 2017) in general, located in acidic soils and low P content. Currently, it is estimated a yield gap higher than 133 tons ha^{-1} for sugarcane, and one of the reasons is the low soil fertility (Marin et al., 2016; Monteiro and Sentelhas, 2017). Therefore, it is clear that the current P management in sugarcane is constraining the sustainable growth of crop production from an economic and environmental point of view.

One strategy to reduce the use of synthetic P fertilizers and increase the P use efficiency is the nutrient recycling from bio-resources (Withers et al. 2015). Agriculture is one of the economic sectors that most contribute to residue generation, which for the most part has not an appropriate destination (Moreira et al., 2021). In the sugarcane industry, particular attention has been drawn to filter cake (FC), a byproduct generated in the purification of sugarcane juice to produce sugar and ethanol. FC has been used in sugarcane fields as supplementation to P fertilization because, in addition to residual nutrients from sugarcane, it also contains Ca and P added by the industry to help with the juice clarification (Rosseto et al., 2018).

Research has shown that the association of FC with mineral fertilizers, has a great potential in increasing the sugarcane yield (Santos et al. 2014; Caione et al. 2015; Moda et al. 2015; Vazquez et al. 2015; Bekheet et al. 2018; Borges et al. 2019; Lima Vasconcelos et al. 2020; Estrada-Bonilla et al., 2021). Also, as an organic material, FC increases the soil´s water holding capacity, aeration, cation exchange capacity, pH, and reduces the Al saturation, (Korndörfer and Anderson 1997; Santos et al., 2014). Considering that sugarcane is a semi-perinnial crop, such effects can be very important to the longevity of the sugarcane field.

The positive effects of the association of organic materials with mineral fertilizers occur because organic amendments decreased the soil P adsorption capacity (Zhang et al., 2022), improving the acquisition by plants (Caione et al., 2015; Withers et al., 2018). The increase in the P use efficiency is possibly related to the ion chelation (Zhu et al., 2018) and the synthesis of a range of exudates, i.e. low molecular weight organic acids (LMWOAs) (Behera et al., 2014). The LMWOAs are organic compounds derived from plants, microorganisms, and decomposition of plant residues with the ability to increase the P availability in the soil through the: competition with P for the same sorption sites in soils; complexation of Fe, Al, and Ca; and dissolution of sparingly soluble P minerals in the soil (Fox et al., 1990; Lan et al., 1995; Ström et al., 2005; Yuan et al., 2016).

The change in the soil pH by LMWOAs is the main process responsible for the dissolution of P minerals, which include calcium phosphate, as apatite (Andersson et al., 2015; Qin et al., 2013; Wang et al., 2021). Thus, the benefits of the association of FC with mineral P fertilizers are expected not only for soluble sources, such as triple superphosphate (TSP) but also for RP, which usually has a low dissolution rate in the soil. In this sense, additional gains can be achieved if FC is applied in the soil combined with RP because hypothetically there may be a dual and gradual P release in the soil and a better synchronism with the P demand by sugarcane.

Several factors can influence P reactions in the soil as the phosphate source, rate of application, plant demand, and the soil´s P sorption capacity (McLaughlin et al., 2011; Weeks and Hettiarachchi, 2019). In soils with high P adsorption capacity and frequent fertilizer inputs, the nutrient tends to accumulate in soils (Withers et al., 2001), initially in low available forms. However, depending on the crop, the type of soil, and the history of land use, this P can efficiently be accessed by plants (Condron et al., 2013). Thus the pools of P, the species formed by the reactions with other soil constituents, and the yield can differ between a site historically cultivated with sugarcane and frequent P input, and another where the crop was recently established. Also, is well known that the biotic and abiotic reactions that guide the P cycle vary from seconds to years (Bünemann and Condron, 2007; Fardeau, 1995; Frossard et al., 2011), highlighting that the effect of the P fertilization must be evaluated in the long-term, instead only in one crop cycle.

Even though sugarcane is a long-cycle crop (cycle of 5-7 years), most of the studies on the effects of FC in the soil were carried out in the short and medium term (Bokhtiar et al., 2008; Fravet et al., 2010; Santos et al., 2010; Almeida Júnior et al., 2011; González et al., 2014; Utami et al., 2012; Arruda et al., 2019; Braos et al., 2020; Soares et al., 2022). Therefore, it is necessary to better investigate the long-term effect of this byproduct associated with TSP and RP on the P dynamics in the soil, especially to assess the residual effect of the P sources. Also, is not clear if FC changes the P release from TSP and RP, and if these changes are consistent in different types of soils.

The general hypothesis of this study is that the use of FC can alter the rate of P release from mineral fertilizers (TSP and RP), increasing its availability in the soil, and leading to enhanced P acquisition by sugarcane plants. The general objective was to evaluate the long-term effect of FC associated with mineral P sources (TSP and RP) on the P availability and dynamics in the soil.

The general objective guided five scientific papers, presented in chapters 2 to 6 of this thesis, each one with a specific objective, as follows: to evaluate the potential of portable X-ray fluorescence (pXRF) to quantify P in sugarcane leaves *in vivo* conditions; to evaluate the release and dynamics of P over time from TSP and RP associated to FC in the soil; to investigate the release, mobility, and lability of P from FC associated or not with mineral fertilizers (TSP and RP) in acidic and alkaline soils; to evaluate the long-term effect of the association of FC with TSP and RP (at rates of 90 and 180 kg ha^{-1} P_2O_5), applied to sugarcane fields, in the stocks and species of P in two distinct soils (sandy and clayey Oxisols); to evaluate the agronomic performance of sugarcane cultivated in two Oxisols (sandy and clayey) fertilized with TSP and RP (at rates of 90 and 180 kg ha^{-1} P_2O_5)in the presence and absence of FC, for five consecutive cropping seasons.

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2. PHOSPHORUS QUANTIFICATION IN SUGARCANE (*Saccharum officinarum*) LEAVES IN VIVO BY PORTABLE X-RAY FLUORESCENCE SPECTROSCOPY

Abstract

This study aimed to evaluate the potential of portable X-ray fluorescence (pXRF) equipment to quantify P in sugar cane leaves *in vivo* conditions. Therefore, sugarcane seedlings were grown in soil fertilized with increasing doses of P, and the leaf P content was measured by pXRF and by inductively coupled plasma optical emission spectrometry (ICP OES). The X-ray tube operational condition, analysis under vacuum or air, the number of points measured per leaf, the acquisition time per spot, and the best quantitative strategy to determine the P content in leaves were investigated. Using three points per leaf, an acquisition time of 60 s per point, voltage and current at 40 kV, and 30 µA, respectively, analysis under vacuum, and an external calibration curve considering the densities of standards and leaves, we found a high correlation between the P content in sugarcane leaves by pXRF and ICP OES. A cross-calibration curve can be equally useful.

Keywords: *Saccharum spp*, Plant P nutrition, Leaf P analysis, Sustainable chemistry

2.1. Introduction

Phosphorus (P) is an essential element for plant growth and development because it is a central component of the synthesis of nucleic acids, proteins, lipids, and sugars, playing a fundamental role in the transformation and regulation of several enzymatic activities and metabolic processes for the adequate functioning of plant cells [1-3]. After nitrogen (N), P is the major plant growth-limiting nutrient, requiring high inputs via mineral and/or organic fertilizers to achieve acceptable levels of agricultural productivity [4-6], which may not be environmentally and economically sustainable.

One of the most efficient ways to evaluate the effect of nutrients on agricultural yield is by leaf analysis because the plant is the best indicator of nutrient availability. The results reflect the momentaneous nutritional status of the plants and indicate zones of P deficiency or surpluses [7,8]. However, a major problem in using leaf analysis to assess nutrient profiles across a large field is the demand for sampling in short periods, in a large number of plants, and in a specific stage of plant development, which may not be feasible using traditional laboratory procedures based on acid digestion. Digestion-based methods for P quantification on tissues cause total destruction of the plant matrix resulting in a solution that must be filtered and analyzed by inductively coupled plasma (ICP) spectrometry, or colorimetric techniques [9,10]. These processes, which require hazardous chemicals, are time-consuming and can lead to element loss due to incomplete solubilization of the material [12].

Ideally, a method for elemental determination should offer adequate limits of quantification, maximum accuracy, reproducibility, and portability allied to minimal sample preparation and interference [13]. For P determination in plant tissue, such requirements can be fulfilled by X-ray fluorescence spectroscopy (XRF). X-ray fluorescence is the emission of characteristic wavelengths that may occur during the relaxation of excited atoms or ions. The excitation can be carried out by X-ray radiation or accelerated particles. The XRF phenomenon is well-understood and allows to access quantitatively and qualitatively several chemical elements in different types of samples [14,15]. X-ray fluorescence spectroscopy measures the energy and flux of the emitted photons. The photon energy, or wavelength, is characteristic of each element, while the photon flux is a direct proportion of the element concentration in the sample [16,17]. The advantages of elementary quantification by XRF over traditional methods are numerous since it requires a small amount of sample and can be performed in powder, pellet, or directly on solid samples, with little or no preparation [13,15,18]. Also, the use of portable equipment (pXRF) allows the analysis of plants in the field and *in vivo* conditions [11, 16]. In this way, the nutritional status of the same plant can be measured over time.

Previous studies have reported significant linear relationships between XRF and acid digestion to quantify P in the leaves of several high commercial value crops such as maize, bean, soybean, sugarcane, wheat, cotton, and eucalyptus [12,19-23]. However, these results were based upon data obtained from leaves ground to powder, pelletized, or detached from plants. In the present study, we intend to adapt the use of pXRF for P quantification on leaves '*in vivo'* rather than on detached ones, which broadens the range of applications of the technique. Usually, the common quantification of elements is done by cross-calibration, that is, adopting an established technique of elementary quantification as a reference to generate calibration curves.

Despite the importance of leaf analysis for soil/plant nutritional management and the advantages of elementary analysis by pXRF compared to traditional methods, this technique needs to be improved so that it can be used routinely for P quantification. We hypothesize that the quantification of P in sugarcane leaves *in vivo* and *in situ* by pXRF is possible after adequate operational conditions calibration and considering the matrix differences between tissue samples and standards. This study aimed to evaluate the potential of pXRF to quantify P in sugarcane leaves *in vivo* conditions. For this, we conducted an experiment in a greenhouse in which sugarcane seedlings, grown in pots with soil fertilized with increasing doses of P, had their leaf content measured directly by pXRF and by ICP optical emission spectrometry (ICP OES) after microwave digestion.

2.2. Material and Methods

2.2.1. Sugar cane cultivation

Sugarcane seedlings (one per pot), approximately 45 days after bud germination, were transplanted into PVC pots filled with 3 kg of soil air-dried, and sieved at 4 mm. The soil used was a *Latossolo Vermelho-Amarelo distrófico* [24] or Hapludox [25] medium texture, sampled in the 0-20 cm layer of a grassland area, in southeast Brazil. Chemical, mineralogical and physical parameters of the original soil are shown in Table 1, highlighting the low available P content [26], primordial for the development of the current study. The sugarcane variety RB96-6928 was chosen because it is one of the most cultivated in the southeast region of Brazil and because of its high responsiveness to soil available P content [27].

Table 1. Chemical, mineralogical and physical parameters of the original soil before the experiment establishment.

pH	**OM**[a]	**P resin**	**Al^{3+}**	**H+Al**	**Ca^{2+}**	**Mg^{2+}**	**K^{+}**	**SB**[b]	**CEC**[c]	**m**[d]	**V**[e]
$CaCl_2$	g kg^{-1}	mg kg^{-1}	--------------- mmol$_c$ kg^{-1} ---------------							%	
4.9	16	5	2	16	13	5	<0.9	18.8	34.8	10	54

Granulometry			**DCB**[f]		**Oxalate**	
Clay	**Silt**	**Sand**	**Fe**	**Al**	**Fe**	**Al**
--- g kg^{-1} ---						
180	90	730	9.11	1.99	0.27	1.47

[a]OM= organic matter; [b] SB= sum of bases; [c] CEC= cation exchange capacity; [d] m= Al saturation; [e] V= base saturation; [f] DCB= dithionite-citrate-sodium bicarbonate.

For adequate plant development, were applied 320 mg kg^{-1} of Ca as $CaCO_3$ and 60 mg kg^{-1} of Mg as MgO, before the transplant of the sugarcane seedlings. After, a nutrient solution was applied twice (15 and 30 days) during the crop growth, providing each time 100 mg kg^{-1} of N as NH_4NO_3, 100 mg kg^{-1} of K, and 45 mg kg^{-1} of S as K_2SO_4. Additionally, a nutrient solution was applied once to provide 0.81 mg kg^{-1} of B as H_3BO_3, 4.0 mg kg^{-1} of Zn as $ZnSO_4.7H_2O$, 3.66 mg kg^{-1} of Mn as $MnSO_4$, 1.3 mg kg^{-1} of Cu as $CuSO_4.5H_2O$, and 0.15 mg kg^{-1} of Mo as $(NH_4)_6Mo_7O_{24}$, as recommended by Novais et al. (1991)[28] for greenhouse experiments.

Increasing doses of P_2O_5 (0, 30, 60, 90, 120, 150, 200, 400, and 500 mg kg^{-1}) were applied as triple superphosphate, as powder, being uniformly mixed to the soil immediately before transplanting the seedlings. Plants were grown for 60 days in a greenhouse with temperature from 30 to 18 °C, day and night, respectively. Pots were watered daily with distilled water maintaining ~70% of the soil's maximum water holding capacity. The experimental design was completely randomized with nine treatments and three repetitions.

2.2.2. pXRF method optimization

Initially, the pXRF instrumental settings were optimized to obtain the highest signal-to-noise ratio (SNR) (Eq. (1)) and the lowest coefficient of variation (CV). The X-ray tube voltage and current settings (15 kV x 55 μA or 40 kV x 30 μA), the analysis under vacuum or air, the number of spots measured per leaf (1-10), and the dwell time per spot (60, 90, 120, 150, 180s) were evaluated.

$$SNR = \frac{I\ (cps)}{\sqrt{\frac{Bg\ (cps)}{t}}} \tag{1}$$

where *I (cps)* is the characteristic X-ray net counting rate of the element, *Bg (cps)* is the element background counting rate, and *t* is the lifetime (seconds).

All parameters were optimized *in vivo* conditions by taking measurements at three distinct points on the middle third of the limb of sugarcane Top VisibleDewlap abaxial surface, or +1 leaf. Each of the three points per leaf was scanned once, with a data acquisition time of 180 s per scan, using a pXRF spectrometer, Tracer III - SD model (Bruker, USA), equipped with a 4 W Rh X-ray tube, and a 10 - mm^2 X - Flash® Peltier-cooled silicon drift detector. The equipment was fixed in an acrylic docking station and a shield for radiation was used during the measurements.

Using eq. (2) and (3) it was observed that > 99% of the P Kα emission from the irradiator was attenuated by the leaf. Therefore, the measurements were performed considering that the sugarcane leaves are infinitely thick samples for P Kα radiation. For this test, an irradiator was made by grounding laboratory-grade salts (22.7% Na_3PO_4; 9.10% Zn $(PO_4)_2$; 18.20% K_3PO_4; 9.10% $CuSO_4.5H_2O$; 13.60% $MnSO_4.4H_2O$; 9.10% $FeSO_4$; 18.20% $CaNO_3$). The material was pressed manually in an X-ray sample cup flask (Chemplex, No. 1530), and sealed with a 5-μm polypropylene film (Spex, No. 3520).

$$A = \frac{1 - H}{-\ln\,(H)} \tag{2}$$

$$H = \left(\frac{I^{\,irrad+sam} - I^{\,sam}}{I^{\,irrad}}\right) \tag{3}$$

where A is the absorption factor (unitless), H is the transmission factor (unitless), ln is the natural logarithm, $I^{\,irrad}$ is the characteristic X-ray intensity of the element from the irradiator (counts per second), $I^{\,sam}$ is the characteristic X-ray intensity of the element from the sample (counts per second), $I^{\,irrad\,+\,sam}$ is the characteristic X-ray intensities of the element from the irradiator, which was partly absorbed by the sample, plus the element i from the sample (counts per second).

2.2.3. pXRF analysis

From the optimization tests described above, the determination of P concentration in sugarcane leaves *in vivo* conditions by pXRF was performed. Before the measurements, all pots were watered with distilled water keeping the soil with ~70% of maximum water holding capacity to ensure that all plants were under the same water condition. The leaves were carefully placed on a 5 μm polypropylene film (Spex Sample-Prep, no. 3520, USA) to make sure that the incident X-rays reached only the leaf blade. In each sugarcane plant, three points (one scan per point) were measured in the Top VisibleDewlap, with a data acquisition time of 60 s per scan, the

Rh X-ray tube at 40 kV and 30 μA, and under vacuum. The data was acquired using the Bruker S1PXRF® software and later processed by the Artax ® software. The pXRF intensity was obtained at the P Kα peak (2.01 KeV) and divided by the dwell time, obtaining the intensity in counts per second.

2.2.4. Comparative method

For comparison and validation, the leaves analyzed by pXRF were removed from the plants, sanitized with deionized water, dried in an oven at 60°C for approximately 48 hours, ground in a mill at 1 mm sieve, and the P concentration was determined by ICP OES after microwave digestion. For this, 0.25 g of the ground sample was transferred to a TFM® flask (MD -1844 Mars XPress) where 2 mL of H_2O_2 (30% w w^{-1}), 2 mL HNO_3 (20% v v^{-1}), and 5 mL of ultrapure water were added. The following heating program was used: a) reaching 80 ºC in 3 min (100% 1600W); b) reaching 150°C in 5 min (100% 1600W); c) reach 180°C 10 min (75% of 1600W); d) stay at 180°C for 5 min; e) reach 25°C in 8 min. The digested material was filtered through Whatman n. 42-paper filter, the extract was diluted to 25 mL and analyzed in ICP OES (iCap 6300 ICP Spectrometer-Thermo Scientific). Subsequently, the elemental concentration (mg kg^{-1}) determined by both methods was compared. The limit of detection of P for ICP OES was 2.49 mg kg^{-1}, while that to pXRF was 220 mg kg^{-1}.

2.2.5. Conversion of the P Kα XRF signal into P concentration in the leaves

Before the comparison of the P content obtained by the two methods, it was necessary to convert the P Kα XRF signal into P concentration in the leaves. Three strategies were employed for that purpose: i) generation of an external calibration curve using standards (cellulose pellets) whose matrix's chemical composition was similar to the samples, ii) generation of an external calibration curve in which the pXRF counting rate was normalized by the ratio between the density of standard cellulose pellets, and the density of sugarcane leaves (DC: DL), which were 1.4×10^{-3} g cm^{-2} and 5.8×10^{-4} g cm^{-2}, respectively, and iii) generation of an external calibration curve by cross-calibration using the reference method (ICP OES). The equations obtained are shown in Figure 1, and the results are discussed in the next section.

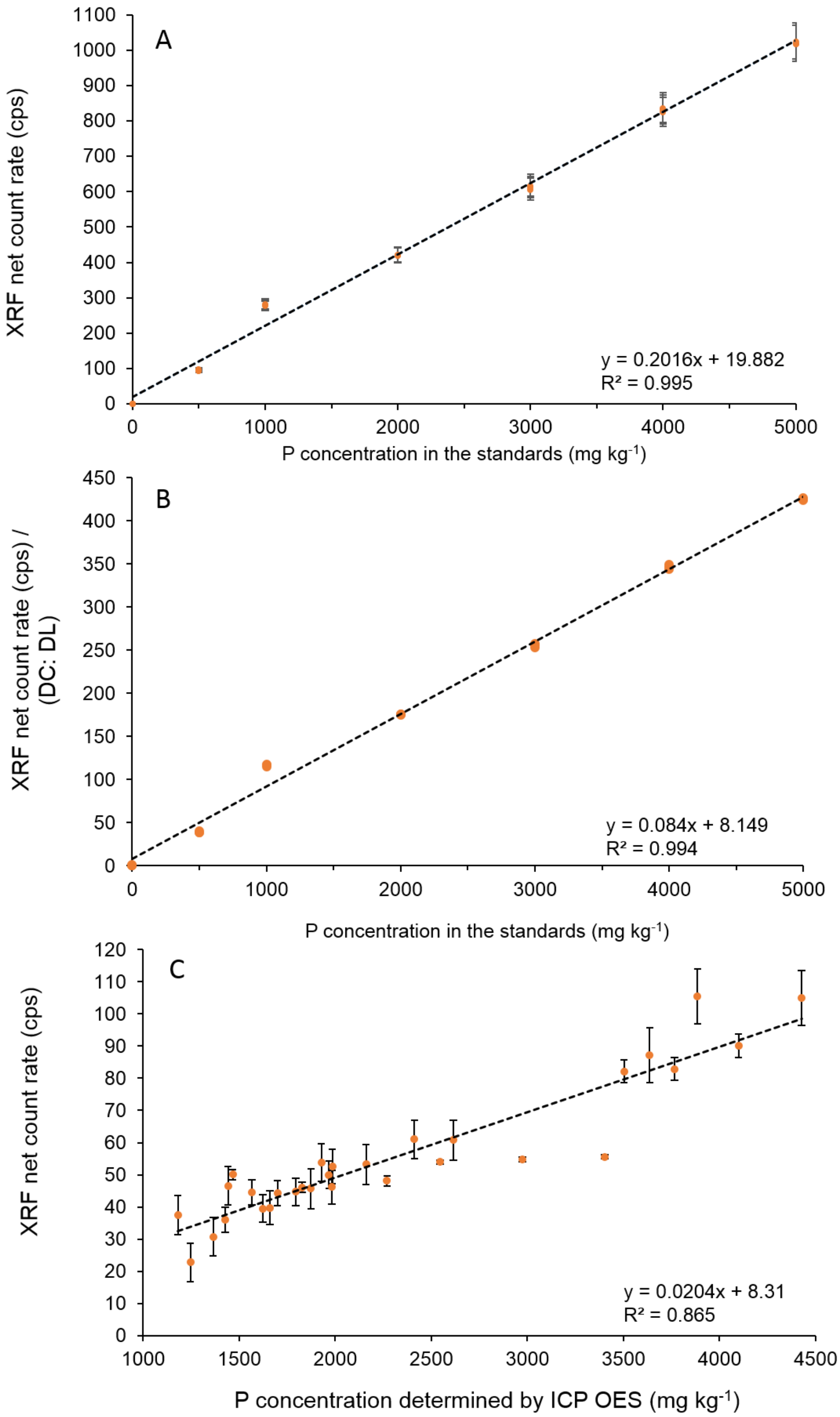

Figure 1. Portable XRF (pXRF) calibration curves: (A) External calibration curve using a standard whose matrix is similar to that of samples. (B) External calibration curve in which the pXRF counting rate was normalized by the ratio between the densities of standard cellulose pellets and the density of sugarcane leaves (DC: DL). (C) P counting rate (cps) in sugarcane leaves determined by pXRF in relation to the P concentration obtained by inductively coupled plasma optical emission spectrometry (ICP OES). Vertical error bars correspond to standard deviation. R²= Coefficient of determination.

Considering that the major organic constituent of leaves is cellulose and its closely related biopolymers, a cellulose powder (particle size < 20µm, Spex, USA) was spiked with sodium phosphate monohydrate ($Na_2HPO_4.H_2O$ – Êxodo Científica) and then pressed yielding pellets with the concentrations of P at 0, 500, 1000, 2000, 3000, 4000, and 5000 mg kg^{-1}, used as standards. The moisture of the pellets was not considered due to the closeness between the average atomic number for cellulose and water, 4 and 4.66, respectively. The measurement condition of the P in the pellets followed the same one used for the sugarcane leaves.

All of the tested strategies converted the P Kα pXRF signal into P concentration on a wet basis since the measurements were made on fresh leaves. However, as the standard pellets and the quantification by ICP OES result in P concentrations on a dry basis, the values were adjusted for water content by eqs (4) and (5). To obtain the dry weight, the leaves were removed from the plants and dried at 60°C in an oven.

$$[P]DB = \frac{[P]\ WB}{1 - w} \tag{4}$$

where *[P]DB* is the P concentration in leaves on a dry basis (milligrams per kilogram), *[P]WB* is the P concentration in leaves on a wet base (milligrams per kilogram), and *w* is the water content of the sample (grams)

$$w = \frac{(wet\ w) - (dry\ w)}{(wet\ sample\ weight)} \tag{5}$$

where *wet w* is the wet sample weight (grams) and *dry w* is the dry sample weight (grams).

2.2.6. Statistical analysis

The pXRF instrumental settings were analyzed based on the SNR and the CV (%). The comparison between the pXRF and ICP OES methods was performed using simple linear regressions and Pearson´s correlation test. Statistical analysis was performed using the Statistical Analysis System software v.9.3 (SAS Institute, INC., Cary, USA).

2.3. Results and Discussion

2.3.1. Optimization of experimental parameters

The effect comparison between the two combinations of voltage and current and the effect of the atmosphere (with or without vacuum) on the SNR is shown in Figure 2. Based on that, it was defined that the best combination of voltage and current was 40 kV and 30 µA. These parameters were used to evaluate the gain obtained with the use of vacuum, which resulted in an improvement of approximately 1.6 times in the SNR, facilitating the detection of P concentration in sugarcane leaves. The optimization of voltage and current allows optimal equipment performance for the analytical problem [29]. In the same way, for P, the use of vacuum increased the SNR and enhanced the elemental sensitivity, which is highly recommended and essential for light elements, especially those of atomic number below 20 [13,30]. The limits of detection in XRF depend upon the atomic number, not only due to the air attenuation but also the X-ray fluorescence yield of light elements, which is low due to the Auger decay phenomenon [31].

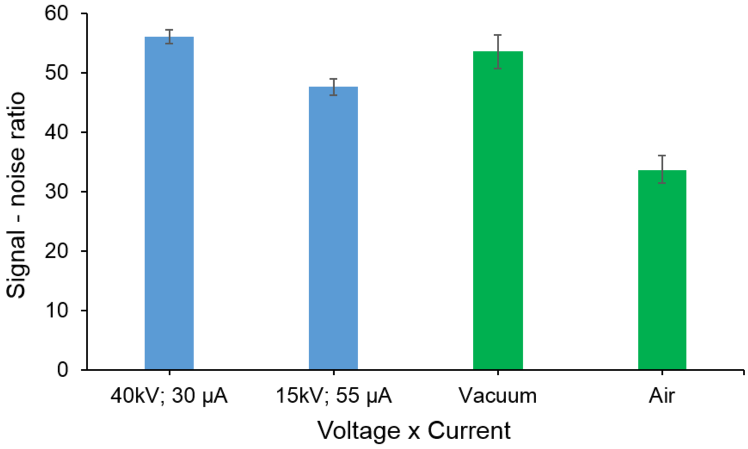

Figure 2. Evaluation of signal-to-noise ratio (SNR) for P detection under two different combinations of voltage and current (40 kV x 30 mA or 15 kV x 55 mA, respectively) and of the effect of the atmosphere (measured using 40 kV x 30 mA).

The number of spots tested per leaf showed that the CV (%) remained below 5% when more than two, and less than nine spots were measured (Figure 3A). Within that range, it was defined that three points per leaf were sufficient to represent the whole leaf considering the lowest CV (%), saving time. The lowest CV (%) was found with a dwell time of 150 s per spot (Figure 3B). However, considering that the

CV (%) for all the dwell times tested was <5 (which was considered an acceptable variation for analysis under *in vivo* conditions), and because the CV (%) of 60 s acquisition time was very similar from the 150 s (1.52 and 1.31%, respectively), the dwell time of 60 s was considered adequate for the P content determination in fresh sugarcane leaves.

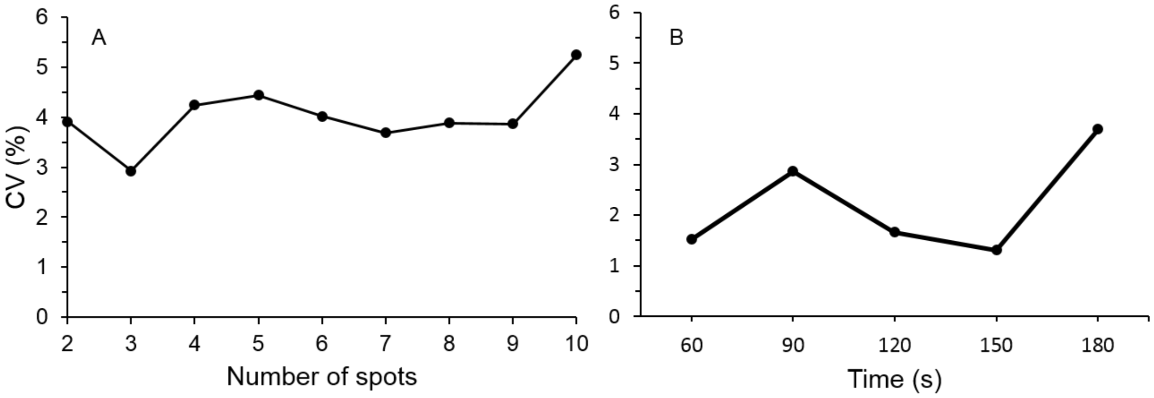

Figure 3. Coefficient of variation (CV) as a function of (A) the number of spots and (B) acquisition time for determination of the P content in sugarcane leaves by pXRF.

2.3.2. Determination of P content in sugarcane leaves

Figure 4A presents the pXRF spectra for a blank and leaf sample, while Figure 4B shows an amplification in the P Kα peak recorded in sugarcane leaves. The spectra were recorded under the optimized conditions, ie. vacuum, Rh X-ray tube at 40 kV and 30 μA, three points per leaf, and 60 s of dwell time. Accordingly, the P Kα peak increase with the dose of P_2O_5 applied to the soil (Figure 4B). The spectral visual inspection suggests a correlation between soil P fertilization and P leaf concentration, and hence it shows the dependence of the pXRF signal on the elemental quantity per unit of volume.

Several factors affect the elemental intensity shown in Figure 4B. As previously mentioned, the elemental sensitivity depends on the atomic number which will depend upon the cross-section, i.e., its capacity to absorb the incoming X-rays used for excitation, and the X-ray fluorescence yield since there are other relaxation channels. Other important parameters are sample thickness and matrix composition.

The relationship between sample thickness and the X-ray fluorescence intensity, or formally in the present count rate, is of major importance for defining the quantitative method. Samples can be considered infinitely thin when the XRF signal of a certain element holds a linear dependence with sample thickness. However, above a certain thickness, the sample starts attenuating the photons produced within itself. Hence, the linear dependence between thickness and XRF signal assumes an asymptotic behavior, this regime is called intermediate thickness. Finally, above a certain thickness, there is no relationship between sample thickness and the XRF signal coming from the sample. In this regime, called infinitely thickness, the XRF signal depends on the concentration of the element present in the sample and on the matrix composition. The tests using equations 2 and 3 indicated that the samples investigated in the present study were infinitely thick for P.

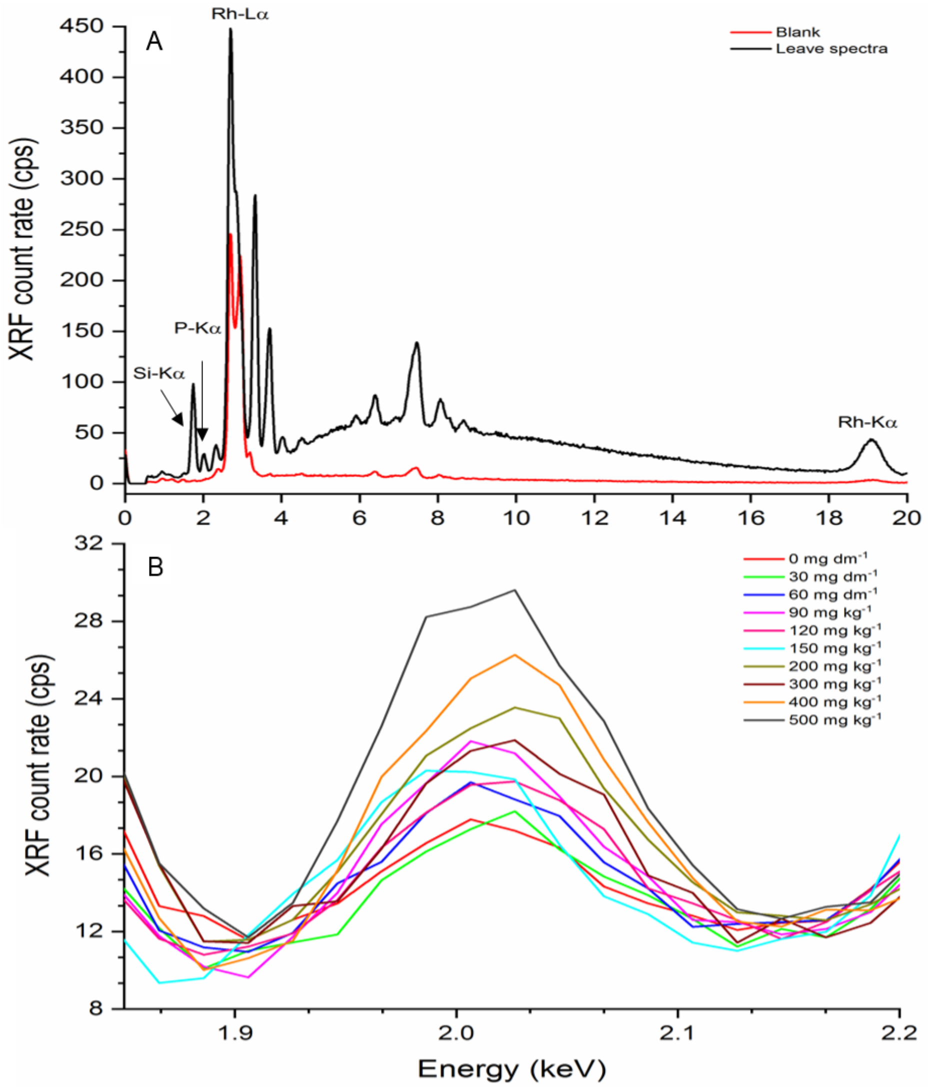

Figure 4. Fragment of portable XRF (pXRF) spectra of sugarcane leaves cultivated in soil with increasing doses of P fertilizer as P_2O_5 (milligrams per kilogram). (A) pXRF spectra for blank and leaf samples and (B) amplification of the P Kα peak recorded at sugarcane leaves.

The linear regression and Pearson's correlation coefficient, $R^2 = 0.833$ and $r = 0.927$ respectively, shown in Figure 5, revealed high dependence between the pXRF intensity (cps) and the doses of P fertilizer as P_2O_5 (mg kg^{-1}) applied to the soil. Thus, it demonstrates the capacity of the technique to identify differences in the content of P in sugarcane leaves *in vivo* conditions.

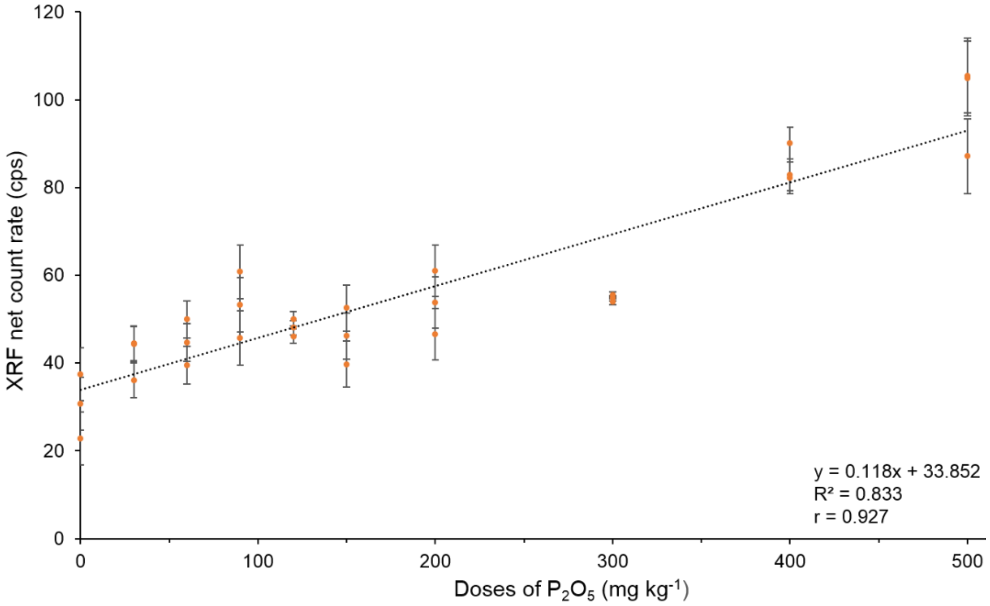

Figure 5. P intensity in sugarcane leaves determined by portable XRF (pXRF) in relation to the P fertilizer doses as P_2O_5 (milligrams per kilograms) applied to the soil. Vertical error bars correspond to standard deviation. R^2 is the coefficient of determination, and *r* is the Pearson´s correlation coefficient.

Data presented in Figure 6A shows that the P concentration (mg kg^{-1}) obtained with the external calibration curve using standards, without the normalization by the ratio DC: DL (Figure 1A) held a high linear relationship, $R^2 = 0.866$ and $r = 0.930$, with the P concentration obtained by the destructive reference method. However, compared to the ICP OES reference method, the external calibration underestimated the P concentration in leaves (Figure 6B).

As a technique for direct analysis of solids, XRF is highly susceptible to matrix effects, hence the composition of sample and standards matrices must be as close as possible [32]. If one considers the density ratio between standard pellets and sugarcane leaves (Figure 1B) to convert the P Kα signal from pXRF into P concentration in the leaves, we found a good linear relationship, $R^2 = 0.798$ and $r = 0.893$, between the P concentration obtained by the ICP OES destructive reference method and pXRF (Figure 7A).

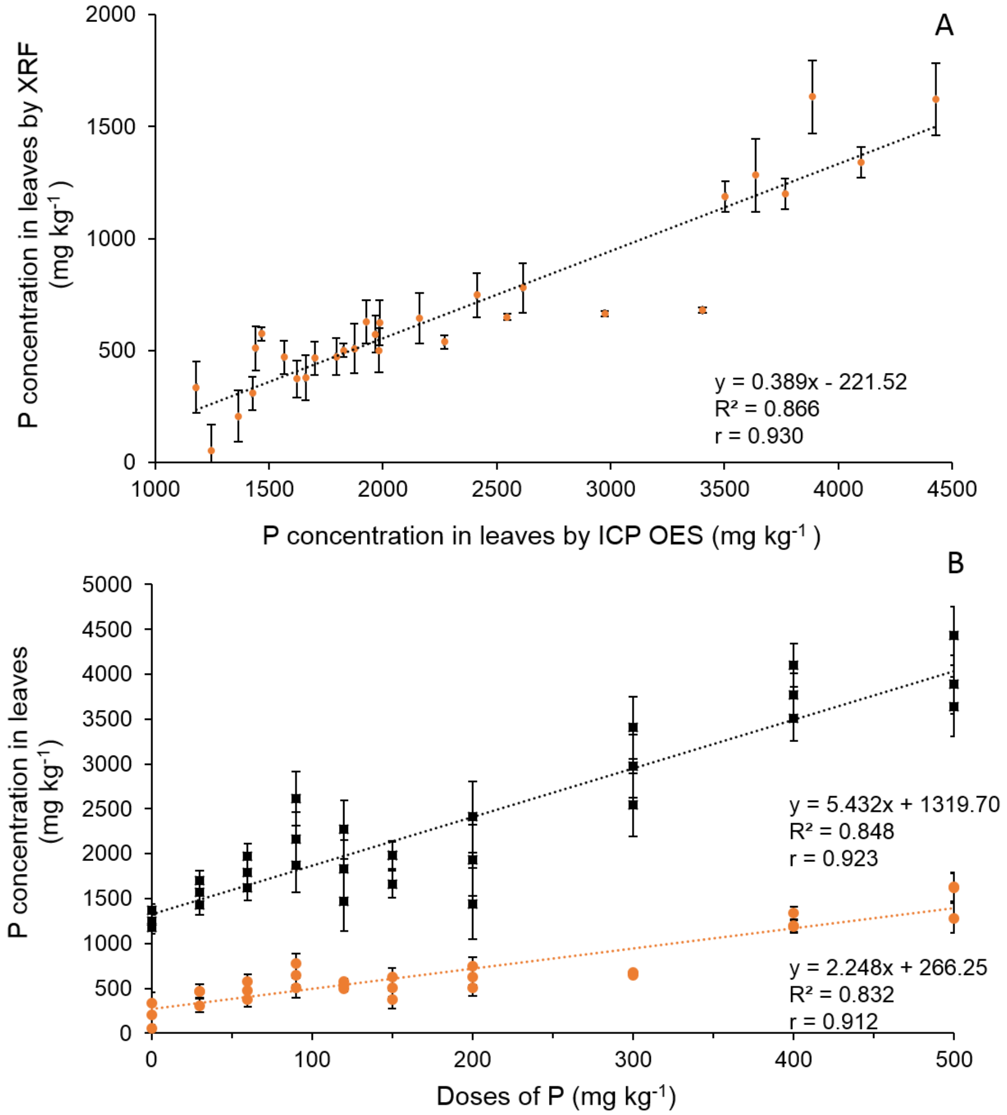

Figure 6. (A) Comparison of the P content in sugarcane leaves obtained by pXRF and by inductively coupled plasma optical emission spectrometry (ICP OES), using an external calibration curve without the normalization by the ratio between the densities of standard pellets and the density of sugarcane leaves (DC: DL). (B) Total P content in sugarcane leaves determined by pXRF and ICP OES in relation to the P concentration applied to the soil: (black ■) linear regression obtained to ICP OES and (orange ●) linear regression obtained to pXRF. Vertical error bars correspond to standard deviation. R² is the coefficient of determination, and *r* is the Pearson´s correlation coefficient.

Nevertheless, a thin underestimation of the P content by pXRF remains (Figure 7B). A factor that may explain it regards the high silicon (Si) content in the leaves, accumulated in the form of amorphous hydrated silica [33, 34]. The concentration of Si in the sugarcane leaves is between 7.0 and 19.0 g kg^{-1} [35], while the P concentration is between 1.5 and 3.0 g.kg^{-1} [11]. Si is a beneficial element for plants, related to the mitigation of different biotic and abiotic stresses, especially those that affect photosynthetic capacity as low P and water availability, Al, Mn, and Fe toxicity, and the incidence of pests and diseases [36-38]. There is also a structural role of Si on tissues because of its hardness property [39,40] and an inversely proportional relationship with the cellulose content on the leaf [41]. Silica is mainly deposited along with the epidermis [33] whilst P is predominated in the mesophyll of the leaves [42].

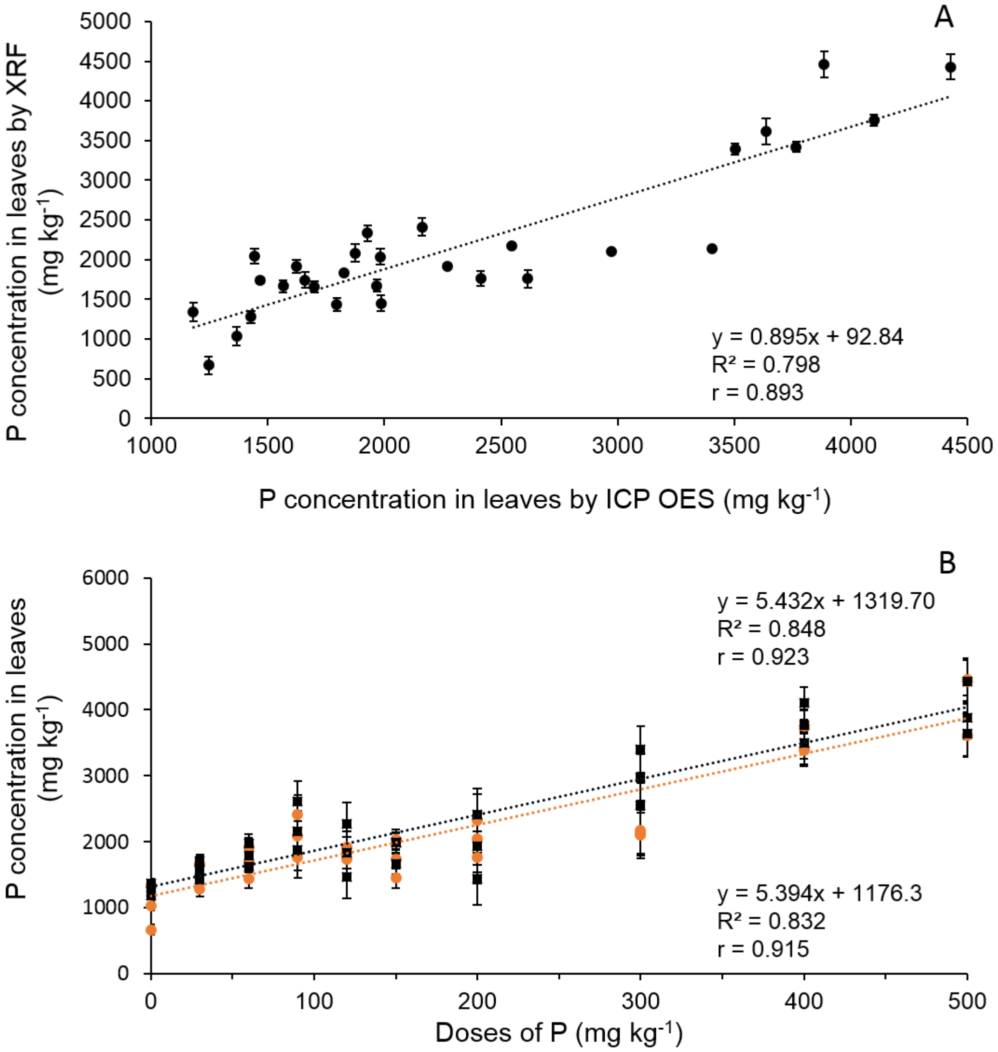

Figure 7. (A) Comparison of the P content in sugarcane leaves obtained by pXRF and by inductively coupled plasma optical emission spectrometry (ICP OES) using an external calibration curve considering the normalization by the ratio between the density of standard cellulose pellets and the density of sugarcane leaves (DC: DL). (B) Total P content in sugarcane leaves determined by pXRF and ICP OES in relation to the P concentration applied to the soil: (black ■) linear regression obtained to ICP OES and (orange ●) linear regression obtained to pXRF considering the normalization by the ratio between the density of standard cellulose pellets, and the density of sugarcane leaves (DC: DL) (B). Vertical error bars correspond to standard deviation. R^2 is the coefficient of determination, and *r* is the Pearson´s correlation coefficient.

This Si probably deposited on the leaf epidermis, especially on the abaxial surface, attenuated the P Kα X-rays produced within the leaf, which would promote an underestimation of P content. In the energy range presented here, the attenuation coefficient is dominated by the photoelectron absorption cross-section and has also a minor contribution of scattering cross-sections. The Si K absorption edge is at 1.83 keV, while the P Kα X-ray emission is at 2.01 keV. The resonance between the X-ray fluorescence produced by P and the edge of Si increases the probability of attenuation. In principle, this effect could be partly corrected by adding similar concentrations of Si to the standards used to prepare the calibration curve. However, the composition heterogeneity of the sugarcane leaf structure that concentrates the Si on the epidermis could not be easily addressed and replicable in laboratory-produced material. Hence, the present study highlights the importance of matching not only the average elemental composition between samples and standards but also pXRF users must pay attention to the physical structure of the leaves.

Costa Jr. et al. (2019) [43] employed pXRF to determine several nutrients in soybean leaves *in vivo* conditions and also concluded that external calibration underestimated the concentration of all elements compared to the reference method (same as used here). In this regard, Dao et al. (2016) [20] found good correlations between the P content in maize leaves by XRF and the conventional tissue analysis method using the water content in the leaves to normalize the fluorescence intensities of P to a common basis. Guerra et al (2018) [44], analyzing fresh fragments of sugarcane leaves with energy-dispersive pXRF spectrometry obtained concentrations of K, Ca, S, and Si close to those found by the conventional method of analysis. However, those authors did not measure P due to the difficulty in adapting the calibration models for this element considering its low concentration in the sample.

An alternative to overcome the uncertainty generated by matrix differences is cross-calibration, in which the P signal obtained in the leaves by pXRF is correlated to the P concentration obtained by a reference method of analysis (Figure 1C). In the present study, this method consisted of P determination through microwave-assisted digestion followed by ICP OES. Thus, in the framework of the present study, this strategy proved to be as good as the use of the external calibration curve in which the pXRF counting rate was normalized by the ratio DC: DL. Once the pXRF method is calibrated, it allows rapid determination *in vivo* conditions. This feature can be explored in a myriad of cases ranging from kinetic studies or nutrient uptake and remobilization up to the precision agricultural determination of the nutritional status of plants.

Despite the difficulties caused by the matrix effect, the quantification of P in sugarcane leaves *in vivo* and *in situ* by pXRF was possible and accurate by using cross-calibration and if the density of the standards pellets is considered to normalize the fluorescence intensities of P. The use of this technique provided a cheaper and faster analysis, with little sample preparation, high spatial resolution, and consistent with the precepts of sustainable chemistry. The ideal situation is that the standards used to generate the external calibration curve, and convert the P intensity into concentration, be chemically and physically similar to the leaf. However, this is a big challenge when it comes to living samples, and should be further evaluated in future studies.

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3. SUGARCANE BYPRODUCT INFLUENCE ON MINERAL FERTILIZERS SOLUBILITY AND PHOSPHORUS DYNAMICS IN THE SOIL

Abstract

This research study aimed to evaluate the release and dynamics of P over time using mineral fertilizers - triple superphosphate (TSP) and rock phosphate (RP) – associated with filter cake (FC), a byproduct of the sugarcane industry. The P concentration in the soil solution, the potential acid phosphatase activity, the P content in the leaves, and the changes promoted in the soil P pools were evaluated in two short crop cycles in a greenhouse experiment. The addition of FC was responsible for increasing RP solubilization and the P content in the leaves. The reduction in the amount of P supplied via TSP, when associated with FC, did not affect the soil solution P concentration, resulting in satisfactory crop P uptake. FC addition affected the labile and moderately labile P pools in the soil positively, resulting in higher P availability for the crop. The association of rock phosphate with filter cake demonstrates considerable potential for expanding the use of rock phosphate in sugarcane fertilization without the need for costly industrial processes. Furthermore, the combination of this byproduct with triple superphosphate enables reductions in the supply of P in the form of soluble mineral fertilizers, with its environmental and economic advantage.

Keywords: Filter cake, Oxisol, P Solubility, Soil P fractions

3.1. Introduction

Phosphorus (P) is a key element in agricultural production, not only because it is essential to the development of plants, but also because of its relative scarcity in terrestrial ecosystems which often limits crop productivity (Sullivan et al., 2014). In general, P is found in soil precipitated with iron (Fe), aluminum (Al), or calcium (Ca), adsorbed to the Fe and Al oxides of the clay fraction, in solution, in organic form, or as part of insoluble compounds. These forms have different P retention and release capacities. The *in situ* formation of Fe and Al phosphates of low solubility may be found in acidic soil conditions, while the increase in pH leads to the precipitation of P as Ca phosphate (Wright, 2009). In addition to pH, the type and amount of minerals in the clay fraction also determines the P species (Kana et al., 2011; Shigaki and Sharpley, 2011).

In tropical soils, the main reactions that control P bioavailability are adsorption to Fe and Al (oxy) hydroxides and 1:1 clay minerals, and precipitation with Al and Fe (Nascimento et al. 2018; Withers et al. 2018). The low P availability resulting from these reactions is usually overcome by the addition of high doses of readily water-soluble phosphate fertilizers, such as triple superphosphate (TSP), which promote rapid increases in P concentration in the soil solution, favoring its absorption by plants. However, they can also be converted into insoluble P forms by displacing other anions adsorbed to the soil solid phase or by precipitation (Menezes-Blackburn et al., 2016a), accumulating P in the non-labile compartment, and decreasing its availability to plants (Menezes-Blackburn et al., 2016b).

In the sugarcane fields in Brazil, only 10 to 20% of the P applied via fertilizers is absorbed in the first crop cycle (Raij, 2004), which for the most part, is related to the inherent soil properties. Satisfactory levels of productivity were achieved with the use of 150 - 180 kg ha^{-1} of P_2O_5, although relatively much less than this is required for strong crop development (Novais et al., 2007). The need for doses higher than those required to achieve expected yields accentuates the low-efficiency level of P fertilizer management in this crop. Thus, strategies that combine reductions in the use of fertilizers derived from rock phosphate (RP), a non-renewable resource, with increasing productivity in a sustainable manner are needed.

Given this framework, reactive RP from sedimentary origin and industrial byproducts such as filter cake (FC) represent promising sources of P for sugarcane. Sedimentary RP has intermediate solubility between water-soluble sources and natural rock phosphates, and a lower cost compared to soluble sources (Renner, 2008). Its high residual effect on the soil can be beneficial to long-cycle crops such as sugarcane. FC is produced in large volume (30 - 40 kg ton^{-1} of processed sugar cane) during the process of clarifying the sugarcane juice (Prado et al., 2013), and its use as fertilizer has expanded as a result of its high content of organic matter (OM) and micro and macronutrients (Dee et al., 2003).

Applied to the soil, RP and FC make their P content gradually available through the slow solubilization of Ca phosphates and the mineralization of OM (Keller et al., 2012), with better synchronism between soil P availability and its demand by plants. In the literature, positive interactions between organic residues and mineral fertilizers are reported, explained by the changes promoted in the community and diversity of soil microbial, increased production of P-solubilizing enzymes, and the action of low molecular weight organic acids (LMWOAs) (Arruda et al., 2019; Borges et al., 2019; Estrada-Bonilla et al., 2021; Gilbert, 2008; Soltangheisi et al., 2018).

Thus, by the combination of analysis that included P in the liquid and solid phases, the potential acid phosphatase activity (AP $_{ASE}$), and the P content in the leaves, we tested the hypothesis that the use of mineral fertilizers (TSP and RP) associated with FC increases soil P availability, leading to its enhanced acquisition by sugarcane plants. The aim was to evaluate the release and dynamics of P over time from these fertilizers in the soil.

3.2. Material and Methods

The effects of mineral and organic phosphate fertilizers were tested in a greenhouse experiment. Approximately 45 days after bud germination, a single sugarcane seedling of the RB 96-6928 variety was transplanted into Polyvinyl Chloride (PVC) pots filled with 6 kg of air-dried and sieved (at 4 mm) soil with low available P content (Raij et al., 2001). The soil used was sampled from the 0-20 cm layer of a grassland area in southeast Brazil and was classified as a *Latossolo Vermelho-Amarelo distrófico* sandy texture (Santos et al., 2013), equivalent to *Oxisol* (Soil Survey Staff, 2014) or *Ferralsol* (IUSS WRB Working group, 2015). The soil's chemical and physical parameters were determined following the methodology described by Raij et al. (2001), and Bouyoucos (1962), respectively (Table 1). The pedogenic Fe and Al oxides (dithionite-citrate-sodium bicarbonate) were determined following the methodology described by Mehra and Jackson (1960), while the low crystallinity Fe and Al oxides (oxalate) were determined by Schwertmann (1964) methodology.

Before transplanting the sugarcane seedlings, 90 mg kg^{-1} of Ca as $CaCO_3$, and 30 mg kg^{-1} of Mg as MgO were applied to the soil. During crop growth, a nutrient solution was applied every 30 days, each time providing 50 mg kg^{-1} of N as NH_4NO_3, 50 mg kg^{-1} of K, and 32.7 mg kg^{-1} of S as K_2SO_4. Additionally, for adequate plant development, a nutrient solution was applied once with 0.81 mg kg^{-1} of B as H_3BO_3, 4.0 mg kg^{-1} of Zn as $ZnSO_4{\cdot}7H_2O$, 3.66 mg kg^{-1} of Mn as $MnSO_4$, 1.3 mg kg^{-1} of Cu as $CuSO_4{\cdot}5H_2O$, and 0.15 mg kg^{-1} of Mo as $(NH_4)_6Mo_7O_{24}$, as recommended by Novais et al. (1991). Greenhouse temperature was maintained between 18 °C (night) to 30 °C (day), and pots were watered daily (distilled water) to maintain the soil moisture ~ 70% of the soil´s maximum water holding capacity (0.26 L kg^{-1} of soil).

Table 1. Pre-experiment soil chemical, mineralogical and physical characterization.

pH	**OM**[a]	**P**	**Al^{3+}**	**H+Al**	**Ca^{2+}**	**Mg^{2+}**	**K^+**	**SB**[b]	**CEC**[c]	***M***[d]	***V***[e]
	($g\ kg^{-1}$)	($mg\ kg^{-1}$)	$mmol_c\ kg^{-1}$							(%)	(%)
4.4	12	4	6	18	11	5	2.5	18.5	36.5	24	52

Granulometry ($g\ kg^{-1}$)			**DCB**[f] ($g\ kg^{-1}$)		**Oxalate**[g] ($g\ kg^{-1}$)		**P_{max}**[h] ($mg\ kg^{-1}$)
clay	**silt**	**sand**	**Fe**	**Al**	**Fe**	**Al**	
101	23	877	15.3	42.3	0.6	1.1	342

[a] OM: organic matter determined by Walkley-Black method; [b] SB: sum of bases; [c] CEC: cation exchange capacity; [d] M%: Al saturation; [e] V%: base saturation of CEC; [f] DCB: Fe and Al extracted by dithionite-citrate-sodium bicarbonate; [g] Fe and Al extracted by ammonium oxalate; [h] P_{max}: maximum phosphorus sorption capacity. pH in $CaCl_2$, with ratio soil: solution of 1: 2.5 m/v (Raij et al., 2001). P, K^+, Ca^{2+}, and Mg^{2+} extracted by ion exchange resin. Al^{3+} extracted by KCl 1 mol L^{-1}. H + Al extracted by SMP (Shoemaker, Mclean, Pratt); granulometry determined by Pippet method.

3.2.1. Treatments

The treatments evaluated were: i) Control (no phosphate source); ii) Filter cake (FC); iii) Triple superphosphate (TSP); iv) Triple superphosphate associated with filter cake (TSP + FC); v) Rock phosphate (RP); and vi) Rock phosphate associated with filter cake (RP + FC). The phosphate fertilizers were applied only once, at seedling transplantation, to assess their residual effect in the soil in the second growth cycle. The amount of organic and mineral fertilizers applied was calculated based on the dose commonly used in sugarcane fields in Brazil, namely: 10 t ha^{-1} of FC (dry basis), and 78.7 mg kg^{-1} of total P (the equivalent of 180 mg kg^{-1} of P_2O_5) as TSP or RP (Raij et al., 1997). When the mineral fertilizers were associated with FC (treatments iv, and vi), the total amount of P applied as TSP or RP (Bayóvar from Peru), was diminished to equilibrate with the P content in FC (0.5%; Table S1).

3.2.2. Soil solution recovery and P quantification

To recover the soil solution from the PVC pots and estimate the dissolution rates of the different phosphate fertilizers, a soil solution sampler device called Rhizon® (Rhizosphere Research Products, Wageningen, Netherlands) was installed in each PVC pot. The material consisted of thin porous Polyethersulfone membranes with pore diameters between 0.12 and 0.18 μm, 5 cm in length, and a 12 cm fiberglass tube. The Rhizons were placed 5 cm deep in the soil, immediately above the fertilizers location.

Soil solution was recovered weekly in the first crop cycle (120 days), by coupling the device in sterile vacuum tubes (Figure S1), and approximately 12 h after the soil irrigation (~70% of the soil´s maximum water holding capacity) the solution was sampled. The total P concentration was determined by colorimetry (limit of detection of 0.02 mg L^{-1} of P) (Murphy and Riley, 1962), and the pH in solution samples was measured by electrode pHmeter.

3.2.3. Quantification of the P content in the sugarcane leaves

The P concentration in the sugarcane leaves was measured every 15 days in the first crop cycle (120 days) using a portable X-ray fluorescence (pXRF) spectrometer model Tracer III-SD (Bruker, USA) with Rh target X-ray tube and silicon bypass detector. The determination started at 35 days after the transplant (DAT) of the seedlings as before that period, leaf development was not sufficient to ensure that the incident X-rays reached only the leaf blade. The measurements were taken under *in vivo* conditions, namely, without detaching the leaf from the plant. Each sugarcane plant had its +1 leaf (Top Visible Dewlap) measured at three distinct points in the middle third of the abaxial surface. The pXRF intensity was obtained at the P Kα peak (2.01 keV) and converted into P concentration (mg kg^{-1}) using an external calibration curve (y = 0.0204x + 8.3047). The generation of the external calibration curve, the measurements, and the data processing were carried out according to the procedure described by Soares et al. (2021). The limit of detection of P in the leaves by pXRF was 0.22 g kg^{-1}.

3.2.4. P sequential fractionation and potential acid phosphatase activity (AP_{ASE}) in the soil

Soil was sampled at 30, 120, and 180 DAT, to assess the effect of treatments on the soil P availability using an auger probe from the surface to the bottom of the PVC pot (15 cm deep), in an effort to reach the total volume of soil fertilized. A portion of each sample was air-dried, sieved at 2 mm, and used to carry out the sequential fractionation of Hedley et al. (1982), with modifications by Rheinheimer (2000). The remainder was kept at 4° C for analysis of the potential acid phosphatase activity (AP $_{ASE}$) by colorimetric estimation of the p-nitrophenol released after soil incubation with a buffered solution (pH 6.5) (Tabatabai and Bremmer, 1969).

From the sequential fractionation, three different pools of P in the soil were determined (Cross and Schlesinger, 1995). The labile pool was obtained by summing the inorganic P (Pi) extracted by anion exchange resin (P $_{AER}$), and the Pi and organic P (Po) extracted by $NaHCO_3$ 0.5 mol L^{-1} (Pi $_{BIC}$ and Po $_{BIC}$). The moderately labile pool was obtained by summing the Pi and Po extracted by NaOH 0.1 mol L^{-1} (Pi $_{HID\ 0.1}$ and Po $_{HID\ 0.1}$), and the P extracted by HCl 1 mol L^{-1} (P $_{HCl}$). The non-labile pool was obtained by summing Pi and Po extracted by NaOH 0.5 mol L^{-1} (Pi $_{HID\ 0.5}$ and Po $_{HID\ 0.5}$), and the residual P (P $_{RES}$) extracted by the soil digestion with concentrated H_2SO_4 + H_2O_2 in the presence of saturated $MgCl_2$.

3.2.5. Statistical analysis

The experiment adopted a completely randomized block design, with six treatments and five repetitions. All data were tested for normality of model residuals, and homogeneity of variance by the Shapiro-Wilk (1965) and Levene (1960) tests, respectively. When necessary, data were transformed using the Box-Cox procedure (Box and Cox, 1964). The data on P concentration in the soil solution and P leaf content were evaluated by one-way ANOVA for repeated measures, while the data of soil P fractionation and AP $_{ASE}$ were analyzed by one-way ANOVA. The differences between the treatments were evaluated by Tukey's posthoc test ($p < 0.05$). All statistical analyses were carried out using SAS 9.3 program (SAS Institute, Inc., Cary, NC, USA) and SISVAR 5.6 analysis system (Ferreira, 2011).

3.3. Results

3.3.1. Solubilization of fertilizers and P availability in the soil solution

The soil solution P concentration resulting from solubilization of the mineral fertilizers and its association with FC is shown in Figure 1. The highest P concentration was seen in the TSP treatments, revealing its high dissolution rate in the first few days after soil fertilization, with a peak of P release at 07 DAT. Although during this period the fertilization with TSP resulted in a higher P concentration than in TSP + FC (~235 and 156 mg L^{-1}, respectively), there was no significant difference between these treatments, which, overall remained as such throughout the subsequent solution assessments.

Throughout the experiment, the P concentration in the soil solution recovered from RP (in the absence of FC) was similar to that obtained in the control treatment (Fig. 1). Interestingly, when RP was associated with FC the soil solution P concentration peaked at 07 DAT (~46 mg L^{-1}) demonstrating behavior similar to that of TSP and TSP + FC, although with a P concentration approximately 5-fold lower than in TSP (~235 mg L^{-1}) and 3.4-fold lower than TSP + FC (~156 mg L^{-1}) (Figure 1). General P concentration provided by these treatments progressively declined from 14 DAT, with the most pronounced drop observed in TSP, with P release in the soil solution equal to RP + FC at 28 DAT (~24 mg L^{-1} to TSP, and 10 mg L^{-1} to RP + FC) and 35 DAT (~31 mg L^{-1} to TSP, and 15 mg L^{-1} to RP + FC) (Fig. 1).

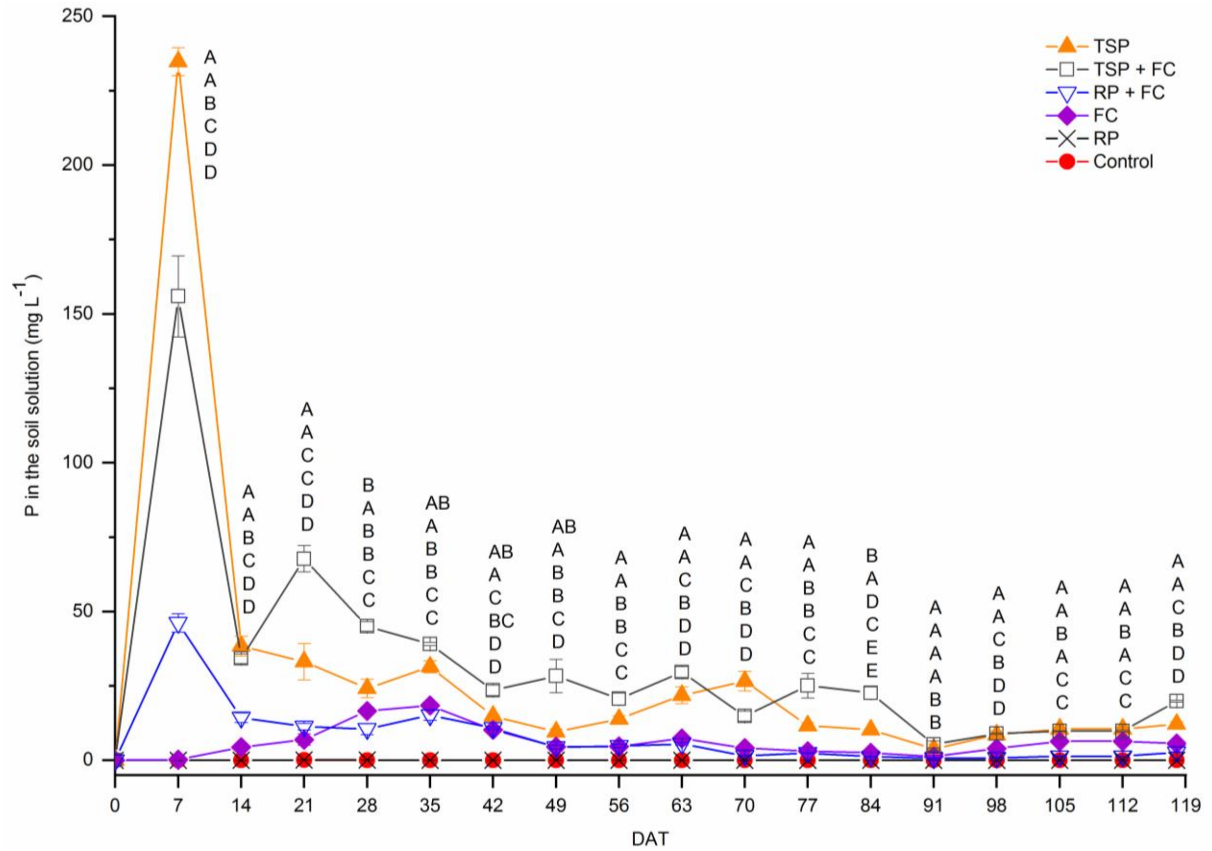

Figure 1. P concentration (mg L^{-1}) in the soil solution recovered weekly for 119 days after transplant (DAT) of seedlings and treatment application. FC: Filter cake; TSP: Triple superphosphate; TSP + FC: Triple superphosphate associated with FC; RP: Rock phosphate; RP + FC: Rock phosphate associated with FC. Vertical error bars correspond to the standard deviation. Means followed by distinct vertical letters (following the same sequence shown in the treatments label) were significantly different according to Tukey´s test ($p < 0.05$)

3.3.2. P content in sugarcane leaves

When comparing the P content in sugarcane leaves and P content in soil solution, we observed that the increases in the foliar P content cause simultaneous depletion in the soil solution, which was more evident from 35 to 77 DAT (Figure 2). This behavior was common to all treatments, except for the control and pure RP, in which the P content in the sugarcane leaves did not differ throughout the experiment, as was also observed in the soil solution. In contrast, plants grown in soil fertilized with RP + FC had a P content in the leaves higher than those grown under the control treatment, similar to that obtained by the addition of TSP (except at 35 DAT).

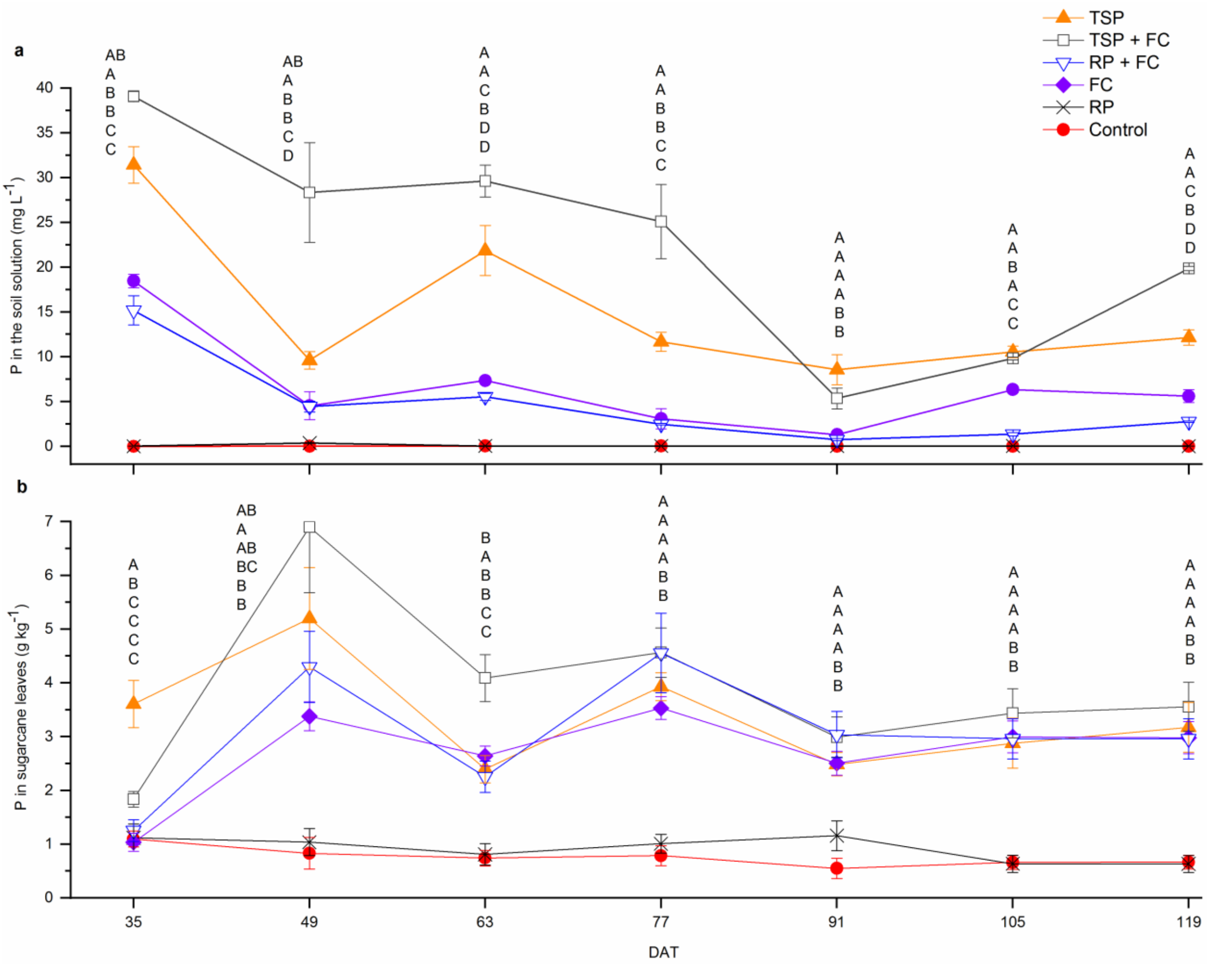

Figure 2. (a) P concentration (mg L^{-1}) in the soil solution recovered every 15 days from 35 to 119 days after transplant (DAT) of seedlings and treatment application. (b) P content in sugarcane leaves (g kg-1) assessed every 15 days from 35 to 119 days after transplant (DAT) of seedlings and treatment application. FC: Filter cake; TSP: Triple superphosphate; TSP + FC: Triple superphosphate associated with FC; RP: Rock phosphate; RP + FC: Rock phosphate associated with FC. Vertical error bars correspond to the standard deviation. Means followed by vertically distinct letters (following the same sequence shown in the treatment labels) were significantly different according to Tukey´s test (p< 0.05)

At the first P quantification in the sugarcane leaves (35 DAT), the highest P content was observed in the TSP treatment (~4 g kg^{-1}). In the subsequent quantifications (49 - 119 DAT) TSP and TSP + FC did not differ from each other in leaf P content (Figure 2).

3.3.3. Potential acid phosphatase activity (AP ASE) and P fractions in the soil

The AP ASE in the soil at 30 DAT was not significantly different between treatments (*p*= 0.146) (Figure 3). At 120 DAT, there was an increase in the AP ASE in all the treatments, especially in the control (~342 mg kg^{-1} h^{-1}), and the soil fertilized with TSP showed the lowest AP_{ASE} (~193 mg kg^{-1} h^{-1}). Lastly, at 180 DAT the AP_{ASE} in the treatments with phosphate fertilization did not differ from the control. However, among the treatments with P fertilization, the AP_{ASE} was higher in RP (~375g kg^{-1} h^{-1}) than in TSP (~199 mg kg^{-1} h^{-1}) and in FC (~263 mg kg^{-1} h^{-1}) (Fig. 3).

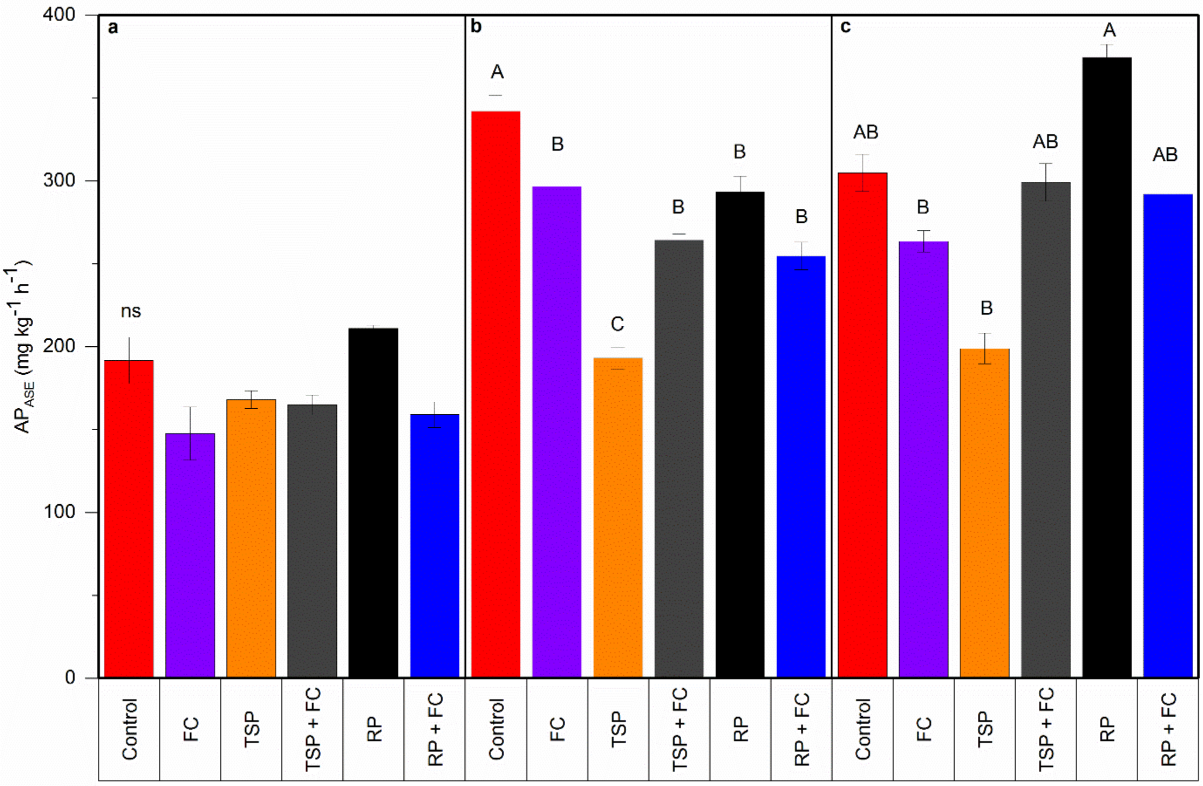

Figure 3. Potential acid phosphatase activity (AP_{ASE}, mg kg^{-1} h^{-1}) evaluated at 30 (a), 120 (b), and 180 (c) days after transplant (DAT) of the seedlings and treatment application. FC: Filter cake; TSP: Triple superphosphate; TSP + FC: Triple superphosphate associated with FC; RP: Rock phosphate; RP + FC: Rock phosphate associated with FC. Vertical error bars correspond to the standard deviation. Means followed by different letters within each evaluated period (30 DAT, 120 DAT, or 180 DAT) were significantly different according to Tukey´s test ($p < 0.05$). ns: Not significant

Considering the soil P fractions, the most significant results were found at 30 and 120 DAT (Figure 4). At 30 DAT the use of TSP individually increased the P content in all the P fractions, which were significantly higher than TSP + FC, except for $Po_{HID\ 0.5}$ (Figure 4 a). By this time, the total labile P pool promoted by TSP (~236 mg kg^{-1}) was approximately 14-fold higher than in TSP + FC (~16 mg kg^{-1}), and 12-fold higher than in RP + FC (~19 mg kg^{-1}). The use of RP + FC resulted in a relative increase in the labile fractions, P_{AER}, and Pi_{BIC} compared to the control, while the use of RP in the absence of FC did not differ from the control in any P fraction at 30 DAT (Figure 4 a).

At 120 DAT there was a marked depletion of the readily available P (P_{AER}) in all treatments (Figure 4 b). In addition, the use of RP + FC was able to keep the Pi_{BIC}, $Pi_{HID\ 0.1}$, and $Pi_{HID\ 0.5}$ fractions at levels equivalent to that promoted by both the TSP treatments (Figure 4 b).

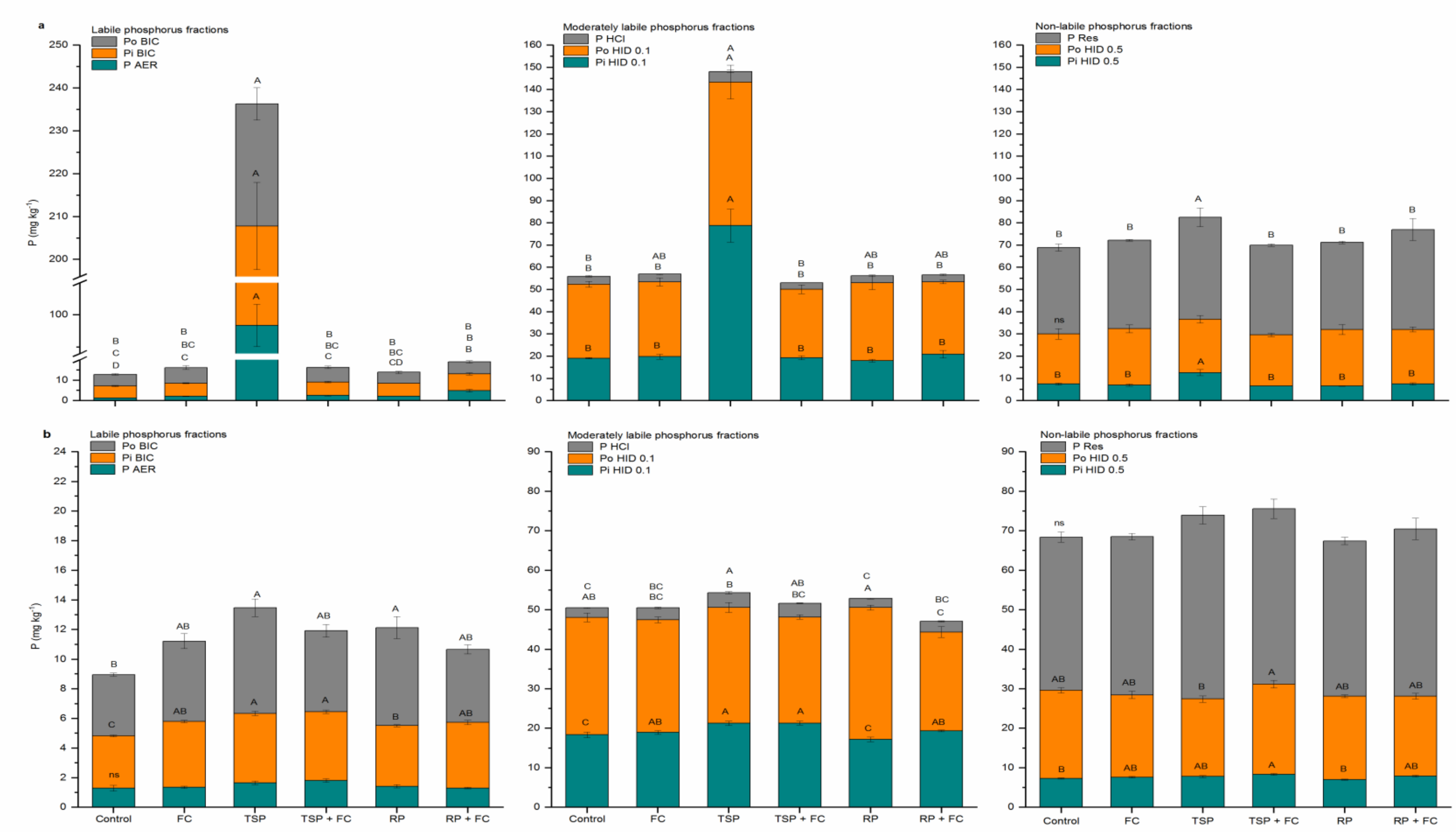

Figure 4. Labile, moderately labile, and non-labile P pools at 30 (a) and120 (b) days after transplant (DAT) of the seedlings and treatment application. FC: Filter cake; TSP: Triple superphosphate; TSP + FC: Triple superphosphate associated with FC; RP: Rock phosphate; RP + FC: Rock phosphate associated with FC. P $_{AER}$: P extracted by anion exchange resin; Pi and Po $_{BIC}$: inorganic and organic P extracted by $NaHCO_3$ 0.5 mol L^{-1}; Pi and Po $_{HID\ 0.1}$: inorganic and organic P extracted by NaOH 0.1 mol L^{-1}; P $_{HCl}$: P extracted by HCl 1 mol L^{-1}; Pi and Po $_{HID\ 0.5}$: inorganic and organic P extracted by NaOH 0.5 mol L^{-1}; P $_{RES}$: Residual P. Vertical error bars correspond to the standard deviation. Within each P fraction, means followed by distinct horizontal letters were significantly different according to Tukey´s test ($p < 0.05$). ns Not significant

3.4. Discussion

The P concentration in the soil solution indicated a slightly higher solubilization rate of TSP than TSP + FC at 07 DAT (Figure 1). This result is consistent with those of Estrada-Bonilla et al. (2021), comparing TSP and TSP mixed with an organic compost. Since most of the P in the FC is found in poorly available forms (Table S1), the mixture of TSP with FC reduced the amount of readily available P applied, which explains the higher soil solution P concentration in TSP.

Of note, the reduction in the amount of P applied as a highly soluble source in TSP + FC did not significantly affect the P concentration in the soil solution throughout the experiment (Figure 1), or in the P content in the sugarcane leaves from 49 DAT (Figure 2 b). These results can be attributed to enhanced P availability in the soil as a consequence of the complexation of H^{+} and Al^{3+} íons by phenolic and carboxylic radicals present in the FC and from the exchange of binders with Fe and Al oxides (Franchini et al., 1999; Pavinato and Rosolem, 2008). This positive effect of FC was proven by Borges et al. (2019) using P X-ray Absorption Near Edge Structure analysis to investigate the species of P in the soil after the addition of a fertilizer with FC in its composition, compared to no FC. The authors reported a decrease in the amount of P associated with kaolinite and oxides/hydroxides of Fe and Al using the fertilizer containing FC.

The most interesting finding was that the curve of P release in the soil solution obtained by RP + FC was similar to that of TSP and TSP + FC, with high P concentration in the first 07 days of assessment and a subsequent gradual decline (Figure 1). Although during this period, the RP + FC was unable to release P with the same concentration as TSP and TSP + FC, our results highlight the positive role of RP + FC in RP solubilization. This effect occurred in two different ways: Initially, between 07 and 14 DAT, there was a direct effect of FC on the RP dissolution reactions, with higher P concentration than in isolated RP and FC treatments. Afterward, from 21 to 56 DAT, the P concentration became equivalent in RP + FC and FC treatments, indicating that the available P came mostly from the mineralization of FC, with no longer effect of FC on the RP dissolution reaction.

Low molecular weight organic acids exuded by plant roots, microorganisms, and by the lysis of OM have been associated with the dissolution of Ca phosphates (Grossl and Inskeep, 1991; Violante and Pigna, 2002; Qin et al., 2013; Wang et al., 2021). Thus, a possible explanation for the results found with the use of RP + FC is that the mineralization of the soluble OM from FC released LMWOAs, which causes the dissolution of the RP, and consequently increased the P in the soil solution. The LMWOAs can also contribute to the dissolution of Ca phosphates by the momentary acidification caused by the proton release in the soil solution (Almeida et al., 2018; Fujii, 2014). In fact, our analysis revealed a marked change in the solution pH from 6.6 to 5.4 in RP + FC treatment at 07 DAT (Figure S2), which was unexpected because the original pH of FC is close to neutral (Table S1), and RPs have no acid dissolution reaction in the soil as TSP has. However, further work is required to establish if the LMWOAs concentration was higher in RP + FC than in the other treatments.

Recently, Helfenstein et al. (2018) demonstrated that there is an inverse relationship between the P concentration in the soil solution and the P turnover (average exchange rate between the phosphate ions in solution and the inorganic phosphate in the solid phase), showing high P turnover values for Oxisols (the type of soil used in our experiment). It means that after P absorption by the roots, this type of soil has a high capacity for restocking the soil solution with phosphate ions by the desorption of P from the solid phase, justifying the opposite behavior of the P concentration curve in the soil solution and the P content in sugarcane leaves, as observed in Figure 2.

The P content in sugarcane leaves and in the soil solution also showed that the use of pure RP is not a feasible strategy since the system was not efficient in providing and restocking P in the soil solution in amounts adequate for plant nutrition. On the other hand, the use of RP associated with FC satisfactorily supplied the sugarcane needs of 1.5 g kg^{-1} of P (Raij et al., 1997), since after 35 DAT the P content in the leaves was similar to the plant grown under the TSP treatment (Figure 2 b). This result supports the positive effect of FC on the greater dissolution of RP, as previously discussed, and implies the possibility of replacing the TSP with RP + FC in crop fertilization.

Although the P content in the leaves was not measured before 35 DAT due to specific limitations of the P quantification using pXRF, the high solubility of TSP at the very beginning of the sugarcane growth was sufficient to keep the foliar P content in plants growing under TSP higher than in TSP + FC up to 35 DAT (Figure 2 b). These differences are consistent with the result of sequential soil P fractionation at

30 DAT in which the P content in the soil fertilized with TSP was higher than in TSP + FC and RP + FC in the three fractions of the labile pool (P $_{AER}$, Pi $_{BIC}$ and Po $_{BIC}$), which represents the P available to plants (Figure 4 a). However, the addition of TSP also increased P content in the moderately labile (especially in Pi and Po $_{HID\ 0.1}$) and the non-labile (Pi $_{HID\ 0.5}$ and P $_{RES}$) pools compared to the other treatments, reflecting the greater interaction of P with Fe^{2+}, Al^{3+}, Ca^{2+} ions, and organic compounds in the solid phase and possible unavailability of P in subsequent periods.

The acid phosphatase enzyme activity is a function of P deficiency in the soil (Richardson and Simpson, 2011). Thus, the non-significant difference in AP $_{ASE}$ between the treatments at 30 DAT (Figure 3 a), suggests that all treatments had the same performance, which contradicts the results of P concentration in soil solution and leaf tissue. However, at 120 DAT the AP $_{ASE}$ in the soil fertilized with TSP was lower than all the other treatments, indicating a high concentration of P in the soil (Figure 3 b).

In the last evaluated period (180 DAT), there seems to be a greater limitation by P than in the previous ones since the AP $_{ASE}$ of all treatments did not differ from the control (Figure 3 c). At this time, the same pattern was observed in the P pools in the soil with a total depletion of Pi in the labile forms (P $_{AER}$ and Pi $_{BIC}$) (Figure S3). Moreover, the low efficiency of RP in providing P at a sufficient rate for plant nutrition was confirmed by the highest AP $_{ASE}$ in the soil treated with pure RP.

Throughout the evaluated period, the results of AP $_{ASE}$ did not show a clear effect of FC, as did in soil solution P and leaves content (Figure 3). Other mechanisms of P acquisition, such as increased root biomass production and the association with arbuscular mycorrhizal fungi have been demonstrated for the variety RB 69-9628 and seem to be more effective than the exudation of acid phosphatases (da Silveira et al., 2014; Arruda et al., 2019). Therefore, considering the sugarcane cultivar used in our study and the similarity of root dry matter between treatments at 180 DAT (Table S2) the isolated evaluation of AP $_{ASE}$ was not a good indicator of the FC effect on the P bioavailability.

The labile pool is the P compartment obtained by the fractionation of the soil that most closely correlates with the P in the solution, especially the P $_{AER}$. Thus, the higher P content in the P $_{AER}$ found in TSP, compared to TSP + FC, was not expected since in the soil solution these treatments did not differ. This divergence demonstrates the diversity of interactions that P undergoes in the soil so that minor variations in the element concentration in the solution result in changes in its dynamics in the solid phase and, consequently, in its availability for plants.

The increased P concentration in the labile, moderately labile, and non-labile pools in the soil provided by TSP at 30 DAT are consistent with those found in other studies with TSP (Gatiboni et al., 2021; Soltangheisi et al., 2018), although it is not desirable because it means that P is being accumulated in less available forms in the short term. This dynamic is one of the main concerns regarding the use of highly soluble fertilizers, such as TSP, since more intense adsorption and precipitation reactions may occur when delivering high P concentrations in solution. Given this framework, the use of RP + FC and TSP + FC seems to be an appropriate alternative. Although the P content in the labile P in these treatments was lower than in TSP (~12 and 14–fold, respectively), the P content in the sugarcane leaves was satisfactory and the P release in the soil solution was more uniform over time.

Our results demonstrate that the FC changes, for the main part, the labile and moderately labile pools, which are the most reactive and dynamic ones (Figure 4). The Pi_{BIC} acts as a source of P when its depletion occurs in the soil solution. At the same time, $Pi_{HID\ 0.1}$ buffers the labile pool when plants and microorganisms consume it (Conte et al., 2003; Tiessen et al., 1984).

3.5. Conclusions

The increases observed in soil solution P content and in the labile and moderately labile P pools promoted by the association of rock phosphate with filter cake provided evidence that this organic byproduct affects the solubilization of rock phosphate, with a direct effect on its dissolution reactions up to 14 days after treatment application. Furthermore, the use of filter cake associated with triple superphosphate did not affect the availability and P uptake by sugarcane, enabling the reduction of P supplied as triple superphosphate. Great efforts have been made to develop slower-release fertilizers to reduce strong and stable P-bonds in the soil. With our results we can conclude that the combination of filter cake with mineral fertilizers in phosphate fertilization management is a feasible strategy for achieving this goal in the sugarcane crop, especially through the possibility of expanding the use of rock phosphate, replacing the triple superphosphate.

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Supplementary material

Figure S1. Soil solution recovered with Rhizon® (Rhizosphere Research Products, Wageningen, Netherlands) and sterile vacuum tube

Table S1. Chemical properties of the filter cake applied used in the experiment

pH [a]	**C (%)** [b]	**P (%)** [c]	**N** [c]	**K_2O** [c]	**CaO** [c]	**MgO** [c]	**SO_4** [c]
			g dm^{-3}				
6.9	36.7	0.55	16.6	7.3	17.4	28.3	9.4
Cu [c]	**Fe** [c]	**Mn** [c]	**Zn** [c]	**H_2O**	**P Pools** [d] **(mg kg^{-1})**		
--------------- mg dm^{-3} --------------				**(%)**	**labile P**	**m-labile P**	**n-labile P**
34	6519	813	75	66.3	858.1	4740.3	5326.5

[a] pH in $CaCl_2$ 0.01 mol L^{-1} at a ratio of 1:2.5 (m/v) (Raij et al., 2011); [b] Determined by loss of mass by ignition; [c] Determined following Malavolta et al., 1989; [d] Determined by Hedley et al., (1982), with modifications by Rheinheimer (2000). Labile P: labile phosphorus pool; m-labile: moderately labile phosphorus pool; n-labile: non-labile phosphorus pool

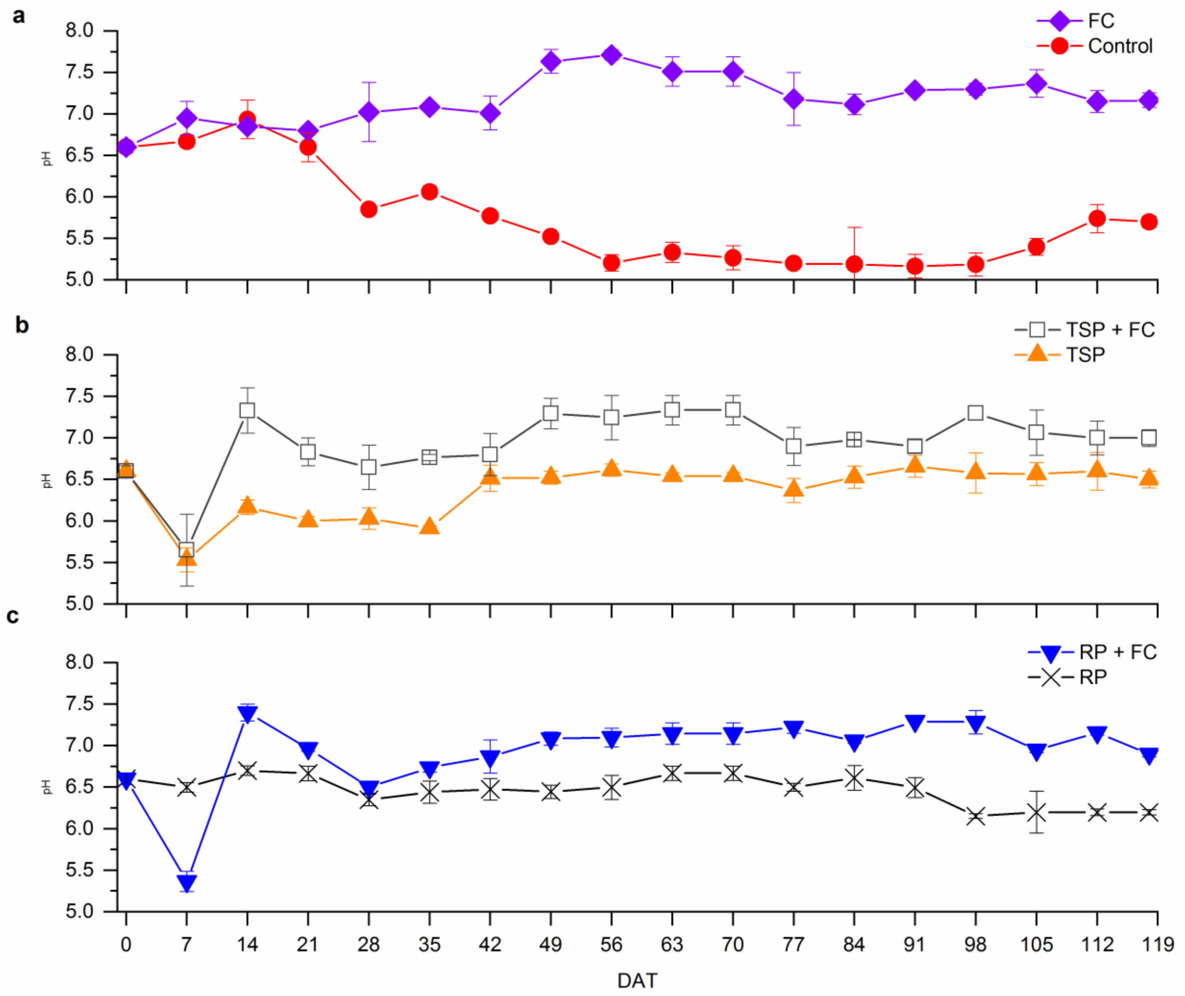

Figure S2. pH in the soil solution recovered weekly for 119 days after transplant (DAT) of seedlings and treatment application. (a) Filter cake (FC) and control treatments; (b) Triple superphosphate (TSP) and Triple superphosphate associated with FC (TSP + FC) treatments; Rock phosphate (RP) and Rock phosphate associated with (RP + FC) treatments. Vertical error bars correspond to the standard deviation

Table S2. Shoot and root dry matter yield in the sugar cane grown for 180 days after transplant (DAT) in response to treatments control, filter cake (FC), triple superphosphate (TSP), triple superphosphate associated with FC (TSP + FC), rock phosphate (RP), and rock phosphate associated with FC (RP + FC).

Treatments	g plant^{-1}	
	Shoot dry matter [a]	Root dry matter
Control	100.7 C	19.2 B
FC	270.3 A	66.4 AB
TSP	261.8 A	68.4 A
TSP + FC	270.0 A	51.2 AB
RP	138.1 B	35.6 AB
RP + FC	285.4 A	44.8 AB

[a] Shoot dry matter is the sum of the dry matter obtained in two crop cycles (at 120 and 180 days after transplant). Means followed by vertically distinct letters were significantly different according to Tukey´s test (p< 0.05)

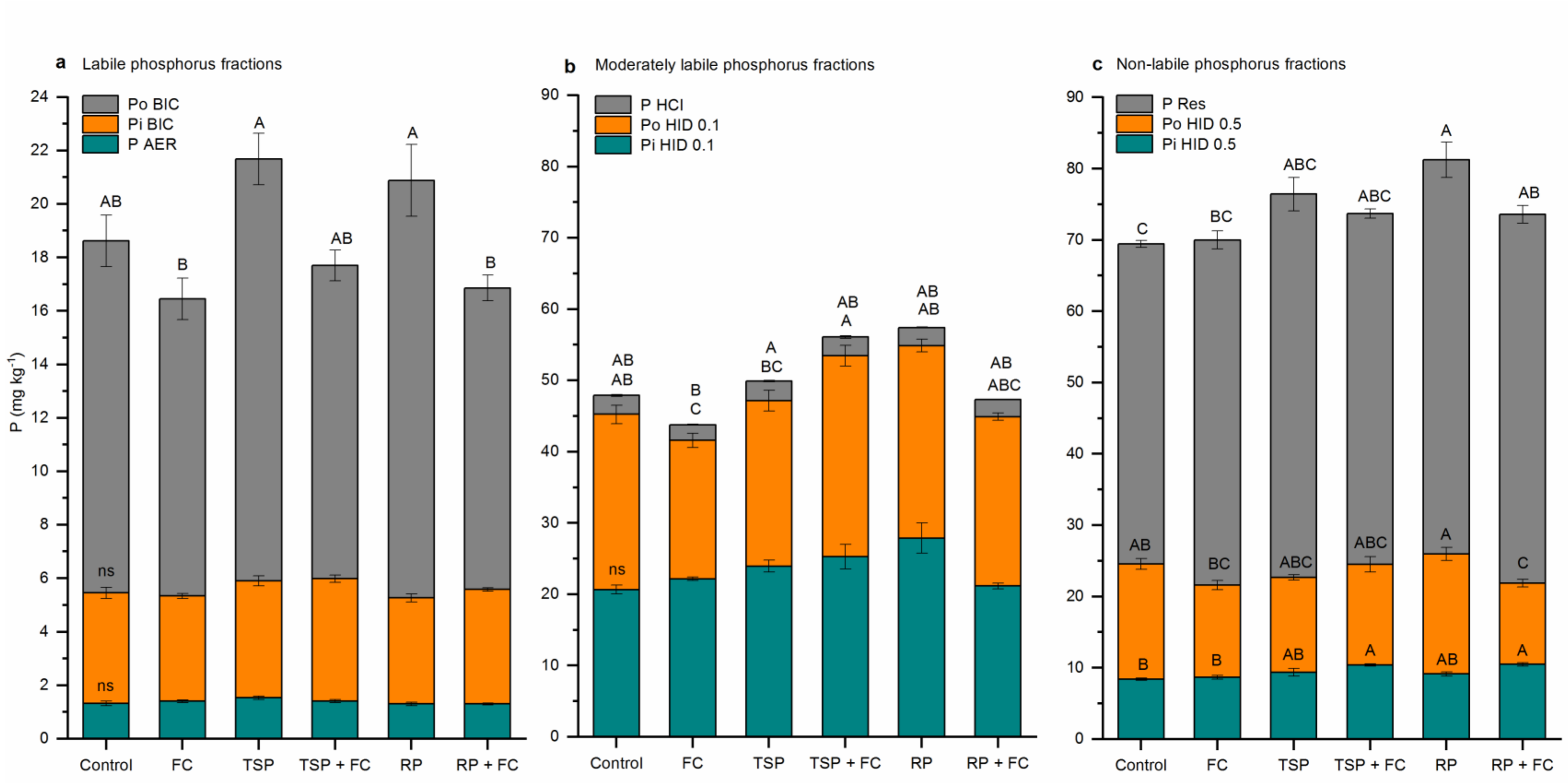

Figure S3. (a) Labile, (b) moderately labile, and (c) non-labile P pools at 180 days after transplant (DAT) of the seedlings and treatment application. FC: Filter cake; TSP: Triple superphosphate; TSP + FC: Triple superphosphate associated with FC; RP: Rock phosphate; RP + FC: Rock phosphate associated with FC. P $_{AER}$: P extracted by anion exchange resin; Pi and Po $_{BIC}$: inorganic and organic P extracted by $NaHCO_3$ 0.5 mol L^{-1}; Pi and Po $_{HID\ 0.1}$: inorganic and organic P extracted by NaOH 0.1 mol L^{-1}; P $_{HCl}$: P extracted by HCl 1 mol L^{-1}; Pi and Po $_{HID\ 0.5}$: inorganic and organic P extracted by NaOH 0.5 mol L^{-1}; P $_{RES}$: Residual P. Vertical error bars correspond to the standard deviation. Within each P fraction, means followed by distinct horizontal letters were significantly different according to Tukey´s test ($p < 0.05$). ^{ns} Not significant

Figure S4. Sugar cane plants cultivated for 180 days. (A) control, (B) filter cake, (C) triple superphosphate, (D) triple superphosphate associated with filter cake, (E) rock phosphate, and (F) rock phosphate associated with filter cake.

4. LABILITY AND DYNAMICS OF P FROM MINERAL AND ORGANIC FERTILIZERS IN ACIDIC AND ALKALINE SOILS

Abstract

The recycling of phosphorus (P) from organic residues is the key to overcoming the low use efficiency and environmental problems related to mineral fertilizer use. Filter cake (FC) is a byproduct from the sugar/ethanol industry used in sugarcane fields to recycle nutrients. This study aimed to investigate the release, mobility, and lability of P from FC associated or not with mineral fertilizers (TSP and RP) in acidic and alkaline soils. The treatments were: pure FC, pure triple superphosphate (TSP), pure rock phosphate (RP), TSP+FC (35:65), and RP+FC (35:65). The dissolution rate was investigated for 10 consecutive days in a column perfusion system. In a 5-wk soil incubation experiment, the total P distribution (%Total P) and the Resin P (%Resin P) were assessed in three soil sections sampled around the fertilizer application point. FC showed rapid mineralization, which was favored in acidic soil. The dual and gradual P release was observed only for TSP. In the non-limed acidic soil, the association of FC with TSP reduced the P diffusion and availability, but not in the limed soil, with the possibility of reducing the use of TSP. FC increased the soil pH, positively affecting the %ResinP in the non-limed acidic soil when it was combined with RP. Due to the alkaline soil chemical characteristics, no effect of FC on P diffusion and availability was observed and only TSP (+ and –FC), was efficient in increasing the available P in the three soil. Thus, FC affected the P diffusion and availability differently in the soils.

Keywords: P recycling; P diffusion; Sugarcane byproduct; Kinetics release

4.1. Introduction

The phosphate fertilization is challenging in soils with high phosphorus (P) sorption capacity, either due to the high content of Fe and Al (oxy) hydroxides in acidic soils (Norrish and Rosser 1983) or due to the high Ca content in neutral to alkaline soils (Lindsay et al., 1989). In these conditions, P can be converted into insoluble forms by surface precipitation with Fe, Al, and Ca, or by inner and out-sphere adsorption reactions into the surface of minerals, especially around the fertilizer application point (Beauchemin et al., 2003). The pH is one of the parameters which determines the soil´s ability to retain P (Kana et al., 2011). The increase in pH leads to the precipitation of P as Ca phosphates, while its reduction induces the formation of Fe and Al phosphates of low solubility (Wright, 2009).

In addition to being a relatively scarce nutrient in most terrestrial ecosystems (Sullivan et al., 2014), the soil P-fixation reactions make the efficiency of mineral phosphate fertilizers usually less than 30% (Norrish and Rosser, 1983). However, finding the best strategy for adding P via mineral fertilizers is not easy. While the rapid dissolution of highly soluble acidulated phosphates, like triple superphosphate (TSP), favors soil fixation reactions (Menezes-Blackburn et al., 2016), the low dissolution of rock phosphates (RP) in soils with pH above 5.5 (Rajan et al., 2004) can lead to P deficiency to crop development. Even acidic soils can show pH above 5.5 due to the addition of agricultural limestone [$Ca(Mg)CO_3$] to reduce the H^+ effect and neutralize the Al toxicity, increasing the nutrient availability and cation exchange capacity (Scott et al. 2003; Baquy et al., 2018).

To reduce the P fixation in tropical soils the best management strategy for phosphate fertilization must take into account the use of a P source with intermediate solubility between highly water-soluble and slow-release fertilizers. It is also necessary to consider that RP which originates mineral fertilizers is a finite resource, which must be preserved. Nutrient recycling from byproducts can fit in this approach with additional gains in environmental performance. Among them, the filter cake (FC) a byproduct of sugarcane industrial processing, used in combination with mineral fertilizers has shown great potential in increasing the sugarcane yield (Santos et al. 2014; Caione et al. 2015; Moda et al. 2015; Vazquez et al. 2015; Bekheet et al. 2018; Borges et al. 2019; Lima Vasconcelos et al. 2020; Estrada-Bonilla et al., 2021). Worldwide, sugarcane production reaches 532 million tons per year (FAOSTAT, 2019), and tends to increase to supply the demand for clean and renewable energy. As consequence, the amount of FC generated will also increase, since, for each ton of sugarcane processed, 30 - 40 kg of FC is generated (Prado et al., 2013).

Based on the knowledge that organic materials can reduce the P-fixing capacity in the soil, increasing its availability for plants (Caione et al., 2015; Withers et al., 2018), in a recent study, we evaluated the FC effect, associated or not with TSP and RP, in the release and dynamics of P in the soil. We conclude that FC associated with RP increased the P content in soil solution, and also the labile and moderately labile P pools were increased when compared with pure RP (Soares et al., 2022). However, the study was carried out only in acidic soil and in the presence of plant, which has their own mechanisms of phosphate solubilization and acquisition (Hinsinger et al., 2011). It was unclear whether the greater P release and availability were related to a direct effect of FC on RP dissolution, or due to the different P release kinetics of these two fertilizers. As the P availability does not depend only on soil properties, but also on the fertilizer´s characteristics, such as its composition (Williams, 1971), rate of P release, and interaction with soil constituents, it is necessary to deeply investigate the solubility, mobility, and lability of P from FC when interacting with mineral fertilizers.

We hypothesized here that the association of FC with mineral fertilizers will change the P kinetics and release, resulting in a dual and gradual nutrient release, and reducing soil P-fixation. As result, P will be more available and diffuse to further distances from the application point. This research aimed to investigate the release, mobility, and lability of P from FC associated or not with mineral fertilizers (TSP and RP) in acidic and alkaline soils**.**

4.2. Material and Methods

4.2.1. Filter cake characterization

The basic chemical analysis of FC is shown in Table 1. To further characterize it, a sample of the material was air-dried, finely ground (< 150 μm), homogenized, spread in a double-side carbon tape, and analyzed by P K-edge X-ray Absorption Near Edge Structure (XANES) at the Soft X-ray Spectroscopy (SXS) beamline of the Brazilian Synchrotron Light Laboratory (Campinas, Brazil). After the monochromator energy adjusting to 2151.7 eV using hydroxyapatite standard, eight scans were collected in the fluorescence mode, using a vacuum chamber (~10-7 mbar). The scans obtained were merged, and the reference energy (E_0) was adjusted to the maximum of the first derivative. Background correction was performed by fitting a linear first-order polynomial to the pre-edge region and a second-order polynomial over the post-edge region, using the software package Athena (Ravel and Newville, 2005). Linear combination (LC) fitting was carried out using previously collected standards of phytate, lecithin, PO_4-hematite (Fe_2O_3), PO_4-goethite (FeOOH), hydroxyapatite [$Ca_5(PO_4)_3OH$], amorphous calcium phosphate and octacalcium phosphate [$Ca_8H_2(PO_4)_6.5H_2O$]. The E_0 was allowed to variate from 0.4 to 0.5 eV and the sum of the weights of the standards was forced to result in one. The choice of the best LC fitting took into account the R^2 and Chi^2 values.

Table 1. Chemical properties of the filter cake used in the experiments

pH [a]	C (%) [b]	P (%) [c]	N [c]	K_2O [c]	CaO [c]	MgO [c]	SO_4 [c]
					$g\ dm^{-3}$		
6.9	36.7	0.55	16.6	7.3	17.4	28.3	9.4
Cu [c]	**Fe [c]**	**Mn [c]**	**Zn [c]**	**H_2O**	**P Pools [d] ($mg\ kg^{-1}$)**		
-------------- $mg\ dm^{-3}$ --------------				**(%)**	**LP**	**MLP**	**NLP**
34	6519	813	75	66.3	858.1	4740.3	5326.5

[a] pH in $CaCl_2$ 0.01 mol L^{-1} at a ratio of 1:2.5 (m/v) (Raij et al., 2011); [b] Determined by loss of mass by ignition; [c] Determined following Malavolta et al., 1989; [d] Determined by Hedley et al., (1982), with modifications by Rheinheimer (2000). LP: labile phosphorus pool; MLP: moderately labile phosphorus pool; NLP: non-labile phosphorus pool

4.2.2. Kinetics of P release by fertilizers

The dissolution/mineralization and P release from fertilizers were evaluated in a column perfusion test following the methodology described by Baird et al. (2019), with modifications. Treatments were tested in 3 repetitions as follows: I) pure FC; II) pure TSP; III) TSP mixed with FC (TSP+FC), in the mineral: organic ratio of 65:35; IV) pure RP (hydroxylapatite for analysis $Ca_5(PO_4)_3OH$, Thermo Scientific™); and V) RP mixed with FC (RP+FC) in the mineral: organic ratio of 65:35. Approximately 0.5 g (weight recorded) of grounded fertilizers (powder) were placed between 0.45 µm nylon membrane filter (Merck Millipore™) and 0.5 g of silanized glass wool (Sigma Aldrich™), in a glass column with 24 mL capacity, 300 mm length, 0.79 cm^3 cross-section area, and 10 mm inner diameter (DWK Life Sciences Kimble Kontes FlexColumns™) (Figure S2). Considering the total P content in each fertilizer (19.6% in TSP, 18% in RP, and 0.55% in FC), the total P applied in each treatment was: 3.02 mg in FC; 100 mg in TSP; 76.08 mg in TSP+FC; 93.10 mg in RP; and 70.67 mg in RP+FC.

Columns were continuously flushed with demineralized water (pH 6.0 ± 0.1) pumped from the bottom to the top using a multi-channel peristaltic pump set to a flow rate of 10 mL h^{-1}. Eluents were collected for up to 240 hs (10 days), at intervals of 1 h in the first 12 h of flushing; 2 h from 12 to 24 h; 3 h from 25 to 72 h; and once a day after 72 h of flushing. The collected samples were immediately filtered through a 0.7 µm glass microfiber filter (Whatman GF/F ™) and the pH measured. The eluents from treatments I, II, and V, were acid digested with nitric acid (1:10), to avoid the presence of microorganisms in the sample. Total P (mg L^{-1}) was determined in all samples by ICP-OES. After, the cumulative P released for each fertilizer was calculated as the percentage of total P (mg) released relative to the total P (mg) add in the column.

4.2.3. Mobility and potential availability of P to plants

The P diffusion and its potential availability in the soil were measured at the end of a laboratory incubation experiment lasting 5 weeks, a minimum period for FC mineralization (Soares et al., 2022). Treatments were the same as described in item 2.2, plus a control (nil-P added). The fertilizers were tested in three contrasting soil conditions: I. acidic sandy loam Oxisol natural (Soil Survey Staff, 2014); II. acidic sandy loam Oxisol limed; III. alkaline silt loam Mollisol (Soil Survey Staff, 2014). The soil used in conditions I and II was sampled in Piracicaba city, São Paulo, Brazil, and the soil used in condition III was sampled in Manhattan city, Kansas, USA. The pre-experimental chemical and physical soils characterization are shown in Table 2.

Soils were sampled at 0 - 20 cm depth, air-dried, sieved to < 2 mm, and tested to the maximum water holding capacity (MWHC) (Jenkinson and Powlson, 1976). Following the chemical properties of the acidic soil, 320 mg kg^{-1} of Ca as $CaCO_3$, and 60 mg kg^{-1} of Mg as $MgCO_3$ were added and mixed to the soil, to reach 20 $mmol_c$ kg^{-1} of Ca, and 8 $mmol_c$ kg^{-1} of Mg. This limed soil was incubated in the dark at 25 °C (Precision Low Temp Incubator, Waltham, MA) for 15 days before the experiment.

Each soil type was pre-moistened with ~10% of the deionized water demanded to reach 60% of the MWHC (which was 0.21 L kg^{-1} of soil acidic, and 0.42 L kg^{-1} of alkaline soil), and carefully packed in plastic Petri dishes (87 mm diameter and 11 mm height), to obtain a bulk density of ~1.2 g cm^{-3}. The remaining 90% of deionized water was slowly dripped into the soil, and then, the Petri Dish was sealed with Parafilm, wrapped in aluminum foil, and kept at room temperature for moisture equilibration. After 24 h, the dishes were opened, the soil in the center section (0-7.5 mm) was removed, and the treatments were applied at the dose of 3.3 mg of P. Due to the different amounts of fertilizer needed to reach this dose, in treatments II and IV, the fertilizers were mixed with silanized glass wool (Sigma Aldrich™). The amount of glass wool needed was calculated considering the same final density for all Petri dishes. Thus, the dishes were sealed and wrapped again and kept incubated in the dark at 25 °C, for 5 weeks. At the end of this period, the soil was sampled in the layers between 7.5–13.5, 13.5–25.5, and > 25.5 mm radius from the fertilizer application point, namely: section one (Sec 1); section 2 (Sec 2); and section 3 (Sec 3) (Figure S1). The soil samples were oven-dried (40° C), weighed, and grounded with an Agate mortar and pestle. The pH was measured in a 1:10 soil: water extract (Watson and Brown, 1998). The potential plant available P (Resin P), was recovered by anion exchange resin (AER) and quantified colorimetrically (Murphy and Riley, 1962). The total P was determined via ICP-OES, after aqua regia digestion (1:3 *v/v* HNO_3:HCl).

Table 2. Chemical and physical characterization of Oxisol (acidic) and Mollisol (alkaline) used in the incubation experiment.

	Oxisol	Mollisol
pH [a]	4.9	7.6
OM (g kg^{-1}) [b]	14	40
P (mg kg^{-1}) [c]	6	342
K^+ ($mmol_c$ kg^{-1})	1 [c]	12.6 [d]
Ca^{2+} ($mmol_c$ kg^{-1})	4 [c]	136.9 [d]
Mg^{2+} ($mmol_c$ kg^{-1})	3 [c]	15.6 [d]
Na^+ ($mmol_c$ kg^{-1})	-	0.6 [d]
Fe^{2+} (mg dm^{-3}) [e]	48	22.8
Al^{3+} ($mmol_c$ kg^{-1}) [f]	2	0
H + Al ($mmol_c$ kg^{-1}) [g]	22	-
SB ($mmol_c$ kg^{-1}) [h]	8	165.7
CEC (mmolc kg^{-1}) [i]	30	170
V (%) [j]	27	97.5
m (%) [k]	20	0
Clay (g.kg^{-1}) [m]	168	220
Silt (g.kg^{-1}) [m]	92	580
Sand (g.kg^{-1}) [m]	740	200

[a] pH in H_2O, with ratio soil: water solution of 1: 10 m/v; [b] OM: organic matter determined by Walkley-Black (1934); [c] extracted by the anion exchange resin (Myers et al., 2005); [d] extracted with ammonium acetate solution (1M, pH 7.0) (Warncke and Brown, 1998); [e] DTPA extraction (Whitney, 1998); [f] extracted by KCl 1 mol L^{-1} (Raij, et al., 2001); [g] extracted by SMP (Shoemaker, Mclean, Pratt, 1971); [h] SB: sum of bases; [i] CEC: cation exchange capacity; [j] V%: base saturation of CEC; [k] M%: Al saturation; [m] granulometry determined by Pippet method (Kilmer and Alexander, 1949).

Even though fertilizers were added in powder form, it is impossible to guarantee the uniformity of total P concentration in the fertilizer sample used. Thus, Resin P in each section was normalized according to eq (1), described by Pierzynski and Hettiarachchi (2018). For the same reason, was calculated the percentage of P derived from fertilizers in each soil section, following eq. 2.

$$\% \, Resin \, Pi = \frac{Resin \, Pi}{Total \, Pi} \; x \; 100 \tag{1}$$

Where *i* is the dish section (1-3), *Resin Pi* is the potential plant available P (mg kg^{-1}) in the dish section, and *Total Pi* is the total P (mg kg^{-1}) concentration in the dish section.

$$\% \, Total \, Pi = \frac{\lfloor (Total \, Pi - Total \, Soil \, P) \; x \; Wi \; \rfloor}{\Sigma i_{\;(1-3)} = \; \lfloor (Total \, Pi - Total \, Soil \, P) \; x \; Wi \rfloor} \; x \; 100 \tag{2}$$

Where *i* is the dish section (1-3), *Total Pi* is the total P (mg kg^{-1}) concentration in the dish section, *Total Soil P* is the total P (mg kg^{-1}) concentration in the native soil, *Wi* is the soil weight of the respective dish section.

4.2.4. Statistical analysis

In the incubation experiment, treatments were arranged in a complete randomized design, with fertilizer type as the main plot, and soil sections as the sub-plot. The least significant difference (LSD) was used to compare the treatment means ($p < 0.05$). In the column perfusion test, treatments were arranged in a complete randomized design with three repetitions, and the cumulative P release was plotted against the time. All statistical analyses were performed using the SISVAR 5.6 analysis system (Ferreira, 2011).

4.3. Results

4.3.1. Kinetics of P release by fertilizers

The LC fitting of the XANES spectroscopy analysis showed that most of the P present in FC was linked to Fe and Ca (Figure 1), agreeing with its chemical characterization (Table 1). However, we must take into account that in the standards used P is bound to Ca and Fe as minerals, which should not be the case for FC. As an organic material, FC has a complex organic composition, with distinct degrees of decomposition. Thus, these results just help to understand the P interaction with other constituents and its dissolution behavior over time but do not precisely quantify each P species.

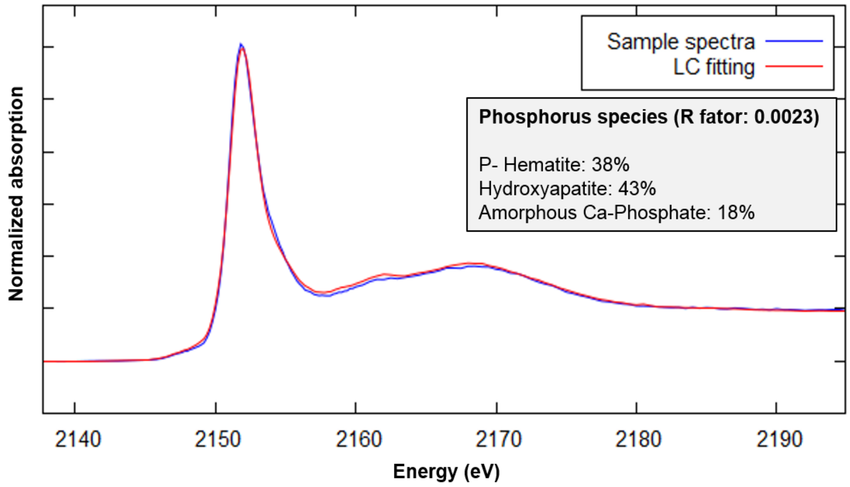

Figure 1. Normalized Phosphorus K-edge XANES spectra with results of the linear combination (LC) fitting for filter cake used in the incubation study.

In Figures 2A and 2B, it is observed that the dissolution of TSP+FC over time was intermediate between TSP and FC, with the cumulative P release (%) lower than in pure TSP alone, but higher than pure FC alone. The cumulative P released in TSP and TSP+FC peaked at 12 h, with ~86%, and 80% of total P released respectively (Figure 2A). When hourly evaluated, both the treatments revealed that more than 30% of the total P was released within 1 h, when the solution pH dropped from 6.0 to < 3.5 (Figure 2B and 2C). The release of P from FC also peaked in the initial time, but it takes 2 h to achieve ~30% of the total P content released (Figures 2A and 2B). A gradual increase in solution pH was observed under FC up to 7 hours, stabilizing nearly pH 7.0 after that (Figure 2C).

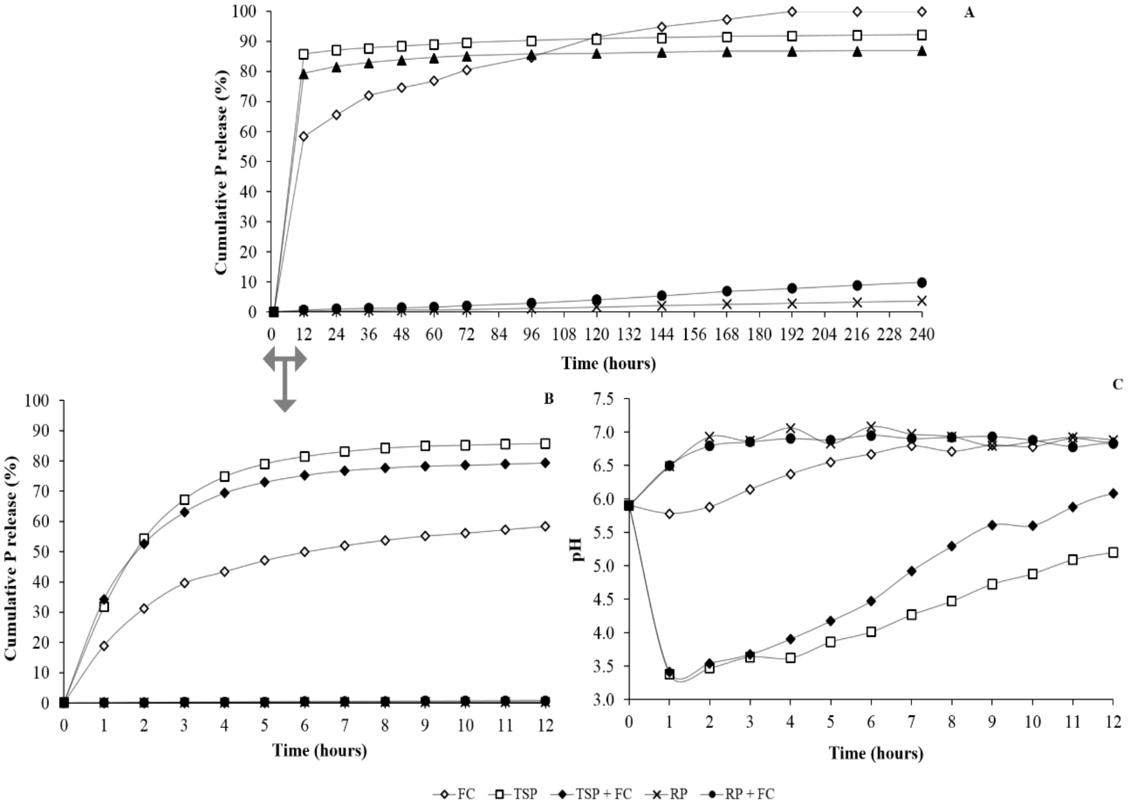

Figure 2. Kinetics of phosphorus release from filter cake (FC), triple superphosphate (TSP), triple superphosphate associated with filter cake (TSP+FC), rock phosphate (RP), and rock phosphate associated with filter cake (RP+FC), for 240 h (A) and in more details up to 12 h (B). Solution pH from FC, TSP, TSP+FC, RP, and RP+FC, for 240 h (C). Values are means of three repetitions.

All the total P from FC was recovered in the solution within 192 h, while TSP and TSP+FC had 92% and 87% of the P content released after 240 h, respectively. The cumulative P released (%) for RP+FC was slightly higher than RP from 96 h to the end of the experiment but did not reach even 10% of the total P added (Figure 2A). In both treatments, the solution pH increased to ~7.0 within 3 h and remained nearly this pH after that (Figure 2C).

4.3.2. Mobility and potential availability of P to plants

The percentage of total P diffused into Sec 1 (7.5-13.5 mm) in the acidic soil was equivalent between the treatments, except for TSP which was lower than FC and RP+FC (Figure 3A). However, in Sec 2 (13.5-25.5 mm) TSP showed the highest %Total P, followed by RP, TSP+FC, RP+FC, and FC. In Sec 3 (> 25 mm), FC showed the highest %Total P. In this acidic soil condition, the available P (%Resin P) in the Sec 1 decreased following the order: TSP > TSP+FC > RP+FC = FC > RP. In Sec 2 (13.5-25.5 mm), TSP combined or not with FC, resulted in a higher %Resin P (~51% and 24% in TSP and TSP+FC, respectively), compared to control (Figure 3B).

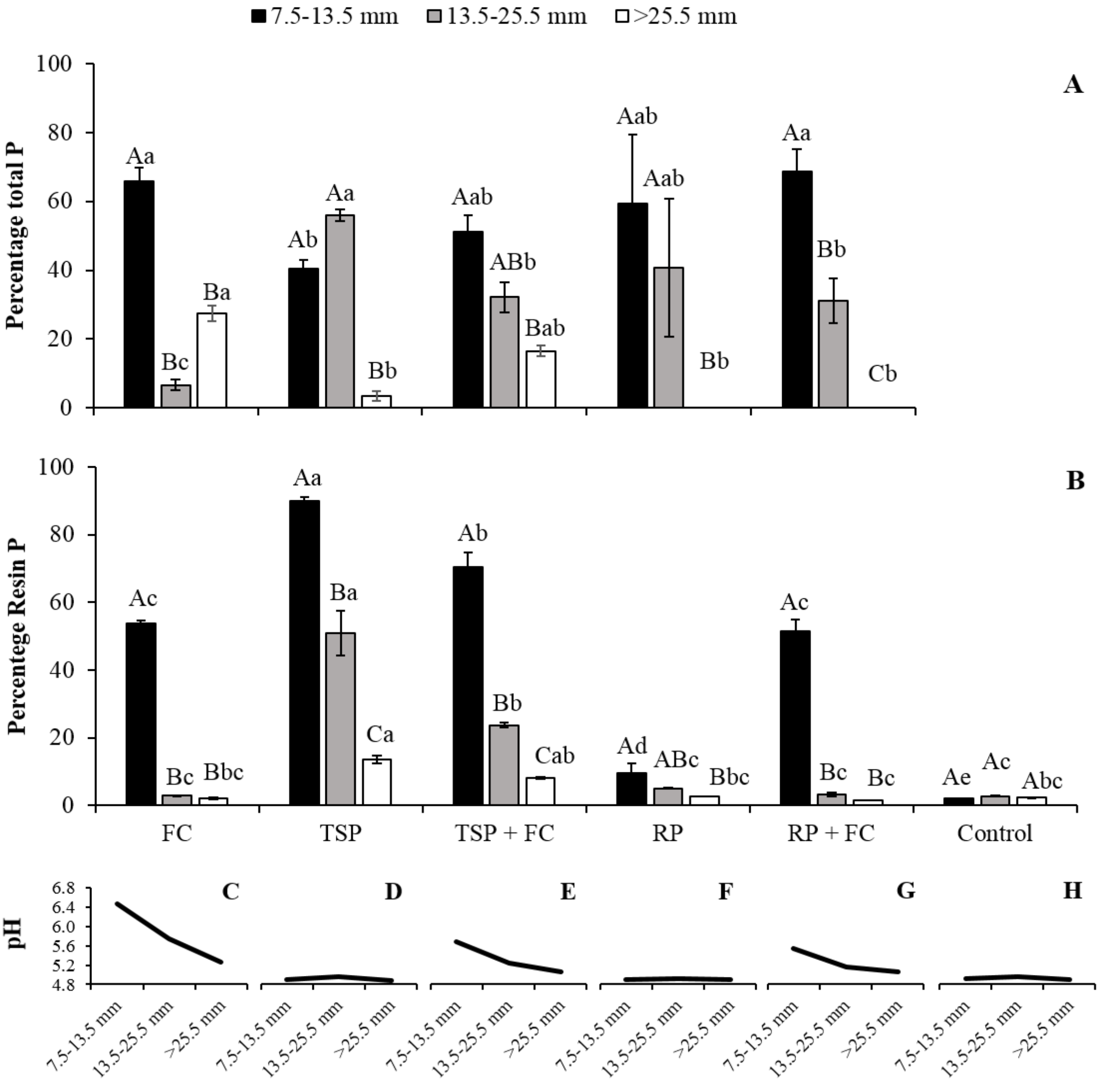

Figure 3. Percentage of total P (%Total P) (A), and percentage of available P (%Resin P) (B) from fertilizers incubated in acidic soil in the Petri dish´s sections (7.5-13.5 mm, 13.5- 25.5 mm, and > 25 mm). Soil pH in each section for FC (C), TSP (D), TSP+FC (E), RP (F), RP+FC (G), and control (H). FC: Filter cake; TSP: Triple superphosphate; TSP+FC: Triple superphosphate associated with FC; RP: Rock phosphate; RP+FC: Rock phosphate associated with FC. Vertical error bars correspond to the standard deviation. Within each treatment, means followed by distinct capital letters differ according to Tukey´s test ($p < 0.05$). Within each soil section means followed by distinct lower case letters differ according to Tukey´s test ($p < 0.05$).

The comparison of sections within each treatment in the acidic soil showed that over 65% of the total P added via FC accumulates in Sec 1 (Figure 3A), and ~54% of the total P in this section was recovered by Resin (Figure 3B). The use of RP+FC showed a similar %Total P and %Resin P than FC in Sec 1, however, P diffusion did not reach Sec 3 as did under FC. For TSP, TSP+FC, and RP, the %Total P in Sec 1 did not differ from that found in Sec 2. In contrast, there was a greater %Resin P in this section compared to Sec 2 for TSP, TSP+FC, and RP.

When the acidic soil was limed, the distribution of P derived from fertilizer was distinct from that without liming (Figure 4A). Therefore, the greater %Total P in Sec 1 was observed with FC and RP addition, followed by TSP+FC, TSP, and RP+FC. As consequence, the opposite behavior was observed in Sec 2, where the %Total P was significantly higher in RP+FC than in the other treatments. None of the treatments was able to significantly increase the %Total P in Sec 3. The addition of RP mixed or not with FC resulted in the lowest %Resin P (< 30%), while FC, TSP, and TSP+FC did not differ from each other (Figure 4B). In Sec 2 the use of TSP increased the %Resin P more efficiently than TSP+FC, similar to the results observed without liming. FC, RP, and RP+FC were not able to increase the %Resin P in Sec 2. Finally, in Sec 3 only TSP increased the %Resin P relative to control.

The comparison between soil sections within treatments highlights that all the fertilizers tested in the acidic limed soil resulted in greater accumulation of Total and Resin P in Sec 1 in detriment to Sec 2 and/or Sec 3 (Figure 4A). The exception was RP+FC, with a higher %Total P in Sec 2, but not in available form as Resin P (Figure 4A and 4B). Any P movement through the soil occurred for pure RP, with 100% of the P being restricted to the section closer to the application point. P from FC also has restricted diffusion in the soil, with only ~5% of the total P found in Sec 2 (Figure 4A).

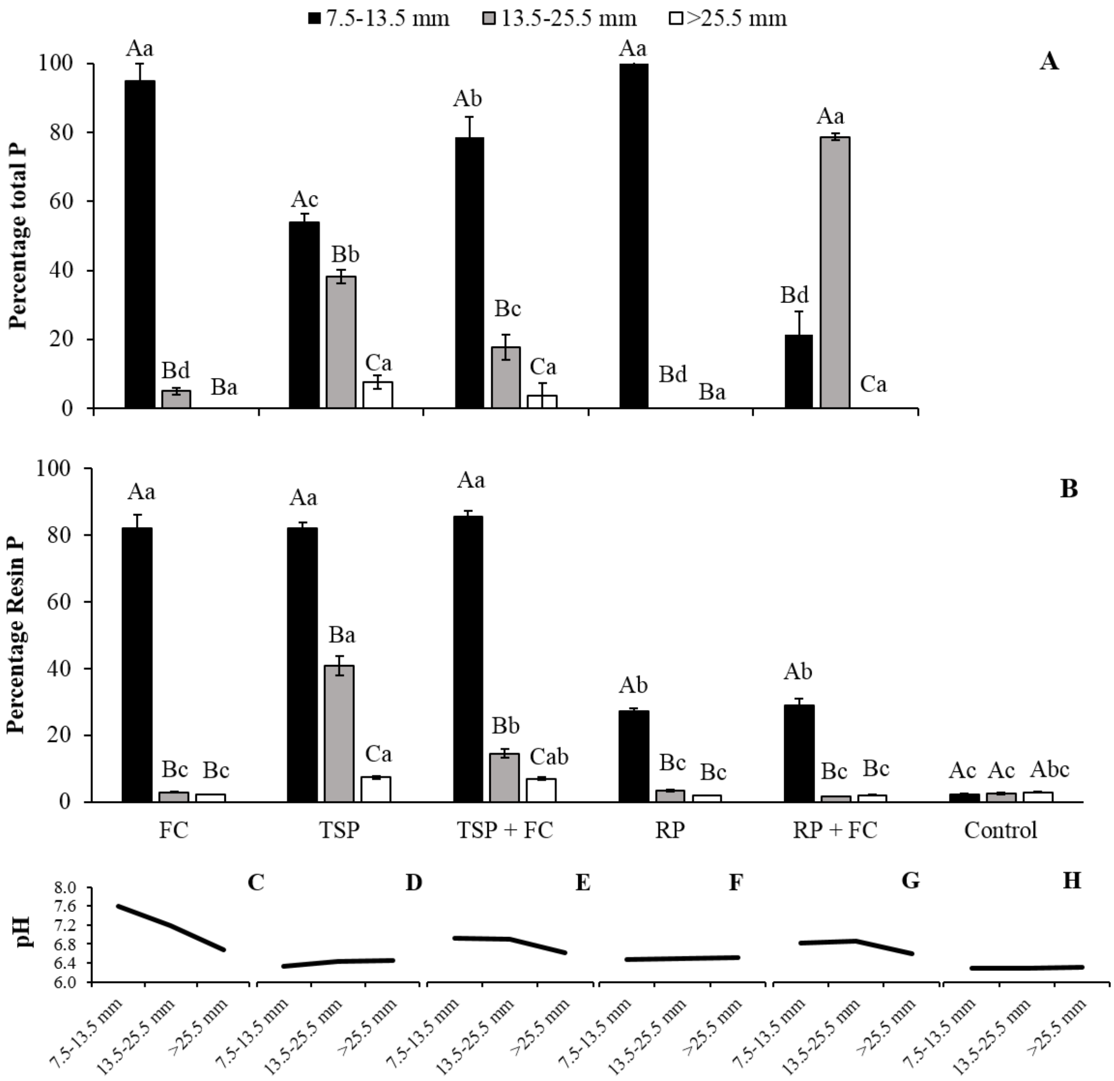

Figure 4. Percentage of total P (%Total P) (A), and percentage of available P (%Resin P) (B) from fertilizers incubated in the acidic limed soil in the Petri dish´s sections (7.5- 13.5 mm, 13.5- 25.5 mm, and > 25 mm). Soil pH in each section for FC (C), TSP (D), TSP+FC (E), RP (F), RP+FC (G), and control (H). FC: Filter cake; TSP: Triple superphosphate; TSP+FC: Triple superphosphate associated with FC; RP: Rock phosphate; RP+FC: Rock phosphate associated with FC. Vertical error bars correspond to the standard deviation. Within each treatment, means followed by distinct capital letters differ according to Tukey´s test ($p < 0.05$). Within each soil section means followed by distinct lower case letters differ according to Tukey´s test ($p < 0.05$).

For the alkaline soil, the comparison within soil sections didn´t show an expressive difference regarding the effect of the fertilizer on the P diffusion (Figure 5A). Except for RP, all the fertilizer sources and combinations resulted in equal %Total P in Sec 1 and 2, and negligible in the last section. Significant differences between treatments were found for %Resin P in Sec 1, with TSP and TSP+FC higher than FC, RP, and RP+FC (Figure 5B). However, RP and RP+FC did not differ from the control. In Sec 2, only TSP treatments increased the %Resin P from the original soil, and TSP was higher than TSP+FC.

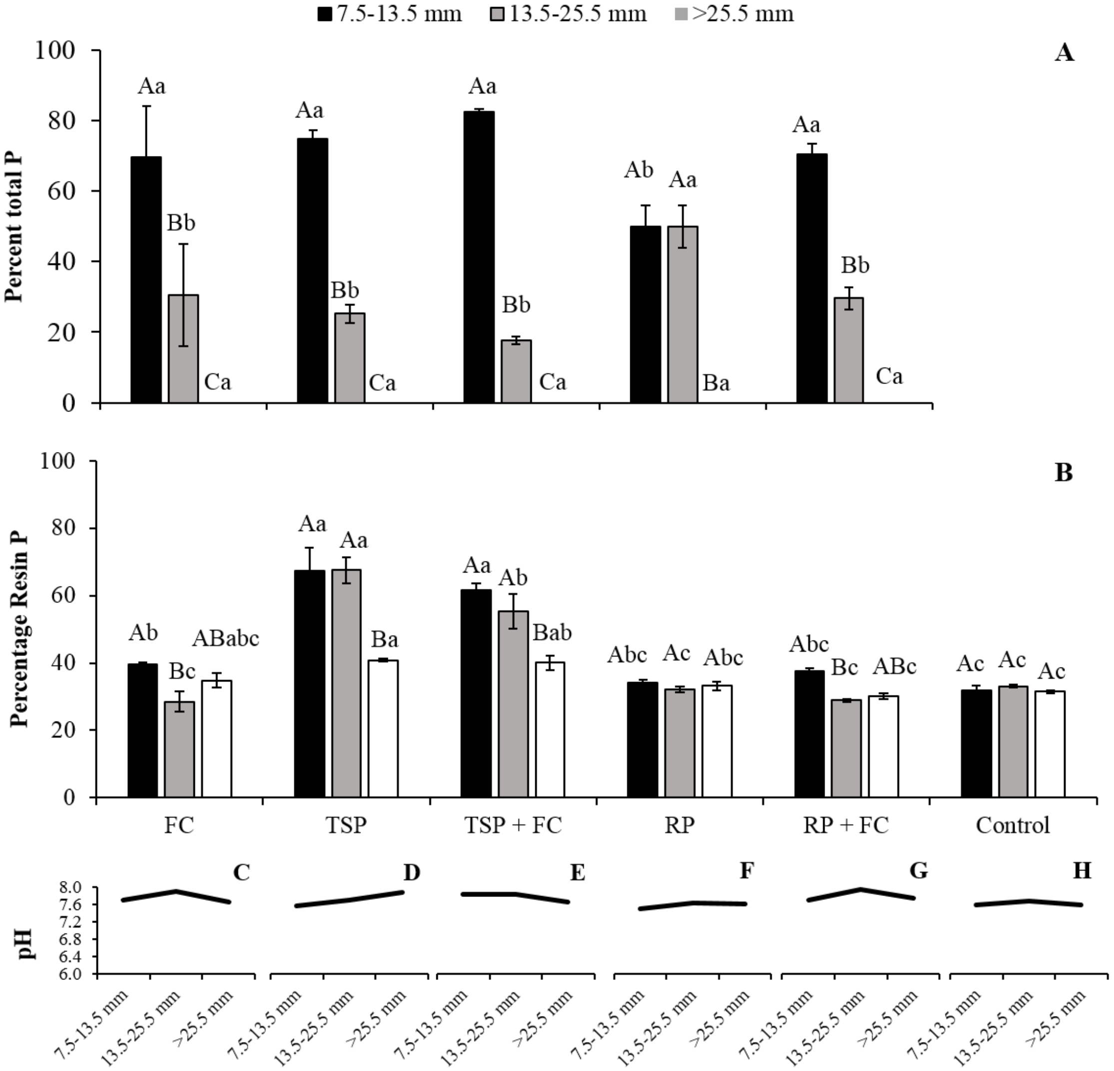

Figure 5. Percentage of total P (%Total P) (A), and percentage of available P (%Resin P) (B) from fertilizers incubated in alkaline soil in the Petri dish´s sections (7.5- 13.5 mm, 13.5- 25.5 mm, and > 25 mm). Soil pH in each section for FC (C), TSP (D), TSP+FC (E), RP (F), RP+FC (G), and control (H). FC: Filter cake; TSP: Triple superphosphate; TSP+FC: Triple superphosphate associated with FC; RP: Rock phosphate; RP+FC: Rock phosphate associated with FC. Vertical error bars correspond to the standard deviation. Within each treatment, means followed by distinct capital letters differ according to Tukey´s test ($p < 0.05$). Within each soil section means followed by distinct lower case letters differ according to Tukey´s test ($p < 0.05$).

As observed for the limed acidic soil, in the alkaline soil the P diffusion for all treatments was significantly different between Sec 1 and 2, with more than 70% of the total P restricted to Sec 1 (Figure 5A). However, RP had the same %Total P in both sections (~50% in each section), which is not so distinct from the acidic soil (Figure 3A), but very distinct from the limed acidic soil (Figure 4A). The most important finding regarding %Resin P was that TSP and TSP+FC were similar in Sec 1, while TSP resulted in a higher %Resin P in Sec 2 compared to TSP+FC (Figure 5B).

4.4. Discussion

4.4.1. Kinetics of P release by fertilizers

Throughout the 240 h of column perfusion test, curves from FC, TSP, and TSP+FC had a similar shape, but different P release rates. Within 12 h, more than 80% of P from TSP and TSP+FC had been released, but less than 60% from FC. These differences are consistent with the high TSP solubility in water, and the varied FC composition, which includes materials in different decomposition stages and water affinity. Despite this lower P release compared to TSP, we can state that FC had rapid mineralization probably because of its low C: N (< 20) and C: P (< 200) ratio, favoring its mineralization (Umrit and Friesen 1994; Alamgir et al., 2012). After 12 h, the P content in the recovered solution from TSP and TSP+FC reached its peak and stabilized, while FC kept releasing P up to 192 h. The type of interactions between P and other components of FC (P fractions) explains this behavior since it revealed that a great amount of P was in the moderately labile pool, which is slowly released after P depletion from the labile P pool. The incomplete TSP dissolution even after 240h (10 days) of water flushing on it is probably due to the presence of some water-insoluble compounds commonly found in these fertilizers (Baird et al., 2019).

In the column system used, the pH variation in the solution was dependent exclusively on the fertilizers composition, dissolution reactions rate, and activity of the products released. The rapid dissolution reaction of TSP and TSP+FC decreased the solution pH to < 3.5, but in the following hours, the pH increased again. The TSP dissolution release H^+ íons through the monocalcium phosphate hydrolysis [$Ca(H_2PO_4)_2 + H_2O = H_3PO_4 + CaHPO_4$], and dissociation of H_3PO_4 to $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-} (FTRC, 2015; Lindsay and Stephenson, 1959). During these reactions, the solution can reach pH 1.5 - 1.0 depending on the fertilizer concentration (Lindsay and Stephenson, 1959). The dicalcium phosphate ($CaHPO_4$) also dissociates, releasing Ca^{2+}, $H_2PO_4^-$ and OH^-, and thus, the previous acidity generated is neutralized (Fernández et al., 1999).

The slower dissolution of FC compared to TSP was proved by its ability to keep solution pH stable longer than a soluble fertilizer. The FC effect on solution pH was a consequence of its neutral pH, and probably due to the decarboxylation of organic anions and the ammonification process that usually occurs during the mineralization of organic materials (Xu et al., 2006). The increasing pH effect under FC was not neutralized because its P release rate was less intense than under TSP. Moreover, plant residues with low N: base ration (as FC), usually have a long-term effect on the soil pH (Pocknee and Sumner, 1997).

The solution pH increase observed under RP treatments in the first hours of the experiment is related to the reactions of Ca phosphates with water, promoting a buffer effect in the solution. In acid pH, the dissolution reaction of apatite is: $Ca_5(PO_4)_3OH = 5Ca^{2+} + 3PO_4^{3-} + OH^-$. Thus, the release of OH^- explains the increasing pH.

4.4.2. Mobility and potential availability of P to plants

The %Total P in soil sections was used as an indicator of P fertilizer's diffusion, considering that the more P was released, the greater the diffusion zone. The P recovered by AER (%Resin P) determined how much of the total P from each soil session was potentially available to plants (Myers et al., 2005). However, the soil labile P pool is not comprised only of P extracted by AER, but also by the organic and inorganic P weakly adsorbed to the soil colloids surface (Hedley et al., 1982). Therefore, our results only partially reflect the availability of P released from fertilizers. Among the treatments tested, the P released by FC showed the highest diffusion zone in the acidic soil. However, when that soil was limed, P from FC was mostly restricted to Sec 1. The greater P movement in the acidic soil indicates that FC mineralization was favored under acidic conditions (initial pH 4.9) because its characteristics such as the neutral pH, the high Ca content (Table 1) and the predominant P bonded to Ca, makes it less stable in acid soil conditions. The lime addition not only increases the soil pH but also the exchangeable Ca^{2+} availability. This element can act as a binder between the organic reactive functional groups and the soil mineral phase (Pereira et al., 2013), which could protect FC from mineralization. Moreover, in residues such as manure and composts, P can be found in acid-soluble organic forms (Sharpley and Moyer, 2000; He et al., 2004; Turner and Leytem, 2004) which, we believe, is the case of FC.

Despite the higher P release and diffusion observed in the acidic soil under pure FC, the nutrient availability was lower than in the limed soil. Liming has a positive effect on P availability by the neutralization of H^+ íons, precipitation of Fe and Al by OH^- addition, and neutralization of the positive charges of Fe and Al oxides and hydroxides (Sanchez and Uehara, 1980). However, at the end of the incubation time, FC raised pH in both soils (Figure 3C), indicating that the different %Resin P was not a direct effect of liming, but a consequence of the lower FC mineralization in the limed soil. Our data showed that over 50% of the P from FC was released after 6 h of its application, following the same trend as TSP, but less intense (Figure 2D). So, it is reasonable to say that, in the same way as water-soluble mineral fertilizers, FC can rapidly increase the soil P content, leading to high rates of P adsorption to (oxy) hydroxides of Fe and Al. Because the amount of P fixed in the soil is proportional to its content in solution, P adsorption and precipitation reactions were favored in Sec 1 of the acid soil. According to Benbi and Gilkes (1987), these reactions are more likely to happen from 1 - 5 mm from the fertilizer application point.

The greater P release from FC in acidic soil explains the better diffusion when TSP+FC was tested in the acidic condition. However, compared to TSP, this treatment reduced the %Total P in Sec 2, regardless of liming, because of the replacement of 35% of the mineral fertilizer by FC. The TSP dissolution rapidly raises the P concentration in the soil solution, which in acidic conditions increases the affinity and binding strength of P with the OM, and Fe and Al (oxy) hydroxides that surround the fertilizer (Beauchemin et al., 2003; Hedley and McLaughlin, 2005; Wang et al., 2017). The remained P can move to an unsaturated zone where it will be more weakly retained through specific adsorption reactions (Benbi and Gilkes 1987), becoming easily available. In our study, as the combination of TSP+FC decreased the P content in the solution, the amount of available P remained was lower than in pure TSP. In the limed soil this was not observed in Sec 1 because the soil pH raised the %Resin P in TSP+FC, equating it with TSP (Figure 4B and 4E).

However, the diffusion dynamics of P from the more saturated to less saturated zones depends not only on the P concentration but also on the volume of soil in which the element will interact. The higher the P concentration, and the smaller the volume of soil, the greater the chances of saturating the maximum P holding capacity of the soil, remaining P in available forms. Hence, in general, all fertilizers showed a higher %Resin P in Sec 1 (high P concentration: soil volume ratio) than in Sec 2 regardless of the liming addition. Other studies analyzing the movement of P from TSP found similar results (Heronemus et al., 2021; Lombi et al., 2005).

The FC effect was also noticed when associated with RP in acidic soil. In Sec 1, the %Resin P in this treatment was similar to that found for FC, and higher than in pure RP, suggesting that the greater P availability could be resultant of the FC mineralization (Figure 3B). However, only 35% of the total P applied via RP+FC was provided by FC, so the release and availability of P in this treatment could not be equal to that obtained by the pure FC. It seems, therefore, that the %Resin P found in RP+FC is not resultant of the P released by FC, but the recognized effect of this byproduct on raising the CEC and soil pH, and reducing the exchangeable Al^{3+} content in the soil (Korndörfer and Anderson, 1997). Otherwise, in the acidic limed soil the positive effects of FC on P availability were not observed under RP+FC, as the initial soil pH was already between 6.0-6.5 (Figures 4F and 4G), a range considered adequate for the maximum availability of P (Havlin et al., 2005). At this pH, RP had low dissolution, with P remaining restricted to the soil section adjacent to the application site (Figure 4A).

In the alkaline soil, the total P was mainly confined to 13.5 mm from the fertilizer application point, except for RP. This low diffusion is consistent with other works testing different sources of P in alkaline soils (Lombi et al., 2005; Weeks and Hettiarachchi, 2020), and is related to its physicochemical properties (Table 2). Soil texture influences the P diffusion zone because it changes the tortuosity of the soil pores, thus the P diffusion zone is lower in fine texture soils (Lawton and Vomocil, 1954). Moreover, the high content of P, Ca, and OM may have affected the dissolution rate and P availability of fertilizers, making this soil unresponsive to phosphate fertilization.

Surprisingly, in the alkaline soil RP showed the same %Total P in Sec 1 and 2, as observed in the acidic soil, which probably favored RP dissolution, optimized at pH < 5.5 (Rajan et al., 2004). As RP is composed of P bound to Ca in the form of apatite, its dissolution in alkaline soils, and high Ca^{2+} exchangeable content is unlikely, as it depends both on the soil acidity and Ca removal from the system (Andersson et al., 2015; Andersson et al., 2016). However, increasing soil pH may favor OM mineralization because of the greater microbial turnover (Adams and Adams, 1983). The release of H^+ ions from organic anions, the nitrification, and the increase in exchangeable H^+ due to the elevation of CEC (Porter et al., 1980; Helyar, 1976; Bolan et al., 1991; Donald and Williams, 1954; Williams and Donald, 1957), are mechanisms that shortly acidify the soil during the OM decomposition. As the soil in question has high OM content, and we suspect high microbial activity during the incubation time, the acidity generated around the RP may have been sufficient to cause some dissolution. However, to confirm this hypothesis, it would be necessary to analyze the degree of humification of soil OM to infer its stability and potential mineralization rate.

Despite the possible acidifying mechanisms mentioned above, which act on the solubilization of phosphates (Qin et al., 2013; Wang et al. 2021), the P availability from RP treatments was very low, as the reactions must have been limited by the soil buffering. A similar situation could be observed in the soil fertilized with FC, in which the soil pH variation was much lower than in the acidic soil with and without liming (Figures 3C, 4C, and 5C).

In alkaline soils fertilized with organic materials, the transformation of labile to moderately labile P is slower than when using mineral fertilizers (Audette et al., 2016). Thus, it is more likely that P-Ca remains relatively soluble in the soil, due to the organic acids that delay the crystallization of the minerals formed (Grossl and Inskeep, 1991; Delgado et al., 2002; Sato et al., 2005). Therefore, over time, in the presence of plants, the P from the FC treatments could be extracted more easily than in the treatments with mineral fertilizers alone. FC addition also did not affect the P diffusion in TSP+FC, compared to TSP. The greater presence of P under TSP in the section closest to the point of application was also observed by Lombi et al. (2004) in alkaline soil and was attributed to the presence of Ca in the TSP. The reduction of %Resin P in TSP+FC in Sec 2 was due to the reduction in the amount of soluble P applied in these treatments compared to TSP, as previously explained for the acidic soil, however, both treatments maintained increased the %ResinP in the three soil sessions.

4.5. Conclusions

By the combination of results, we can state that our hypothesis was partially confirmed. The dual and gradual P release hypothesized was observed only for TSP, and after 5 wks of soil incubation, the association of FC to mineral fertilizers affected the diffusion and availability of P from mineral fertilizers differently in the three soils tested. As a consequence of the chemical composition of FC, its mineralization rate is rapid and facilitated in acidic soil. When combined with TSP, this byproduct reduced the P diffusion and availability in the acidic soil, while in the limed soil, the reduction in the amount of soluble P applied did not interfere with the P availability in Sec 1. From a practical point of view, it represents the possibility of reducing the use of soluble fertilizers. FC also positively affected the P availability in the non-limed acidic soil when it was combined with RP because of its effect on raising the CEC and soil pH, and reducing the exchangeable Al^{3+} content in the soil. The same effect was not observed in the limed acidic soil, as the original pH was already favorable for RP dissolution, but not its availability. For the alkaline soil, with a high sum of bases and content of OM, Ca, and P, it was not detected any effect of FC on P diffusion, and only TSP, in the presence and absence of FC, was efficient in increasing the available P in the three soil. As in the acidic non-limed soil, the combination of TSP and FC in the ratio 35: 65 negatively affects the %ResinP. Interestingly, the %Total P under RP in this soil was similar to the non-limed acidic soil, which could be related to an acidifying effect of the native soil OM, but we need to expand our investigation to confirm this theory.

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Supplementary information

Figure S1. Representation of the fertilizer placed in the center of the Petri Dish, and the three soil sections sampled after 5 weeks of incubation.

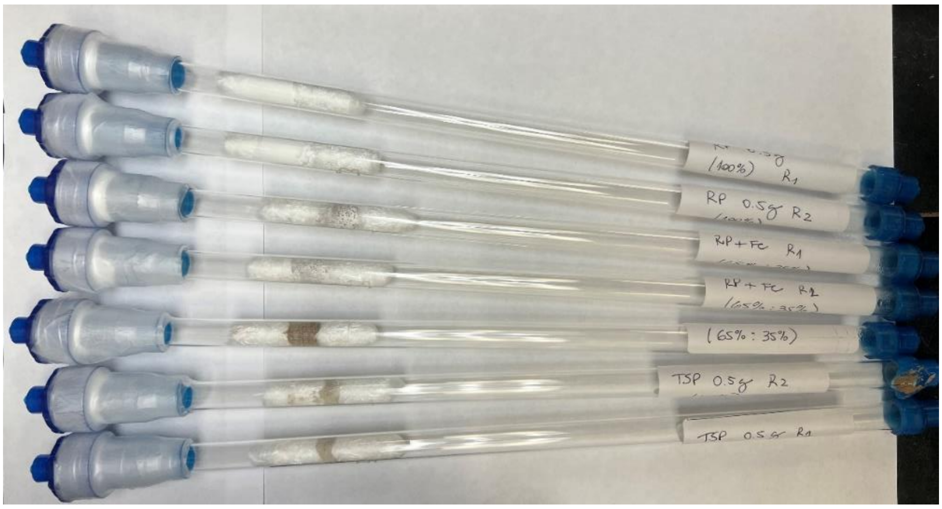

Figure S2. Grounded fertilizers – filter cake, triple superphosphate, triple superphosphate associated with filter cake, rock phosphate, and rock phosphate associated with filter cake -placed between nylon membrane filter and silanized glass wool in the glass columns (DWK Life Sciences Kimble Kontes FlexColumns ™).

Figure S3. Setup of the column perfusion system used to evaluate the phosphorus release from fertilizers.

Table S1. Total phosphorus (mg kg^{-1}) in the acidic soil (with and without liming), and alkaline soil sections (7.5- 13.5, 135- 25.5, and >25.5 mm) in the Petri dish after 5 weeks of fertilizer placement and incubation.

	Section		
Treatment	7.5 - 13.5 mm	13.5 - 25.5 mm	> 25.5 mm
	Acidic soil		
Control	180 Ad	175 Ac	183 Aa
FC	451 Ab	187 Bc	190 Ba
TSP	501 Aa	309 Ba	182 Ca
TSP + FC	451 Ab	229 Bb	184 Ca
RP	190 Ad	193 Ac	177 Aa
RP + FC	343 Ac	191 Bc	171 Ba
	Acidic soil with liming		
Control	169 Ac	172 Ab	160 Aa
FC	378 Ab	154 Bb	168 Ba
TSP	544 Aa	248 Ba	167 Ca
TSP + FC	412 Ab	181 Bb	160 Ba
RP	157 Ac	184 Ab	165 Aa
RP + FC	201 Ac	195 Ab	156 Ba
	Alkaline soil		
Control	1067 Ad	1045 Aa	1039 Aa
FC	1224 Ac	1076 Ba	1036 Ba
TSP	1576 Aa	1100 Ba	1055 Ba
TSP + FC	1395 Ab	1053 Ba	1044 Ba
RP	1044 Ad	1068 Aa	1024 Aa
RP + FC	1405 Ab	1070 Ba	1031 Ba

Note: Within each treatment, means followed by distinct horizontal capital letters were significantly different according to Tukey´s test (*p*< 0.05). Within each soil section means followed by distinct lower case vertical letters were significantly different according to Tukey´s test (*p*< 0.05).

Table S2. Resin phosphorus (mg kg^{-1}) in the acid (with and without liming), and alkaline soil sections (7.5- 13.5, 135- 25.5, and >25.5 mm) in the Petri dish after 5 weeks of fertilizer placement and incubation.

	Section		
Treatment	7.5 - 13.5 mm	13.5 - 25.5 mm	> 25.5 mm
	<u>Acid soil</u>		
Control	4 Ae	5 Ac	4 Ab
FC	242 Ac	5 Bc	4 Bb
TSP	451 Aa	156 Ba	25 Ca
TSP + FC	316 Ab	54 Bb	15 Cab
RP	19 Ae	10 Ac	5 Ab
RP + FC	178 Ad	6 Bc	3 Bb
	<u>Acid soil with liming</u>		
Control	4 Ae	4 Ab	5 Aa
FC	312 Ac	5 Bb	3 Ba
TSP	448 Aa	102 Ba	12 Ca
TSP + FC	352 Ab	26 Bb	11 Ba
RP	50 Ad	5 Bb	3 Ba
RP + FC	58 Ad	3 Bb	3 Ba
	<u>Alkaline soil</u>		
Control	354 Ad	346 Ac	326 Aab
FC	485 Ac	308 Bc	361 Bab
TSP	1066 Aa	744 Ba	431 Ca
TSP + FC	858 Ab	538 Bb	419 Cab
RP	357 Ad	344 Ac	340 Aab
RP + FC	529 Ac	300 Bc	311 Bb

Note: Within each treatment, means followed by distinct horizontal capital letters differs according to Tukey´s test ($p< 0.05$). Within each soil section means followed by distinct lower case vertical letters differs according to Tukey´s test ($p< 0.05$).

5. SOIL PHOSPHORUS STOCKS AND SPECIES UNDER RECYCLED FILTER CAKE ASSOCIATED WITH MINERAL PHOSPHATE SOURCES

Abstract

In sugarcane fields in Brazil, phosphate fertilizers are applied mainly at crop establishment, leading to low phosphorus (P) use efficiency over the crop cycles. Filter cake (FC), a byproduct of the sugarcane industry, associated with mineral fertilizers (triple superphosphate (TSP) or rock phosphate (RP)) has the potential of increasing soil P availability. This study aimed to evaluate the long-term effect of the association of FC with TSP and RP (at rates of 90 and 180 kg ha^{-1} P_2O_5), applied to sugarcane fields, in the stocks and species of P in two distinct soils (sandy and clayey Oxisols). Thus, two experiments were carried out for 5-yrs in commercial sugarcane fields. Changes in soil P pools were investigated using Hedley´s procedure and the P species were defined by P K-edge XANES spectroscopy. In both sites, FC reduced the conversion of labile P to less available forms. In the sandy soil, the association of FC with TSP and RP (180 kg ha^{-1}) resulted in a beneficial residual effect, also increasing RP solubilization. Similarly, in the clayey soil TSP+FC (180 kg ha^{-1}) also showed a good residual effect, and RP was quickly solubilized. The association of FC with mineral sources changed differently the species and dynamics of P in the soil. After 5-yrs, in the sandy soil RP+FC (180 kg ha^{-1} of P_2O_5) was as efficient as TSP (+ and - FC), in increasing the labile P pool, while in the clayey soil, RP+FC was less efficient than TSP+FC (180 kg ha^{-1} of P_2O_5).

Keywords: Pools of P, Triple superphosphate, Bayóvar, Gafsa, Sugarcane, Byproduct, XANES, Hedley´s fractionation

5.1. Introduction

As the second element that most constrains crop yield (Vance, 2003), P has been widely studied in agricultural environments. The lack of available P in the soil is the result of its complex dynamics and diversity of species that can occur, in simple and complex environmental matrices (Kruse et al., 2015). In tropical soils, the P availability is mainly guided by adsorption and/or precipitation reactions of its inorganic (Pi) and organic (Po) forms to (oxy) hydroxides of Fe and Al, and by the adsorption to 1:1 clay minerals and organic matter (OM) (Nascimento et al., 2018; Wang et al., 2014; Withers et al., 2018).

In sugarcane cultivation, the soil P supplementation has special importance because, in addition to being essential for the photosynthetic activity, macromolecule production, and root and tiller development, it also influences sucrose production and accumulation (Alexander, 1973; Lima Vasconcelos et al., 2017), which is one of the most important industrial quality parameters. In addition, this crop is produced on a large scale in Brazil (8,423 thousand ha) (CONAB, 2021), representing a great portion of the phosphate fertilizers consumption in the country.

Most mineral P fertilizers are manufactured from Ca-phosphate minerals - rock phosphates (RP) - of low solubility, which after acidification originate products soluble in water such as triple superphosphate (TSP). These fertilizers have a rapid but not persistent dissolution rate, favoring the P conversion into poorly available forms (McLaughlin et al., 2011). Therefore, rarely more than 25% of the applied P is recovered by crops (Cornish, 2009; Roberts and Johnston, 2015). In sugarcane fields, it may be worse because the majority of P fertilizers are applied at the crop establishment, supplying P beyond that required by plants at that time, reducing the competition between the plant drain and the soil drain, and potentially promoting more P fixation.

One strategy to avoid the P fixation resultant from water-soluble sources in the soil is using filter cake (FC), a byproduct of the sugarcane industry which associated with mineral fertilizers can increase the P use efficiency and sugarcane yield (Bekheet et al., 2018; Borges et al., 2019; Caione et al., 2015; Estrada-Bonilla et al., 2021; Lima Vasconcelos et al., 2020; Moda et al., 2015; Santos et al., 2014; Vazquez et al., 2015; Yang et al., 2013). The rate of P release from FC is intermediate between TSP and RP (Chapter 4), and we hypothesize that FC combined with a slow-release fertilizer - as RP - can deliver P to the soil solution in a synchronized way according to the plant's demand, reducing the P fixation. Additionally, FC use can increase the TSP efficiency due to the release of low molecular weight organic acids in soil solution, that can complex metallic cations and compete with P for the soil adsorption sites (Menezes-Blackburn et al., 2016; Qin et al., 2013), potentially reducing the dose of TSP needed for adequate plant nutrition.

The P release rates and reactions of the above-mentioned organic and mineral fertilizers can affect differently its distribution and availability in the soil. Also, the strength of P retention energy influences the stability and availability of the formed compounds. Thus, from a practical point of view, it is possible to group the element in pools like labile, moderately labile, and non-labile (Hedley et al., 1982). For example, at a high P concentration in solution, such as around the TSP granule, P is more likely to be retained by precipitation reactions (McLaughlin et al., 2011), converting it into stable and recalcitrant forms of the non-labile pool. By prolonging the time required for the release of P from the fertilizer, the concentration in the solution is reduced and specific adsorption is more likely to occur with low binding energy and unbalanced charges (McLaughlin et al., 2011), and most of the P remains in the labile and moderately labile fractions. Despite some uncertainties (Barrow et al., 2019), P fractionation is the most common and efficient methodology to quantify P in different soil pools. However, the result infers about P lability, but not the P chemical species in the extracted compounds (Rivard et al., 2016), which can be obtained using P K-edge X-ray Absorption Near Edge Structure (XANES), including mineral, amorphous, and adsorbed phases (Kelly et al., 2008). To better understand the FC contribution to the P availability in tropical soils fertilized with TSP and RP, it is necessary to investigate how it may affect the distribution and species of P in the soil, which can be achieved by a combination of both techniques.

Based on the hypothesis that the use of mineral fertilizers in association with FC will change the dynamic and species of P in the soil differently than the use of these fertilizers individually, we aimed to evaluate the long-term effect of the association of FC with TSP and RP (at rates of 90 and 180 kg ha^{-1} P_2O_5), applied to sugarcane fields, in the stocks and species of P in two distinct soils (sandy and clayey Oxisols).

5.2. Material and Methods

5.2.1. Site description and soil characterization

Two experiments were established and conducted over 5 successive sugarcane seasons in the Cerrado biome, in São Paulo State, Brazil. The first experiment was carried out in a Latossolo Vermelho Amarelo distrófico sandy texture (Santos et al., 2018) or Hapludox (Soil Survey Staff, 2014), and the second in a Latossolo Vermelho Eutrófico chernossólico clayey texture (Santos et al., 2018) or Hapludox (Soil Survey Staff, 2014). Soils previous to the trial establishment were evaluated following the chemical and physical characterization (Table 1) of soil samples collected at 0-10, 10-20, 20-40, 40-60, and 60-80 cm layers. The main minerals constituents of the soil were identified by X-ray diffraction (XRD) in the clay fraction of 0-20 cm soil layer (Jackson, 2005). Accordingly, in both selected areas, there was low available P (Resin P), and the predominance of kaolinite, gibbsite, and hematite (Figures S1 and S2). However, in sandy soil, it was observed a greater presence of kaolinite, while in clayey soil there was a predominance of hematite and gibbsite.

Experiment one was located in Agudos (22°33’22” S; 49°06’15” W, at 715 m a.s.l.), with 21 ºC as the mean annual temperature and 1119.8 mm as the mean rainfall. Before the establishment, this area had a history of extensive degraded pastureland for more than twenty years. Experiment two was located in Macatuba (22° 29’ 39” S; 48° 41’ 14” W, at 515 m a.s.l.), where the mean annual temperature is 20.8 ºC, and the mean rainfall is 1103.4 mm. This second area has been used as a sugarcane field for approximately fifty years. The pre-experiment vegetation in both areas was desiccated using glyphosate® (4 L ha^{-1} in experiment one, and 6 L ha^{-1} in experiment two), and incorporated into the soil by deep plowing (0.00- 0.50 m). After, it were added and incorporated by disc harrow (~0-20 cm depth), 3 and 3.5 Mg ha^{-1} of lime ($CaCO_3$ + $MgCO_3$), and 1.5 and 2 Mg ha^{-1} of gypsum ($CaSO_4$), in the experiments one and two, respectively.

Table 1. Pre-experiment soil chemical and physical characterization from sandy soil (Agudos) and clayey soil (Macatuba)

																DCB [f]		Oxalate [g]		
Layer	pH	OM [a]	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H + Al	SB [b]	CEC [c]	V [d]	M [e]	Clay	Silt	Sand	Fe	Al	Fe	Al	P max [h]
cm	$CaCl_2$	g kg^{-1}	mg kg^{-1}	----------------- $mmol_c$ kg^{-1} -----------------							%		--------------------- g kg^{-1} ------------------------							(mg kg^{-1})
									Sandy soil											
0-10	4.3	14.3	4	1.5	7	5	2	25	13.2	38.2	35	11	-	-	-	-	-	-	-	-
10-20	4.2	10.7	3	1.7	5	3	3	28	10.3	38.3	27	21	-	-	-	-	-	-	-	-
0-20	-	-	-	-	-	-	-	-	-	-	-	-	112	4	884	15.3	42.3	0.6	1.4	342
20-40	3.9	-	3	1.9	3	2	6	38	6.8	44.8	15	48	138	27	835	-	-	-	-	-
40-60	4	-	2	0.8	2.8	0.8	7	34	4.3	38.3	11	63	151	17	832	-	-	-	-	-
60-80	3.9	-	1	0.9	2.8	0.9	9	32	3.8	35.8	11	70	151	24	825	-	-	-	-	-
									Clayey soil											
0-10	5.6	31.0	11	1.9	54	25	0.9	22	80.9	103	79	1	-	-	-	-	-	-	-	-
10-20	5.3	28.5	9	1.7	44	22	0.9	28	67.7	96	71	1	-	-	-	-	-	-	-	-
0-20	-	-	-	-	-	-	-	-	-	-	-	-	629	152	219	110.9	53.4	4.4	1.1	1557
20-40	5.2	-	8	1.2	30	12	0.9	25	43.2	68	63	26	667	122	211	-	-	-	-	-
40-60	5.1	-	5	0.7	23	9	2	25	32.7	58	57	6	-	-	-	-	-	-	-	-
60-80	4.9	-	3	0.5	27	13	2	34	40.5	74	54	5	-	-	-	-	-	-	-	-

[a] OM: organic matter determined by Walkley-Black (1934); [b] SB: sum of bases; [c] CEC: cation exchange capacity; [d] V%: base saturation of CEC; [e] M%: Al saturation; [f] DCB: Fe and Al extracted by dithionite-citrate-sodium bicarbonate; [g] Fe and Al extracted by ammonium oxalate; [h] P_{max}: maximum phosphorus sorption capacity. pH in $CaCl_2$, with ratio soil: solution of 1: 2.5 m/v (Raij et al., 2001). P, K^+, $Ca^{2+,}$ and Mg^{2+} extracted by ion exchange resin. Al^{3+} extracted by KCl 1 mol L^{-1}. H + Al extracted by SMP (Shoemaker, Mclean, Pratt, 1971); granulometry determined by Pipette method.

5.2.2. Treatments

The experiments were set up as split-split plot design with three replications (blocks) and 10 treatments. The treatments consisted of two mineral P sources (TSP and RP), tested in three doses (0, 90, and 180 kg ha^{-1} of P_2O_5 – soluble basis), in the presence (+ FC) or absence (- FC) of 10 ton ha^{-1} of FC (dry basis). Individual plots consisted of six 10 m long rows, distant 1.5 m from each other (90 m^2). In Agudos, the source of RP used was Gafsa (28.5% of total P_2O_5, and 9.6% soluble in citric acid 2%), while in Macatuba it was used Bayóvar (29% of soluble citric acid 2%, and 14% soluble in citric acid 2%). For easy reading of the manuscript, the treatments were named as: Control, FC, TSP 90, TSP 180, TSP+FC 90, TSP+FC 180, RP 90, RP 180, RP+FC 90, and RP+FC 180. Considering the total P content in the FC (Table 2), treatments with FC addition received 55 kg ha^{-1} of P in Agudos, and 113 kg ha^{-1} of P in Macatuba, via FC.

Treatments were applied only in the experimental establishment (in 2014 in Agudos, and 2015 in Macatuba), at the planting furrow (~20 to 25 cm deep from the soil surface). After the fertilizers placement, 18 to 20 sugarcane buds per meter were manually planted at the base of the planting furrow, and covered with a ~10 cm layer of soil. Urea and KCl, at the doses of 60 and 90 kg ha^{-1} of N and 150 and 135 kg ha^{-1} of K_2O, were also applied in Agudos and Macatuba, respectively. After each season´s harvest, the sugarcane ratoons were fertilized with urea and KCl, as topdressing in both locations (doses of 90 kg ha^{-1}of both N and K_2O in Agudos, and of 100 kg ha^{-1} of both N and K_2O in Macatuba).

Table 2. Chemical parameters of the filter cake applied at the establishment of experiments in the sandy soil (Agudos) and clayey soil (Macatuba).

pH[a]	C[b]	P[c]	N[c]	K_2O[c]	Ca[c]	Mg[c]	S[c]	OM[b]	LP[d]	MLP[d]	NLP[d]
	-------------------------- % --------------------------								--------------- mg kg^{-1} ---------------		
Agudos site (Sandy soil)											
6.7	14.3	0.57	2.3	0.14	2	0.16	0.19	28.6	858.1	4740.3	5326.5
Macatuba site (Clayey soil)											
7.4	27.6	1.13	3.5	1.07	4.1	0.33	0.28	53.3	-	-	-

[a] pH in $CaCl_2$ 0.01 mol L^{-1} at a ratio of 1:2.5 (m/v) (Raij et al., 2011); [b] Determined by loss of mass by ignition; [c] Determined following Malavolta et al., 1989; [d] Determined by Hedley et al., (1982), with modifications by Rheinheimer (2000). Labile P: labile phosphorus pool; m-labile: moderately labile phosphorus pool; n-labile: non-labile phosphorus pool

5.2.3. Soil sampling and P measurements

The 4 central rows of each experimental plot were considered a useful area for the soil sampling. Immediately after the first, third, and fifth sugarcane harvests, each row had 4 sub-samples collected in the 0-10, 10-20, and 20-40 cm depth, using an Auger probe. Then, the samples of the 4 rows were mixed as a composite sample for each depth, air dried, and sieved through a 2 mm mesh.

Changes in the soil P pools were determined by Hedley´s sequential fractionation (Hedley et al.,1982), with modifications by Rheinheimer (2000). Following this method, different extractants were used to remove P fractions in the soil. Within each fraction, were measured the concentrations of organic (Po), inorganic (Pi), and total P (Pt). The extractants sequentially added to a 0.5 g of soil sample were: anion exchange resin (P_{AER}), 0.5 mol L^{-1} solution of sodium bicarbonate ($NaHCO_3$) (Pi_{BIC} and Po_{BIC}), 0.1 mol L^{-1} solution of sodium hydroxide (NaOH) ($Pi_{0.1\ HID}$ and $Po_{0.1\ HID}$), 1 mol L^{-1} of hydrochloric acid (P_{HCl}), and 0.5 mol L^{-1} NaOH ($Pi_{0.5\ HID}$ and $Po_{0.5\ HID}$). The remaining soil was dried at 40 °C, grounded, and digested using sulfuric acid (H_2SO_4), hydrogen peroxide (H_2O_2), and magnesium chloride ($MgCl_2$) (Brookes and Powlson, 1981), obtaining the residual P (P_{RES}). A sample of each alkaline extract was digested in an autoclave with H_2SO_4 and ammonium persulfate (USEPA, 1971), obtaining the Pt. The Pt and the Pi (orthophosphate) in the acid extracts were quantified colorimetrically using the Murphy and Riley (1962) method. The Pi in the alkaline extracts was quantified by Dick and Tabatabai (1977) method. The Po in the extracts was the difference between Pi and Pt. For a more comprehensive discussion, the results were grouped into three pools of P: labile P pool (LP), moderately labile P pool (MLP), and non-labile P pool (NLP). The LP pool was obtained by the sum of P_{AER}, Pi_{BIC}, and Po_{BIC}. The MLP pool is the sum of $Pi_{0.1\ HID}$, $Po_{0.1\ HID}$, and P_{HCL}. Finally, the NLP pool is the sum of $Pi_{0.5\ HID}$, $Po_{0.5\ HID}$, and P_{RES}. We believe that this is the best way to picture the effect of the treatment in the P pools in the long-term experiment.

The effect of the treatments on soil P species was analyzed by XANES at the Soft X-ray Spectroscopy (SXS) beamline of the Brazilian Synchrotron Light Laboratory (Campinas, Brazil). Soil samples from the depth of 0-20 (mixture of 0-10 and 10-20 cm) were finely ground (< 150 µm) with an agate mortar and pestle and homogenized. Only the samples collected after the first harvest of treatments using 180 kg ha^{-1} P_2O_5 (in the presence or absence of FC) were analyzed.

Each sample was spread as a thin film in a double-sided carbon tape, mounted in a sample holder, and placed in a vacuum chamber (~10-7 mbar). After energy calibration using a calcium phosphate standard (E_0= 2151.7 eV), the spectra were acquired in the energy range of 2123-2300 eV via four-steps procedure (1.0 eV from 2120 to 2145 eV; 0.2 eV from 2145.2 to 2180 eV; 1.0 eV from 2182 to 2220 eV and 3.0 eV from 2223 to 2300 eV). Spectrum acquisition time was 1s per data point, totaling ~12 min per scan. The scans were taken in fluorescence mode, using a Si double crystal, and the detector dead time was monitored throughout data collection to not exceed 8%. For each sample, 6 to 8 scans were collected and merged. Subsequently, the reference energy (E_0) of each spectrum was adjusted to the maximum of the first derivative. Then, normalization was performed by subtracting the background data by fitting a first-order polynomial to the pre-edge region (E_0 -29.43 to E_0 -5.9 eV) and a second-order polynomial over the post-edge region (E_0 +25 to E_0 +64 eV), using the software package Athena (Ravel and Newville, 2005). The same procedure was performed on a FC sample. For Control treatments, the normalization was performed by subtracting the background signal by fitting a first-order polynomial to the pre-edge region (E_0 -23.97 to E_0 -8.91 eV) and a second polynomial over the post-edge region (E_0 +27.14 to E_0 +61.29 eV).

Since various soil constituents can be potentially relevant for P sorption, the main species of Po and Pi found in tropical soils were selected as standards for SXS, namely: phytate, lecithin, PO_4-kaolinite (Al_2O_3), PO_4-gibbsite [$Al(OH)_3$], PO_4-non-crystalline Al-(hydr)oxide ($AlPO_4*2H_2O$), PO_4-hematite (α-Fe_2O_3), PO_4-goethite (α-FeOOH), PO_4-ferrihydrite, P-hydroxyapatite [$Ca_5(PO_4)_3OH$], amorphous calcium phosphate and octacalcium phosphate [$Ca_8H_2(PO_4)_6.5H_2O$]. Phytate, lecithin, amorphous calcium phosphate, and octacalcium phosphate standards were obtained from commercial suppliers. Gibbsite, kaolinite, and hematite standards were obtained from Dr. Audrey Gamble (University of Delaware). The other standards were prepared as described by Abdala et al. (2005). The spectra obtained from the standards were used against the spectra of the unknown samples, via the linear combination (LC) fitting procedure, over the energy E_0 -15 to E_0 +35 eV. It allowed the determination of the relative proportion of P in soil samples. The choice of P species standards used in the LC fitting was based, initially, on the visual comparison of the pre and post-edge spectral characteristics (Figures S3 and S4). A maximum of 4 standard spectra were used for each LC fitting so that combinations negative or lower than 5% were excluded. The variation of E_0 allowed was between 0.4 and 0.5 eV and the sum of the weights of the LC fitting was forced to result in 1. The choice of the best combination took into account the values of R^2 and Chi^2. The Manceau combo method (Manceau et al., 2012) was also used to validate our data fitting procedure.

5.2.4. Statistical analysis

As the soil was sampled every 2 years the assumption of residual independence is not valid, and mixed models were run. The appropriate model was selected by the likelihood ratio test and, when necessary, Akaike information criterion (AIC) and Bayesian information criterion (AIC) were used as information criteria. To verify the significance of the fixed effects the Wald-F test was performed ($p < 0.05$). In all cases, the predicted mean values and their corresponding confidence intervals were obtained. The treatments comparison was carried out by Fisher's unprotected LSD ($p < 0.05$) and can be verified through the confidence intervals, so that, if there is an intersection between two intervals, we conclude that the means are not significantly different. Otherwise, there is evidence of a significant difference between means. All the analyzes were performed by R software (R Core Team, 2021).

5.3. Results and Discussion

5.3.1. Labile P pool in the soil

Considering the fertilizer placement and that P has low mobility in the soil, the most significant results of Hedley´s procedure were found in the soil sampled at 10-20 cm depth. Thus, only the results of this soil layer and its discussion were presented.

The LP pool was significantly affected by the interaction FC x mineral P source x dose of P in the first and third crop cycles at Agudos and the first and fifth at Macatuba (Tables S1 and S2). In the fifth year, at Agudos there was a significant interaction between FC and mineral P source, and in the third year at Macatuba, there was no significant interaction between FC and either mineral fertilizers or doses

(Tables S1 and S2). By convention, in this pool P is operationally related to forms readily diffused into the soil solution and weakly adsorbed on the surface of mineral compounds and low recalcitrant organic materials (Tissen and Moir, 1993).

After the first sugarcane harvest at Agudos, the association of FC with the mineral fertilizers applied at a rate of 180 kg ha^{-1} of P_2O_5 led to a reduction in the LP compared to no FC addition (-FC) (Figures 1A and B). This finding was not expected and does not support previous research about the kinetics of P release in the soil solution by FC in controlled conditions, which indicated a rapid FC mineralization (Chapter 4), and higher LP when FC was combined with RP compared to pure RP (Soares et al., 2022). This inconsistency can be due to the pre-experiment soil's chemical and physical characteristics - sandy texture, low OM content, low water retention capacity - in which FC mineralization may have been slower than observed in controlled conditions.

It has commonly been assumed that OM can coat native minerals from the soil, changing its characteristics like P sorption capacity and solubility (Wei et al., 2014). Likewise, we suspect that with the increased residence time of FC in the soil, this material may have coated the mineral fertilizers, reducing its solubilization rate, and inducing a residual effect of the fertilizers in the sandy soil. In this case, an increase in the MLP and NLP would also be expected for the treatments under + FC. However, in the first year of the experiment, FC did not affect the MLP (Table S1). Thus, was not possible to confirm this hypothesis by investigating only the P stocks in the soil, and the analysis of the P fractions individually could be useful.

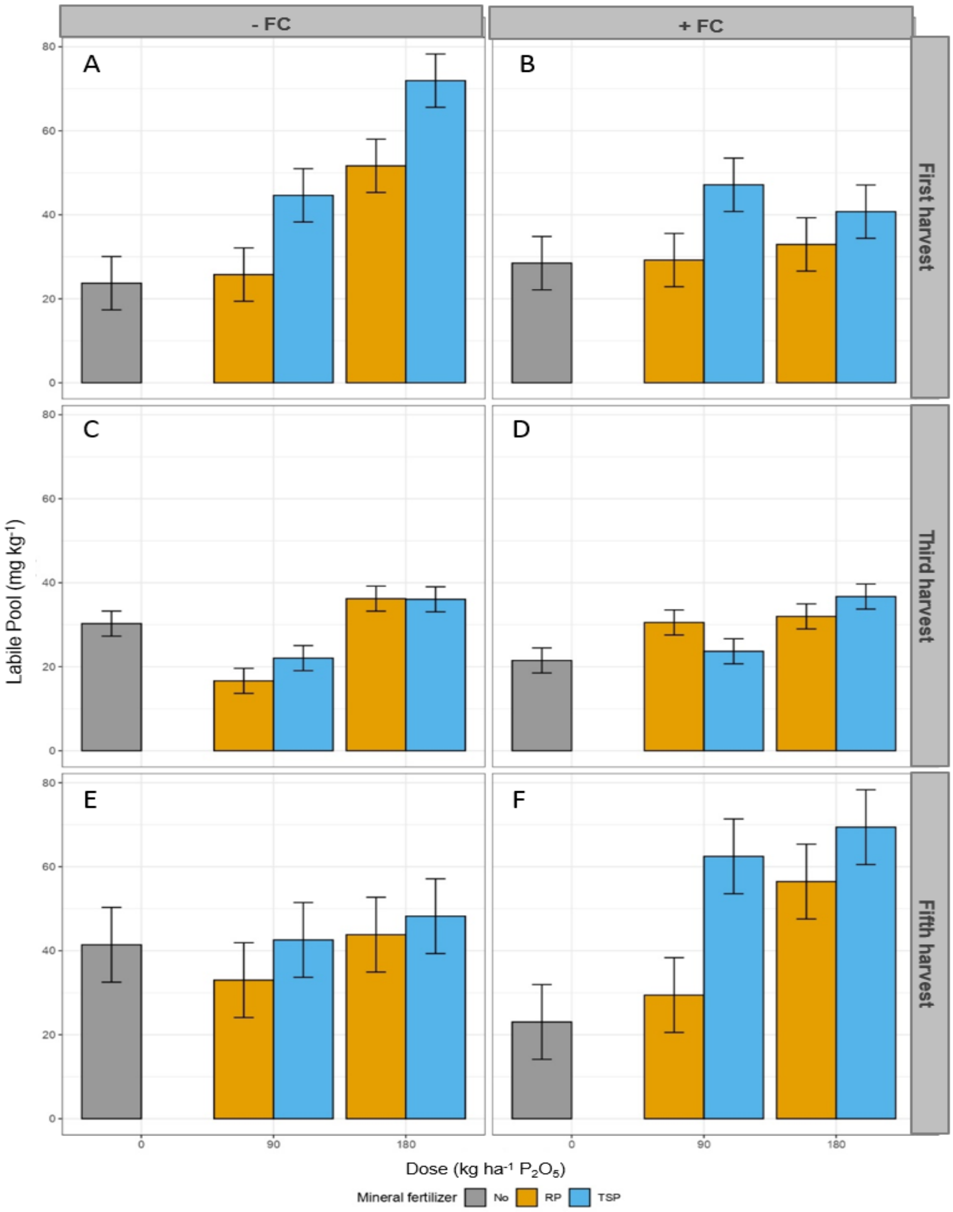

Figure 1. Labile P pool in the soil in the first (A and B), third (C and D), and fifth (E and F) sugarcane cycles affected by the addition of triple super phosphate (TSP) and rock phosphate (RP), at the doses of 90 and 180 kg ha^{-1} of P_2O_5, in the presence (+ FC) and absence of filter cake (- FC), at Agudos site. Treatments comparison (p< 0.05) can be verified through confidence intervals. Means with intersections between two intervals are not significantly different.

Overall, at Agudos there was a decrease in the LP pool for all the treatments in the third year (Figures 1C and 1D), followed by an increase mainly in TSP+FC (both doses) and RP+FC at the highest dose in the fifth year (Figures 1E and 1F). The reduction was possibly due to soil and plant drains, while the increase can be related to the buffering effect of the MLP and NLP pools, as a response to insufficient available P for plant uptake (Gatiboni et al., 2008). In this sense, this increased P content for TSP+FC and RP+FC (Figure 1F), supports our suspicion that the use of FC promoted a residual effect of fertilizers, which usually is not observed for TSP, due to its rapid solubilization in the soil, and frequently conversion into poorly available forms as mentioned in the introduction. Thus, the slower P release rate found for TSP+FC and RP+FC in the first crop cycle is considered beneficial, especially for semi-perennial crops.

Apparently, the soil condition at Macatuba was favorable for rapid mineralization of FC. In the first crop cycle, the use of this byproduct individually was as efficient as TSP 180 (+ FC and - FC) in rising the LP. The mineral sources under + FC were also efficient in increasing the LP compared to the control (- FC) (Figures 2A and 2B). In the same crop season, the largest P stock was obtained for TSP in the highest dose, irrespective of the presence of FC (Figures 2A and B), but only under + FC in the last season (Figures 2E and 2F). Therefore, it is reasonable to assume that there was a residual effect of TSP under + FC. Although this result is similar to the one obtained in the sandy soil, is important to point out that at Macatuba it does not look to be related to the FC effect in delaying the fertilizer solubilization. The increase of the LP over time is supported by research carried out in soils with a history of annual P input (Gatiboni et al., 2008; Guardini et al., 2012; Houben et al., 2019) as in this site.

The comparison between the mineral P sources under + FC and - FC in the first year of Macatuba´s experiment showed distinct behavior in the LP pool. The association of FC with TSP (regardless of the dose) did not increase P availability but did when it was associated with RP (regardless of the dose) signalizing a positive interaction between FC and RP (Figures 2A and 2B). There are two possible explanations: one is based on the assumption that FC can accelerate the RP solubilization through reactions promoted by organic acids released during the mineralization of organic materials (Lu et al., 2014); the second considers that the solubilization rate of RP was the same in RP+FC and pure RP, and the higher P provided by RP+FC was the result of the greater amount of P applied in RP+FC than in pure RP. If the second option is right, differences between TSP+FC and pure TSP also should have been observed, and the MLP must have been equally enhanced by RP and RP+FC, which was not the case (Figures 4A and 4B).

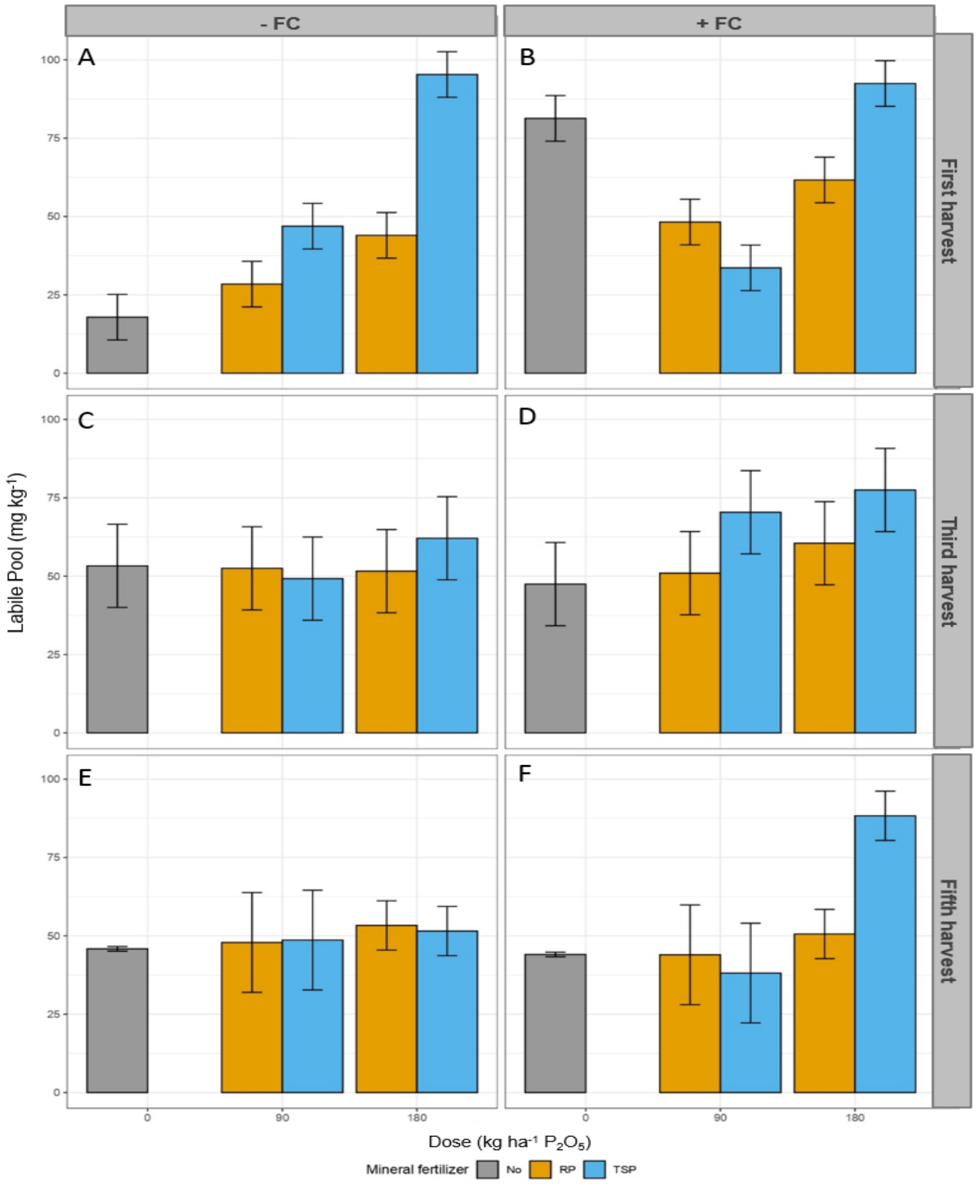

Figure 2. Labile P pool in the soil in the first (A and B), third (C and D), and fifth (E and F) sugarcane cycles affected by the addition of triple super phosphate (TSP) and rock phosphate (RP), at the doses of 90 and 180 kg ha^{-1} of P_2O_5, in the presence (+ FC) and absence of filter cake (- FC), at Macatuba site. Treatments comparison (*p*< 0.05) can be verified through confidence intervals. Means with intersections between two intervals are not significantly different.

Regarding the dose effect, in general, in both experiments when mineral P sources were applied in the pure form, the dose of 180 kg ha^{-1} P_2O_5 performed better than 90 kg ha^{-1} P_2O_5. In + FC, the increase in the dose benefitted the desirable residual effect for both sources at Agudos and TSP at Macatuba site.

5.3.2. Moderately-labile P pool in the soil

The MLP pool in the soil sampled after the first harvest at Agudos was not directly affected by the use of FC, but it was by the mineral P source and doses individually. In the subsequent evaluations (third and fifth years), there was significant FC x mineral P source interaction (Table S1). Overall, the largest stock of MLP was recorded by the use of RP, being greater when applied at a rate of 180 kg ha^{-1} P_2O_5 under - FC (Figure 3). Presumably, this pool comprises apatite and other Ca- phosphates with fewer solubility that are extracted by HCl, besides P adsorbed with intermediate energy by clays minerals, humic acids, and Fe and Al oxide-hydroxides (Hedley et al., 1982; Linquist et al., 1997), what explain those results.

The average over treatments and crop cycles showed that the addition of FC reduced by ~33% the MLP at Agudos site. Reductions in the MLP when RP and TSP were associated with FC were also observed by Borges et al., (2019) testing an organo-mineral fertilizer produced with FC, and by Soltanghiesi et al. (2019) testing it as raw material. As an organic material, the superficial charges and the low molecular weight organic acids released during the decomposition of FC can interact with the soil solid phase and compete with P adsorption sites limiting the retention of P or keeping it in weakly sorbed forms (Pavinato and Rosolem, 2008). Also, organic acids can dissolve insoluble forms of phosphate (Nahas, 1996), such as Ca-phosphates, and organometallic stable complexes with Fe and Al (Sposito, 1989). For RP+FC, the reduction in MLP pool indicates that less insolubilized fertilizer remained in the soil, which means a greater RP solubilization rate under + FC. Indeed, when this fertilizer was applied + FC the MLP was at least 40% lower than under - FC, regardless of the dose, which ultimately can result in greater P uptake by sugarcane.

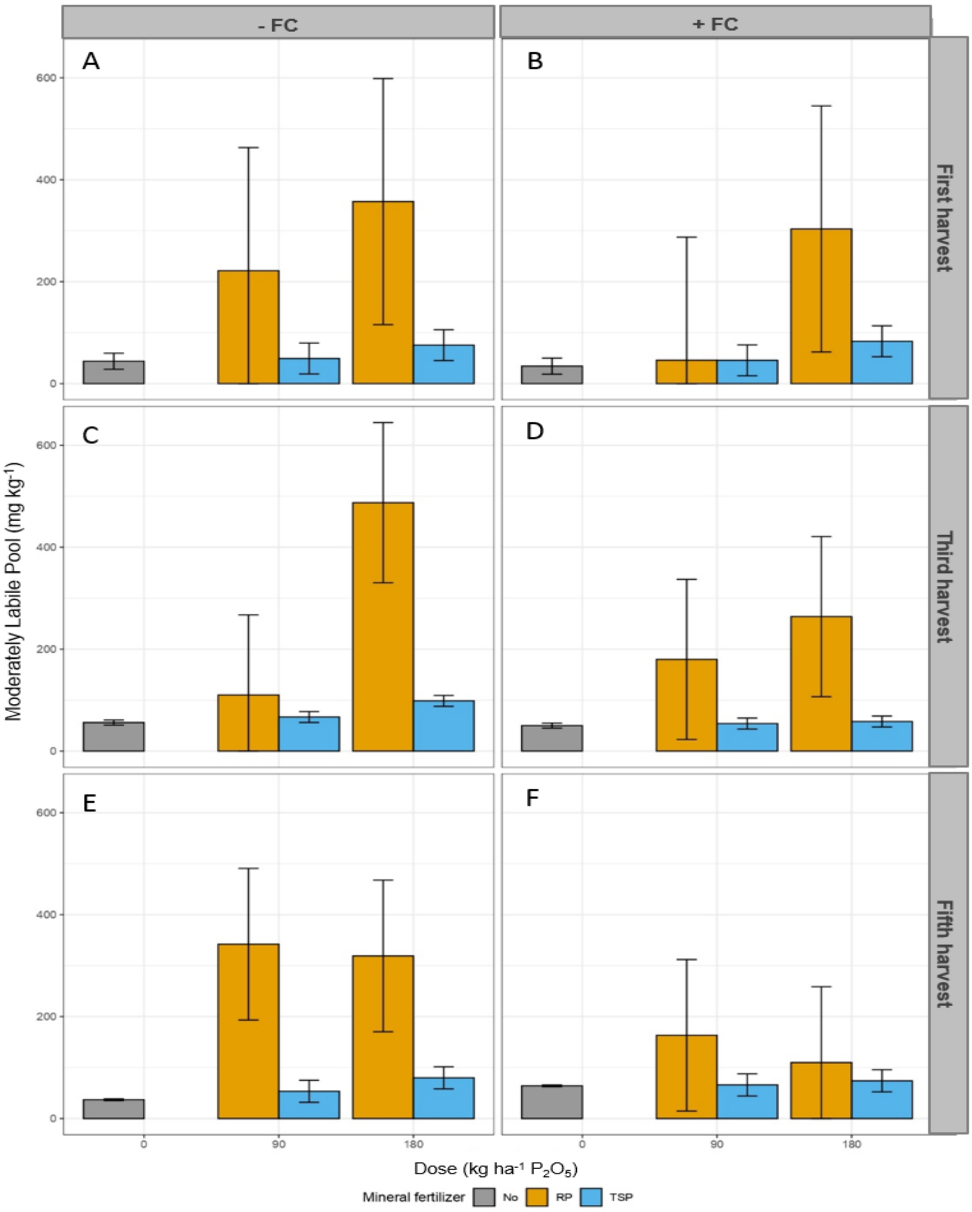

Figure 3. Moderately-labile P pool in the soil in the first (A and B), third (C and D), and fifth (E and F) sugarcane cycles affected by the addition of triple super phosphate (TSP) and rock phosphate (RP), at the doses of 90 and 180 kg ha^{-1} of P_2O_5, in the presence (+ FC) and absence of filter cake (- FC), at Agudos site. Treatments comparison ($p< 0.05$) can be verified through confidence intervals. Means with intersections between two intervals are not significantly different.

At Macatuba site, the difference in the contribution of TSP and RP to the MLP pool was lower than at Agudos. Only in the first year and in the highest dose there was a significant difference between pure RP and RP+FC, and again the use of RP reduced the MLP (Figures 4A and 4B). The changes in the LP and MLP pools by this mineral P source showed that the soil´s physical-chemical characteristics were favorable for the solubilization of RP, explaining its poor contribution to this pool of P.

In general, the increase in the MLP from the first to the third year and the subsequent reduction in the fifth year (Figure 4), explain the result obtained for the LP that suffered low fluctuation from the third to the fifth year, what was expected because of the MLP buffering the LP (Tiessen et al., 1984).

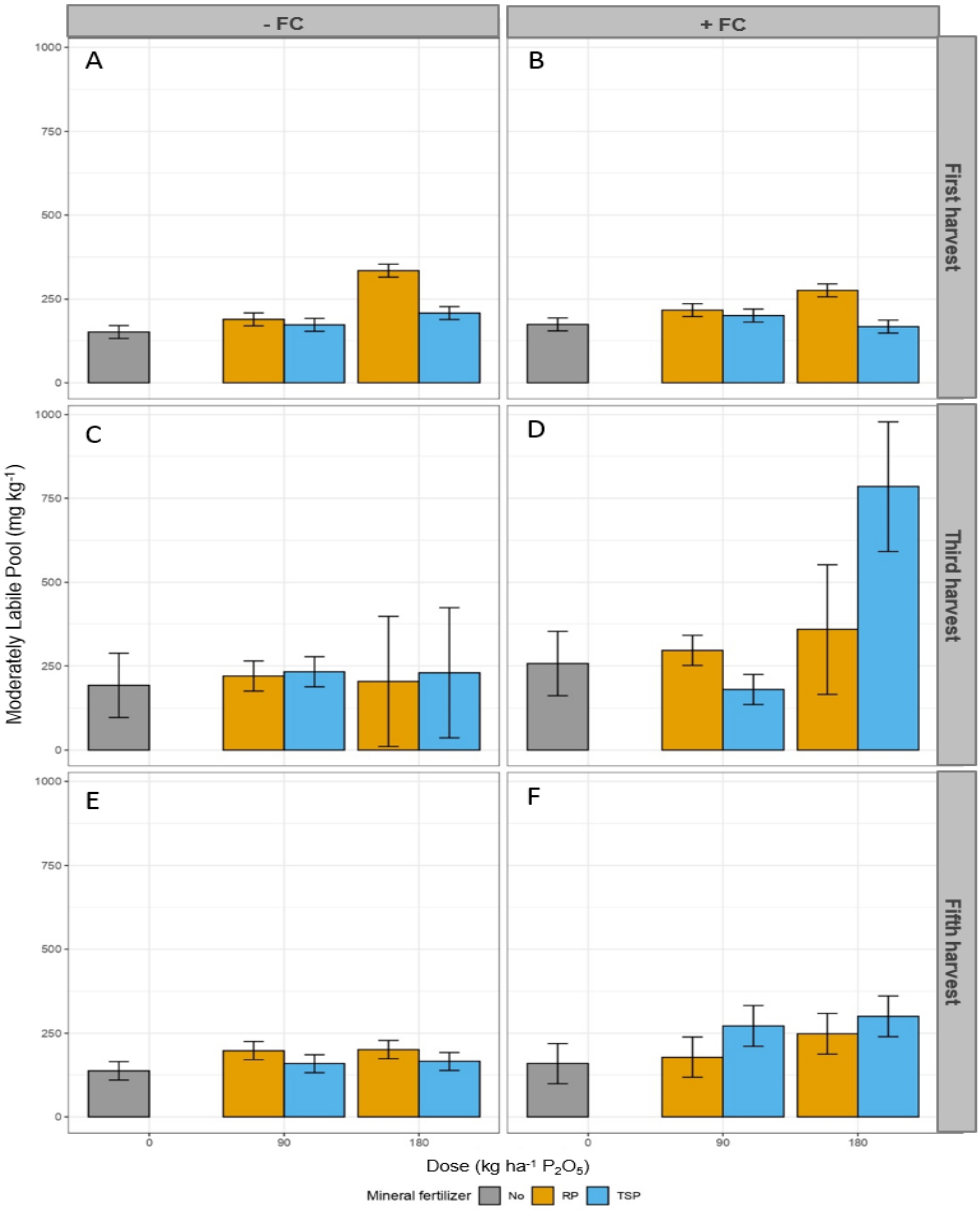

Figure 4. Moderately-labile P pool in the soil in the first (A and B), third (C and D), and fifth (E and F) sugarcane cycles affected by the addition of triple super phosphate (TSP) and rock phosphate (RP), at the doses of 90 and 180 kg ha^{-1} of P_2O_5, in the presence (+ FC) and absence of filter cake (- FC), at Macatuba site. Treatments comparison ($p < 0.05$) can be verified through confidence intervals. Means with intersections between two intervals are not significantly different.

5.3.3. Non-labile P pool in the soil

The greater increments in the NLP pool in the sandy soil (Agudos) were observed under RP addition, which is consistent with its low solubility (Hou et al., 2018). In general, regardless of the association with FC, in all the evaluations this fertilizer increased the NLP compared to TSP and control treatments (Figure 5). In the first and third crop cycles, there was a significant FC x mineral P source x dose of P interaction (Table S1), but no difference was observed between the treatments under + FC and - FC (Figures 5A to 5D). In the first cycle, RP+FC 180 resulted in the highest P content, while under - FC even the lower dose of RP increased the NLP. However, in the third cycle, the dose effect of RP in the NLP was observed only in - FC. Finally, in the last evaluation (fifth year) only the mineral P sources and doses individually affected the NLP (Table S1). Thus, it was not possible to observe any tendency in the effect of the doses of RP over time for the NLP.

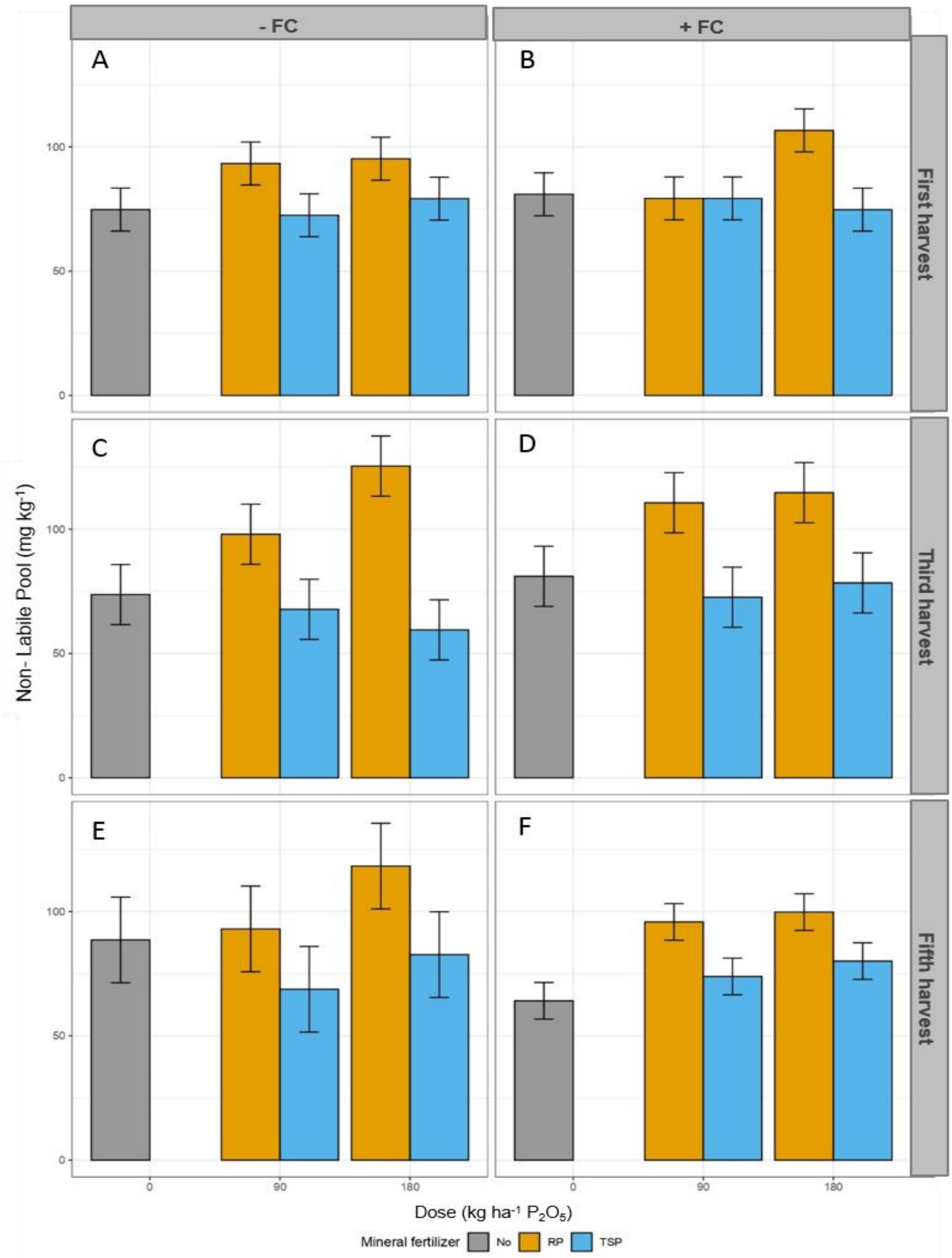

Figure 5. Non- labile pool of P in the soil in the first (A and B), third (C and D), and fifth (E and F) sugarcane cycles affected by the addition of triple super phosphate (TSP) and rock phosphate (RP), at the doses of 90 and 180 kg ha^{-1} of P_2O_5, in the presence (+ FC) and absence of filter cake (- FC), at Agudos site. Treatments comparison (*p*< 0.05) can be verified through confidence intervals. Means with intersections between two intervals are not significantly different.

At Macatuba, the NLP pool was affected by the use of FC in the first and fifth years (Table S2). In those years, all the treatments associated with FC accumulated NLP in the same amount as the control (Figures 6B and 6F). In contrast, under - FC significant differences were found between the treatments (Figures 6A and 6E). In this condition, in the first year, the largest amount of P in the NLP pool was verified for TSP 180, which was also higher than TSP+FC 180. In the fifth year, lower conversion of P into non-available forms was also found for RP+FC, compared to pure RP (regardless of the dose). Interestingly, in the LP no difference for TSP (first year) and RP (fifth year) in the presence or absence of FC was observed, highlighting the role of FC in delaying the conversion of LP into NLP forms. Whereas under - FC the P delivered by TSP followed the same trend commonly reported for this source in tropical soils with low P content; being quickly sorbed by high-energy binding sites (Gatiboni et al., 2021; Rodrigues et al., 2016). This delay, even if temporary, can be of high value for plant nutrition.

In both sites, the NLP was the most stable pool throughout the years due to its low activity and bioavailability (Costa et al., 2016). Nonetheless, after the assessment of the soil P pools, it is clear that the P sources and FC use affect the nutrient dynamic differently depending on soil characteristics. At Macatuba it was noticed a P flux toward the NLP pool over time, regardless of the source and dose of P, leading to a decrease in the P stocks in the MLP and LP in most treatments. On the other hand, in the sandy soil of Agudos, the variation of the P content in the NLP over time was lower than at Macatuba, suffering reductions to restock the more available P pools. The reason for this difference between the sites may be related to the higher Pmax and OM content at Macatuba than at Agudos (~4.5 and 2.5 fold higher, respectively) since this P pool consists of stable Po associated with OM and Pi forms assumed to be with low solubility (Condron et al., 1985; Costa et al., 2016).

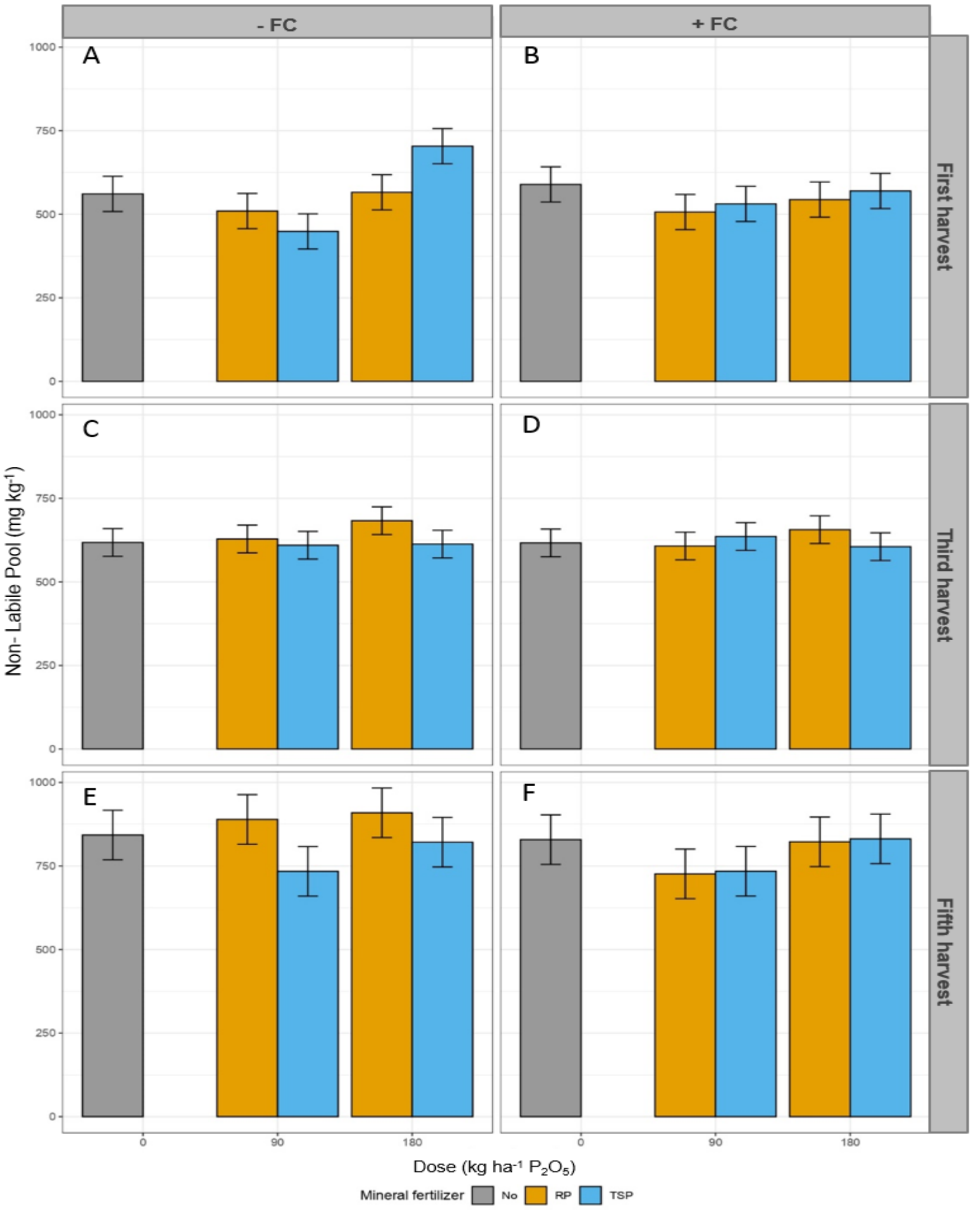

Figure 6. Non-labile P pool in the soil in the first (A and B), third (C and D), and fifth (E and F) sugarcane cycles affected by the addition of triple super phosphate (TSP) and rock phosphate (RP), at the doses of 90 and 180 kg ha^{-1} of P_2O_5, in the presence (+ FC) and absence of filter cake (- FC), at Macatuba site. Treatments comparison ($p < 0.05$) can be verified through confidence intervals. Means with intersections between two intervals are not significantly different.

5.3.4. Speciation of P by XANES in the soil

The P species formed after the addition of phosphate fertilizers was investigated by P K-edge XANES spectroscopy. Is important to highlight, however, that even though fertilization management can cause changes in the P interaction with the minerals in the soil, the association of the results obtained by this technique with the implication of fertilization in the P availability must be carefully done. Gérard

(2016) identified divergences in works evaluating the P sorption capacity of minerals, because of differences in experimental conditions (i.e. pH, ionic strength, variation in the specific surface area of the synthetic mineral used, and dose of P). The P sorption capacity of a mineral can be highly changed by its physical contact with other soil constituents (Wei et al., 2014; Gérard, 2016). Furthermore, the crystallinity and chemical composition of a specific mineral can significantly vary from one site to another (Singh and Gilkes, 1992). Thus, in this work, the discussion related to the role of the treatments in changing the P availability based on its association with soil minerals is only hypothetical and must be interpreted considering the changes in the P pools obtained by Hedley´s fractionation.

Regardless of the soil texture and pre-experimental land use history, before the application of the treatments, the predominant species of P in the soil were associated with Fe (P-hematite), followed by Al (P-gibbsite) and organic compounds as phytate (Table 3). However, it was observed a shift from Fe-bound P to Al-bound P after the addition of TSP, and TSP+FC. The increased contribution of P-Al species compared to P-Fe species is a common result after P fertilization (Börling et al., 2001; Eriksson et al., 2016; Pen et al., 2005), and is explained by the high affinity between P and Fe hydroxides. Initially, P trends to be associated preferentially with Fe, but with soil increasing P availability and saturation of Fe minerals, P shows greater affinity with Al minerals (Khare et al., 2004).

The fertilization with TSP (+ and - FC) led to the association of P with amorphous Al in both experimental locations (Table 3). It is well known that the rapid increase in P concentration in the soil solution by soluble fertilizers induces P retention by different minerals, in different forms and energies (Klotzbücher et al., 2019). The main mechanisms of orthophosphate retention are the displacement of other anions adsorbed to the soil solid phase (chemisorption) and surface precipitation (Menezes-Blackburn et al., 2016). In the specific case of P-amorphous Al, the retention is commonly assumed to be by surface precipitation, which is more stable with time (Prietzel et al., 2016). Thus, we can infer that the high solubility of TSP favored the formation of low available P species in the soil, which was confirmed by the Hedleys´s fractionation, but only under - FC at Macatuba.

The best fits obtained for the soil samples from Macatuba after mineral fertilizers addition (regardless of FC) were found when goethite was used as P-Fe reference. However, at Agudos, similar results were observed for the mineral P sources only under + FC. A possible explanation is that the presence of goethite over hematite is favored by low pH, in addition to high OM content, and moisture in the soil (Kämpf and Schwertmann, 1983; Bortoluzzi et al., 2015), which are naturally high at Macatuba and can be improved by the use of FC at Agudos. The P partitioning as goethite in the topmost layer in soils amendment with pig slurry was also found by Abdala et al. (2020). Some studies have reported that the low molecular weight organic acids from OM are capable to promote the preferential dissolution of hematite by chelation processes, and Fe can be reprecipitated as less crystalline minerals, represented by goethite in our study (Schwertmann, 1991; Jeanroy et al., 1991). Once the presence of goethite has been increased in the soil, the proportion of P associated with this mineral probably will be higher than with hematite, since the less crystallinity and higher specific surface area of goethite (Almeida et al., 2003). However, it is difficult to state to what extent the increase in soil OM content by FC addition can affect the presence of goethite in the soil.

Table 3. Relative proportions of phosphate species (weight %) obtained by the Linear combination fitting of P K-edge XANES data of the soil samples from Agudos and Macatuba, at the first sugarcane harvest, after the addition of 180 kg ha^{-1} of P_2O_5 of triple super phosphate (TSP) and rock phosphate (RP), in the presence (+ FC) and absence of filter cake (- FC) at planting.

Treat.	Phy*	Lec	Kao	Gib	Non-cryst. Al	Hem	Goet	Hyd. apat	Am CaP	Octa CaP	R factor
					Agudos site (Sandy soil)						
Control	20	-	-	23	-	57	-	-	-	-	0.001
TSP	13	-	-	40	26	21	-	-	-	-	0.004
TSP+FC	11	-	-	24	42	-	23	-	-	-	0.003
RP	24	-	-	23	-	30	-	23	-	-	0.009
RP+FC	15	-	-	22	-	-	62	-	-	-	0.002
					Macatuba site (Clayey soil)						
Control	6	-	-	18	-	76	-	-	-	-	0.002
TSP	-	17	-	9	41	-	33	-	-	-	0.002
TSP+FC	12	-	-	26	41	-	21	-	-	-	0.003
RP	16	42	-	11	-	-	31	-	-	-	0.006
RP+FC	15	-	-	27	-	-	58	-	-	-	0.002

*Phy: phytate; Lec: lecithin; Kao: PO_4-kaolinite; Gib: PO_4-gibbsite; Non-Cryst Al: PO_4-Non-crystalline Al Hydroxide; Hem: PO_4-hematite; Goet: PO_4-goethite; Hyd. Apat: hydroxyapatite; Am CaP: amorphous calcium phosphate; OctaCaP: octacalcium phosphate.

It was interesting that P-Ca as hydroxyapatite did not show a good fitting with the soil´s spectra under RP (+ and - FC) at Macatuba or for RP+FC at Agudos, but did it for RP alone at Agudos (Table 3). This finding agrees with Hedley´s fractionation data that suggested a greater RP solubilization rate under + FC, with less insoluble apatite remaining in the MLP pool at Agudos. It also indicates that possibly at Macatuba there was a greater RP solubilization than at Agudos, because of the higher specific surface area of the soil from the former location. This result also highlights that is important to consider the soil properties to define the best fertility management strategy.

Monoester orthophosphates (R-O-PO_3), represented here by phytate, are adsorbed with high energy into the soil due to their residual charge, giving it stability in the solid phase by the formation of organo-mineral complexes (Anderson et al., 1974). Diester phosphates (R-O-PO_2-O-R) in turn, represented by lecithin, have their charges satisfied by the molecule's components, which reduces its adsorption by soil colloids, becoming more susceptible to microbial degradation. Lecithin was not found under + FC at Macatuba because this byproduct adds nutrients and energy promoting the increase of microbial biomass, which can easily mineralize diester phosphates. Besides, changes in soil pH affect the proportion of diester and monoester phosphates in the environment (Turner and Blackwell, 2013). The increase in soil pH after organic materials addition leads to a greater presence of monoester phosphates (Borges et al., 2019; Gatiboni et al., 2013; Turner and Engelbrecht, 2011), and, as reported in Chapters 2 and 4, the use of FC increases the soil pH, explaining our results. Probably, the same result was not observed at Agudos because of its low initial OM content. Indeed, soils under pastureland have shown a majority of high molecular weight polymeric organic P (McLaren et al., 2014; McLaren et al., 2017), more stable to degradation.

5.4. Conclusions

The association of FC with the mineral P sources tested changed the P species and dynamics at different levels in distinct soils (sandy soil from Agudos and clayey soil from Macatuba), confirming our hypothesis and emphasizing that the mineral phosphate management associated with FC must be carried out by taking into account the type of soil and history of land use. In both locations, the FC reduced the conversion of labile P into more stable forms in the soil when combined with RP. At Agudos site, was assumed that FC favored the solubilization of RP, as represented by the reduction of MLP pool and P-Ca. In this location, the association of FC with the mineral P sources was also beneficial for promoting a positive residual effect, leading to a better distribution of the P supply for sugarcane over time. At Macatuba, the association of FC with TSP at the dose of 180 kg ha^{-1} of P_2O_5 resulted in a high LP pool throughout the entire experiment, while its combination with RP was beneficial only in the first year. Based on the P content in the labile, moderately labile, and non-labile P pools and on the investigation of P species in the soil we can conclude that at Agudos the use of RP in association with FC is as efficient as TSP (with and without FC) in supplying P for sugarcane. Otherwise, at Macatuba after 5-yrs of the experiment, RP+FC was less efficient than TSP+FC at the dose of 180 kg ha^{-1} of P_2O_5.

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Supplementary material

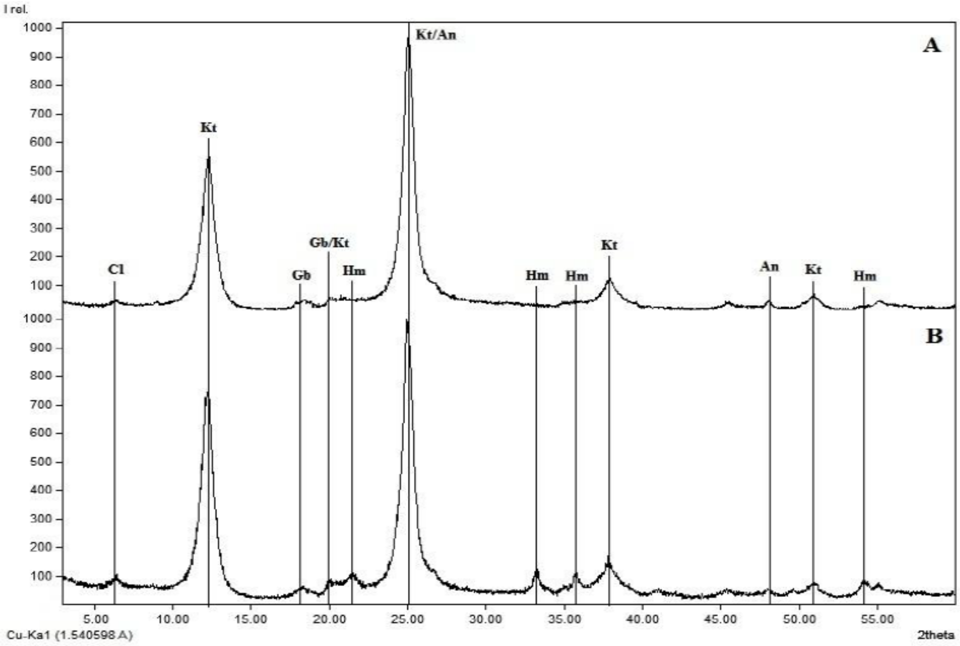

Figure S1. X-ray diffraction of the clay fraction from the 0-20 cm layer of soil sampled at Agudos (sandy soil), treated with (A) and without (B) citrate bicarbonate-dithionate, to extract Fe. An= anatase; Cl= chlorite; Gb= gibbsite; Hm= hematite; Kt= kaolinite.

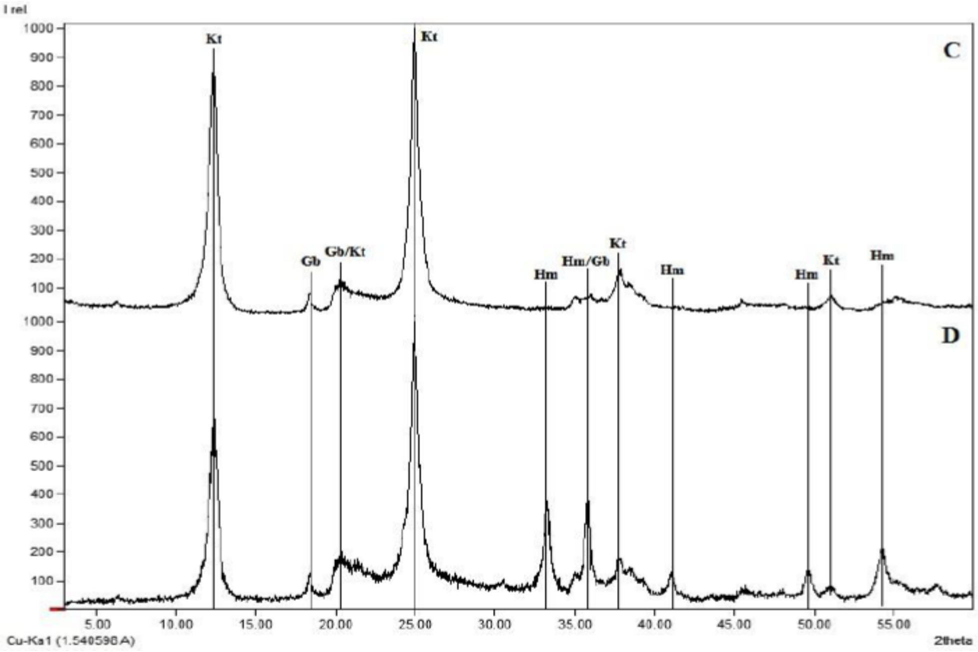

Figure S2. X-ray diffraction of the clay fraction from the 0-20 cm layer of soil sampled at Macatuba (clayey soil), treated with (A) and without (B) citrate bicarbonate-dithionate, to extract Fe. An= anatase; Cl= chlorite; Gb= gibbsite; Hm= hematite; Kt= kaolinite.

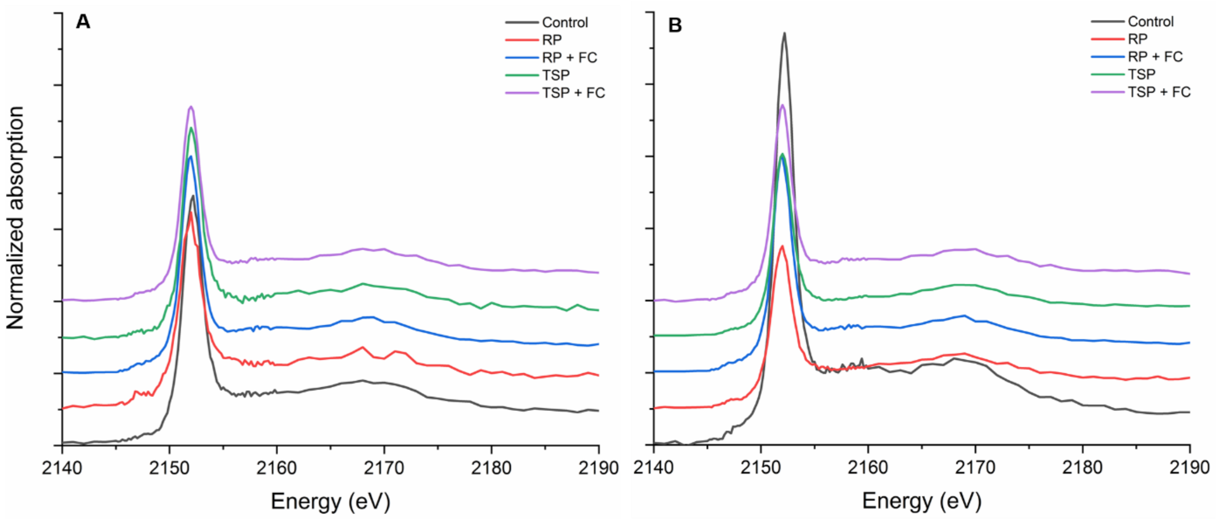

Figure S3: P K-edge XANES spectra of soil samples from the experiments carried out at Agudos (A) and Macatuba (B) after one sugarcane cycle fertilized or not (Controle) with triple superphosphate (TSP) and rock phosphate (RP) in the presence and absence of filter cake (FC).

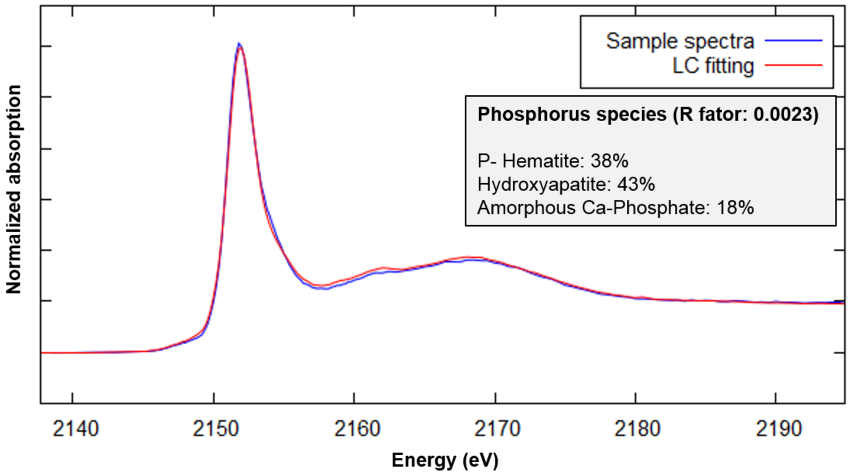

Figure S4. Normalized Phosphorus K-edge Absorption Near Edge Structure (XANES) spectra with results of linear combination (LC) fitting for filter cake used in the study.

Table S1. Statistical significance for the fixed effects ($p < 0.05$) of byproduct (presence or absence of FC), mineral P source (triple superphosphate or rock phosphate), dose (0, 90, and 180 kg ha^{-1} of P_2O_5), and its interactions in the experiment carried out in Agudos.

Fixed effect	Labile P	Moderately- labile P	Non-labile P
	---------------	p> F	---------------
		First year	
Byproduct (FC)	< 0.01	0.486	0.028
Mineral P source (fertilizer)	< 0.01	0.021	< 0.01
Dose	< 0.01	< 0.01	< 0.01
FC x Fertilizer	< 0.01	< 0.01	< 0.01
FC x Dose	< 0.01	< 0.01	0.161
Fertilizer x Dose	< 0.01	0.150	0.049
FC x Fertilizer x Dose	< 0.01	< 0.01	0.229
		Third year	
Byproduct (FC)	0.467	< 0.01	< 0.01
Mineral P source (fertilizer)	< 0.01	< 0.01	< 0.01
Dose	0.033	< 0.01	0.122
FC x Fertilizer	0.499	0.011	< 0.01
FC x Dose	0.638	0.016	0.348
Fertilizer x Dose	0.157	0.014	0.420
FC x Fertilizer x Dose	0.614	0.087	0.928
		Fifth year	
Byproduct (FC)	0.657	0.086	0.074
Mineral P source (fertilizer)	< 0.01	< 0.01	< 0.01
Dose	0.014	0.091	0.039
FC x Fertilizer	0.584	0.424	0.088
FC x Dose	0.238	0.574	0.117
Fertilizer x Dose	0.031	0.051	0.981
FC x Fertilizer x Dose	< 0.01	0.034	0.448

Table S2. Statistical significance for the fixed effects ($p< 0.05$) of byproduct (presence or absence of FC), mineral P source (triple superphosphate or rock phosphate), dose (0, 90, and 180 kg ha^{-1} of P_2O_5), and its interactions in the experiment carried out in Macatuba.

Fixed effect	Labile P	Moderately- labile P	Non-labile P
	---------------	p> F	---------------
		First year	
Byproduct (FC)	< 0.01	0.072	0.08
Mineral P source (fertilizer)	< 0.01	0.024	< 0.01
Dose	< 0.01	0.126	< 0.01
FC x Fertilizer	< 0.01	0.090	< 0.01
FC x Dose	< 0.01	0.796	< 0.01
Fertilizer x Dose	< 0.01	0.534	0.027
FC x Fertilizer x Dose	< 0.01	0.375	0.015
		Third year	
Byproduct (FC)	< 0.01	0.109	< 0.01
Mineral P source (fertilizer)	< 0.01	0.225	< 0.01
Dose	< 0.01	< 0.01	0.303
FC x Fertilizer	< 0.01	0.097	< 0.01
FC x Dose	< 0.01	< 0.01	0.172
Fertilizer x Dose	< 0.01	0.011	0.859
FC x Fertilizer x Dose	< 0.01	0.014	0.476
		Fifth year	
Byproduct (FC)	0.551	0.627	0.035
Mineral P source (fertilizer)	0.102	0.127	0.072
Dose	< 0.01	0.187	< 0.01
FC x Fertilizer	0.468	0.496	0.044
FC x Dose	< 0.01	0.484	0.401
Fertilizer x Dose	0.011	0.030	0.501
FC x Fertilizer x Dose	0.012	0.623	0.515

6. SUGARCANE PERFORMANCE UNDER RECYCLED FILTER CAKE ASSOCIATED WITH MINERAL PHOSPHATE SOURCES

Abstract

Brazil is the major worldwide sugarcane producer, and phosphorus (P) is one of the nutrients that most affect its yield and quality. The nutrient is usually supplied using water-soluble fertilizers added in the planting furrow at crop establishment. The association of filter cake (FC), a byproduct of the sugarcane industry, with mineral P sources is a strategy to increase the P use efficiency. This study aimed to evaluate the agronomic performance of sugarcane cultivated in two Oxisols (sandy and clayey) fertilized with triple superphosphate (TSP) and rock phosphate (RP) (doses of 90 and 180 kg ha^{-1} P_2O_5) in the presence and absence of FC, for five cropping seasons. Two experiments were conducted at Agudos (sandy soil) and Macatuba (clayey soil) sites. In every harvest, the dry matter (DM) production, P uptake, and yield were measured. After 5-yrs, in Agudos, RP+FC 90 kg ha^{-1} of P_2O_5 was enough to increase the cumulative DM and P uptake, but double the dose was required to increase the yield, regardless of the FC addition. In Macatuba, the use of RP+FC at the highest dose resulted in the same cumulative DM and P uptake as TSP, but FC did not affect the yield, and only TSP, in both doses, differs from the control in the last harvest. Collectively, the results showed that the P fertilization with FC and mineral sources had different effects depending on the soil attributes and previous land use, and RP is a feasible strategy to partially replace TSP in Agudos.

Keywords: Sugarcane yield, Triple superphosphate, Byproduct, Bayóvar, Gafsa

6.1. Introduction

Brazil is the major worldwide sugarcane producer, representing approximately 41% of the world's production volume (FAOSTAT 2019). To meet the increased demand for bioenergy, sugarcane fields have expanded to degraded pasture land in the southeast of the country (Taniwaki et al. 2017), a region that concentrates 63% of the national production (production of 371.5 from a total of 592 million of tons) (CONAB 2021).

Phosphorus (P) is one of the nutrients that most affect sugarcane development, metabolism, productivity, and quality since it is an essential element for the photosynthetic activity, synthesis of macromolecules, root development, tillering, and sucrose production (Alexander 1973; Lima Vasconcelos et al. 2017). However, the proper management of phosphate fertilization in tropical soils is challenging because of its naturally high P adsorption capacity caused by the expressive amounts of Fe and Al (oxy) hydroxides and hydrous oxides (Mabagala and Mng'ong'o 2022; Pierzynski and Hettiarachchi 2018).

In this sense, to avoid P insufficiency limiting the sugarcane yield high doses of mineral fertilizers, especially water-soluble ones, have been used (Soltangheisi et al. 2019). However, this practice is proved inefficient, resulting in low P solubility, once a great part of the P applied to the soil turns into surface precipitated or inner-sphere complexed to the soil oxides (Beauchemin et al. 2003; Hedley and McLaughlin 2005; Khatiwada et al. 2012; Wang et al. 2017). This inefficient use of P is a matter of concern since the world consumption of phosphate has increased, but only a few countries (China, Marocco, United States of America, and Russia) hold 75% of the world's rock phosphate (RP) mining (USGS 2022).

The P recycling from bio-resources and the recovery of P in wastes are two of the five strategies listed by Withers et al. (2015) to increase the P use efficiency. In sugarcane fertilization, this strategy can be put into practice by the use of filter cake (FC), a byproduct of sugarcane clarification and filtration. FC is usually applied at the rate of 15-30 ton ha^{-1} (wet base) at the planting furrow (van Raij et al. 1997) in an attempt to increase P use efficiency and partially replace mineral phosphate fertilizers (Almeida Junior et al. 2011; Caione et al. 2015; Santos et al. 2011; Yang et al. 2013; Santos et al. 2014).

Because P is poorly mobile in the soil, the agronomic and operational viability of adding phosphate fertilizers over the straw in the sugarcane ratoon remains inconsistent (Cantarella et al. 2008; Zambrosi et al. 2020). Thus, phosphate fertilizers, as well as FC, are predominantly applied in the planting furrow at crop establishment, supplying both the plant cane and the next ratoons. Even though sugarcane is a semi-perennial crop (cycle of 5-7 years), most of the studies on the effects of FC were carried out up to three cropping seasons in the field (Bekheet et al. 2018; Bokhtiar et al. 2008; Caione et al. 2015; Fravet et al. 2010; Lima Vasconcelos et al. 2020; Moda et al. 2015; Santos et al. 2010; Santos et al. 2014; Vazquez et al. 2015) or in greenhouse experiments (Almeida Júnior et al. 2011; Arruda et al. 2019; Borges et al. 2019; Braos et al. 2020; González et al. 2014; Soares et al. 2022; Utami et al. 2012). Therefore, the long-term effect of this byproduct on the sugarcane yield still missing consistent information.

Given this framework, we hypothesize that the addition of FC associated with mineral fertilizers (triple superphosphate (TSP) or rock phosphate (RP)) at sugarcane establishment can increase the P availability to plants, resulting in higher sugarcane stalk yield and P accumulation in the plant over the crop cycle (five years). The study aimed to evaluate the agronomic performance of sugarcane cultivated in two Oxisols (sandy and clayey) fertilized with TSP and RP (at rates of 90 and 180 kg ha^{-1} P_2O_5) in the presence and absence of FC, for five consecutive cropping seasons.

6.2. Material and Methods

6.2.1. Sites description

The effect of FC and mineral fertilizers were tested for 5-yrs in two field experiments carried out in sites with low available P in the soil (Table 1). The first experiment was conducted from 2013 to 2017, in a Latossolo Vermelho Amarelo distrófico sandy texture (Santos et al., 2013) or Hapludox (Soil Survey Staff, 2014), located in Agudos, São Paulo State, Brazil (22°33’22” S; 9°06’15” W). Previously, the site was covered for approximately twenty years by a degraded pasture. The second experiment was conducted from 2014 to 2018 in a Latossolo Vermelho Eutrófico chernossólico clayey texture (Santos et al., 2013) or Hapludox (Soil Survey Staff, 2014), located in Macatuba, São Paulo State, Brazil (22°29’39” S; 48°41’14” W). This area was used as sugarcane field for about fifty years before the experiment.

Soil characterization of both locations is given in Table 1. Before the establishment, both areas had the pre-experiment vegetation desiccated using glyphosate® (4 L ha^{-1} and 6 L ha^{-1}, respectively) and incorporated into the soil by deep plowing (0 - 50 cm). At this moment, it was also added, and incorporated by disc harrow (~0 - 20 cm depth), 3 and 3.5 ton ha^{-1} of lime ($CaCO_3$ + $MgCO_3$), and 1.5 and 2 ton ha^{-1} of gypsum ($CaSO_4$), at Agudos and Macatuba sites, respectively.

Table 1. Pre-experiment soil chemical, and physical characterization from sandy soil (Agudos) and clayey soil (Macatuba)

																DCB [f]		Oxalate [g]		
Layer	pH	OM [a]	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H + Al	SB [b]	CEC [c]	V [d]	M [e]	Clay	Silt	Sand	Fe	Al	Fe	Al	P max [h]
cm	$CaCl_2$	g kg^{-1}	mg kg^{-1}	----------------- mmolc kg^{-1} -----------------							%		--------------------- g kg^{-1} ------------------------							(mg kg^{-1})
									Sandy soil											
0-10	4.3	14.3	4	1.5	7	5	2	25	13.2	38.2	35	11	-	-	-	-	-	-	-	-
10-20	4.2	10.7	3	1.7	5	3	3	28	10.3	38.3	27	21	-	-	-	-	-	-	-	-
0-20	-	-	-	-	-	-	-	-	-	-	-	-	112	4	884	15.3	42.3	0.6	1.4	342
20-40	3.9	-	3	1.9	3	2	6	38	6.8	44.8	15	48	138	27	835	-	-	-	-	-
40-60	4	-	2	0.8	2.8	0.8	7	34	4.3	38.3	11	63	151	17	832	-	-	-	-	-
60-80	3.9	-	1	0.9	2.8	0.9	9	32	3.8	35.8	11	70	151	24	825	-	-	-	-	-
									Clayey soil											
0-10	5.6	31.0	11	1.9	54	25	0.9	22	80.9	103	79	1	-	-	-	-	-	-	-	-
10-20	5.3	28.5	9	1.7	44	22	0.9	28	67.7	96	71	1	-	-	-	-	-	-	-	-
0-20	-	-	-	-	-	-	-	-	-	-	-	-	629	152	219	110.9	53.4	4.4	1.1	1557
20-40	5.2	-	8	1.2	30	12	0.9	25	43.2	68	63	26	667	122	211	-	-	-	-	-
40-60	5.1	-	5	0.7	23	9	2	25	32.7	58	57	6	-	-	-	-	-	-	-	-
60-80	4.9	-	3	0.5	27	13	2	34	40.5	74	54	5	-	-	-	-	-	-	-	-

[a] OM: organic matter determined by Walkley-Black (1934); [b] SB: sum of bases; [c] CEC: cation exchange capacity; [d] V%: base saturation of CEC; [e] M%: Al saturation; [f] DCB: Fe and Al extracted by dithionite-citrate-sodium bicarbonate; [g] Fe and Al extracted by ammonium oxalate; [h] P_{max}: maximum phosphorus sorption capacity. pH in $CaCl_2$, with ratio soil: solution of 1: 2.5 m/v (Raij et al., 2001). P, K^+, $Ca^{2+,}$ and Mg^{2+} extracted by ion exchange resin. Al^{3+} extracted by KCl 1 mol L^{-1}. H + Al extracted by SMP (Shoemaker, Mclean, Pratt, 1971); granulometry determined by Pippete method.

6.2.2. Experimental design and treatments

The experimental areas consisted of plots of 90 m^2, with six 1.5 m distant rows, 10 m long each. The experiments comprised 10 treatments and three replicates, arranged in a split-split-plot design with the presence (+ FC) or absence (-FC) of 10 Mg ha^{-1} of FC (dry basis) as the main plot, and the mineral P sources - TSP (46% soluble P_2O_5) or RP - tested in three doses (0, 90 and 180 kg ha^{-1} of P_2O_5), as sub-plots. In the first experiment, the source of RP was Gafsa (9.6% soluble in citric acid 2%; 28.5% total P_2O_5), while in the second experiment, it was used the Bayovar RP (14% soluble in citric acid 2%; 29% total P_2O_5). The chemical properties of FC applied in both locations are shown in Table 2.

Table 2. Chemical parameters of the filter cakes applied in the establishment of the experiment at Agudos and Macatuba.

pH [a]	C [b]	P [c]	N [c]	K_2O [c]	Ca [c]	Mg [c]	S [c]	OM [b]	LP [d]	MLP [d]	NLP [d]
	%								mg kg^{-1}		
					Agudos						
6.7	14.3	0.57	2.3	0.14	2	0.16	0.19	28.6	858.1	4740.3	5326.5
					Macatuba						
7.4	27.6	1.13	3.5	1.07	4.1	0.33	0.28	53.3	-	-	-

[a] pH in $CaCl_2$ 0.01 mol L^{-1} at a ratio of 1:2.5 (m/v) (Raij et al., 2011); [b] Determined by loss of mass by ignition; [c] Determined following Malavolta et al., 1989; [d] Determined by Hedley et al., (1982), with modifications by Rheinheimer (2000). Labile P: labile phosphorus pool; m-labile: moderately labile phosphorus pool; n-labile: non-labile phosphorus pool

The choice of the FC and mineral P fertilizers doses was based on official recommendations for sugarcane fields in Brazil (Raij et al. 1997). Both were applied at the bottom of the planting furrow (~20 - 25 cm deep from the soil surface). After the sugarcane stalks were manually distributed at the planting furrow, eighteen to twenty buds per meter, and subsequently covered mechanically with soil. Because of the differences in chemical and physical soil properties and productive potential between the sites, the variety RB 867515 was used in Agudos, while the CTC-16 in Macatuba. In addition to the treatments, at the sugarcane establishment, it was also applied urea and KCl, at the doses of 60 e 90 kg ha^{-1} of N and 150 e 135 kg ha^{-1} of K_2O in Agudos and Macatuba, respectively. Also, every year after the sugarcane harvesting the ratoons (topdressing) were fertilized with 90 and 100 kg ha^{-1} of N as urea, and 90 and 100 kg ha^{-1} of K_2O as KCl in Agudos and Macatuba, respectively.

6.2.3. Sugarcane agronomic performance measurements

Sugarcane harvest was performed manually every twelve months after the planting. The yield was determined by weighing the stalks from the 4 central rows of each sub-plot (60 m^2). The top leaves, dead leaves, and stalks from 1 m of each central line (total of 4 linear meters) were sampled, dried in an oven at 60 °C for approximately 72 h, and weighed to determine the dry matter (DM) production. The total DM per hectare, in each harvest, was estimated using eq. (1). The cumulative DM (t ha^{-1}) is the sum of the total DM (t ha^{-1}) in the 5 harvests.

$$Total\ DM\ (\mathrm{t\ ha^{-1}}) = (DM\ top\ leaves\ + DM\ dead\ leaves\ + DM\ stalks) x\ 6.6667\ m \quad (1)$$

These sampled materials were ground in a Willey mill and had the P content determined according to Malavolta et al. (1997), after acid digestion. The total P uptake (kg ha^{-1}), in each harvest, was calculated by the sum of the proportional amount of P in each plant part (top leaves, dead leaves, and stalks). The cumulative P uptake is the sum of the total P uptake (kg ha^{-1}) in the 5 harvests.

6.2.4. Statistical analysis

As the assumption of homogeneity of variances wasn´t confirmed, mixed models were run. The selection of the appropriate model was made by the likelihood ratio test and, when necessary, Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used as information criteria. The Wald-F test was performed ($p< 0.05$), to verify the fixed effects. In all cases, predicted mean values and their corresponding confidence intervals were obtained. Treatments comparison ($p< 0.05$) can be verified through the confidence intervals, so that, if there is an intersection between two intervals, we conclude that the means are not significantly different. In addition, Fisher's unprotected LSD test was performed to compare the pairwise treatment effects. All the analyzes were performed by R software (R Core Team, 2021).

The evaluated measures (y_{ijkl}), cumulative DM, cumulative P uptake, and cumulative yield in each site independently, were analyzed according to the model given in eq. (2).

$$y_{ijkl} = \mu + \alpha_i + b_j + \beta_k + (\alpha\beta)_{ik} + \gamma_l + (\alpha\gamma)_{il} + (\beta\gamma)_{kl} + (\alpha\beta\gamma)_{ikl} + \varepsilon_{ijkl} \quad (2)$$

where μ is a constant, αi is the i-th fixed FC effect; b_j is the j-th fixed block effect; β_k is the k-th fixed mineral P sources effect; $(\alpha\beta)_{ik}$ is the fixed interaction effect between the i-th FC and the k-th mineral P sources; γ_l is the l-th fixed dose of mineral P sources effect; $(\alpha\gamma)_{il}$ is the fixed interaction effect between the i-th FC, and the l-th dose of mineral P sources; $(\beta\gamma)_{kl}$ is the fixed interaction effect between the k-th mineral P sources, and the l-th dose of mineral P sources; $(\alpha\beta\gamma)_{ikl}$ is the fixed interaction effect between the i-th FC, k-th mineral P sources, and the l-th dose of mineral P sources; ε_{ijkl} is the residual effect, such as $\varepsilon \sim \mathrm{N}(0, \sigma^2)$.

The evaluated measure (y_{ijklm}), stalk yield in each harvest and each site independently, were analyzed according to the model given in eq. (3).

$$\begin{aligned} y_{ijklm} &= \mu + \alpha_i + b_j + \beta_k + (\alpha\beta)_{ik} + \gamma_l + (\alpha\gamma)_{il} + (\beta\gamma)_{kl} + (\alpha\beta\gamma)_{ikl} + \delta_m + \\ &+ (\beta\delta)_{km} + (\alpha\beta\delta)_{ikm} + (\gamma\delta)_{lm} + (\alpha\gamma\delta)_{ilm} + (\beta\gamma\delta)_{klm} + (\alpha\beta\gamma\delta)_{iklm} + \varepsilon_{ijkl} \qquad (3) \end{aligned}$$

where μ, αi, b_j, β_k, $(\alpha\beta)_{ik}$, γ_l, $(\alpha\gamma)_{il}$, $(\beta\gamma)_{kl}$, $(\alpha\beta\gamma)_{ikl}$, are described as in Eq 2. δ_m is the m-th fixed harvest effect; $(\alpha\delta)_{im}$ is the fixed interaction effect between the i-th FC and the m-th harvest; $(\beta\delta)_{km}$ is the fixed interaction effect between the k-th mineral P sources and the m-th harvest; $(\alpha\beta\delta)_{ikm}$ is the fixed interaction effect between the i-th FC, the k-th mineral P sources, and the m-th harvest; $(\gamma\delta)_{lm}$ is the fixed interaction effect between the dose of mineral P sources and the m-th harvest; $(\alpha\gamma\delta)_{ilm}$ is the fixed interaction effect between the i-th FC, l-th dose of mineral P sources, and the m-th harvest; $(\beta\gamma\delta)_{klm}$ is the fixed interaction effect between the k-th mineral P sources, l-th dose of mineral P sources, and the m-th harvest; $(\alpha\beta\gamma\delta)_{iklm}$ is the fixed interaction effect between the i-th FC, k-th mineral P sources, l-th dose of mineral P sources, and the m-th harvest; ε_{ijklm}, is the residual effect, such as $\varepsilon \sim N(0, \sigma_m^2)$.

6.3. Results

6.3.1. Cumulative dry matter and P uptake

Significant FC x mineral P source ($p < 0.001$) and mineral P source x dose ($p < 0.001$) interactions were founded for cumulative DM (t ha^{-1}) at Agudos site. Moreover, it was observed significant difference between the doses of RP ($p < 0.01$), regardless of the presence or absence of FC. In the presence of FC, the Fisher's LSD test ($p < 0.05$) showed that the addition of 90 kg ha^{-1} of RP resulted in the highest cumulative DM, while the dose of 180 kg ha^{-1} performs similarly to TSP applied at the dose of 90 and 180 kg ha^{-1} (Figure 1a). There was no difference between the doses of TSP tested with or without FC. (Figures 1a and 1b). Overall, the mineral P fertilizer sources and doses tested (except TSP 180 kg ha^{-1}) resulted in higher accumulated DM when applied in the presence of FC (Figures 1a and 1b).

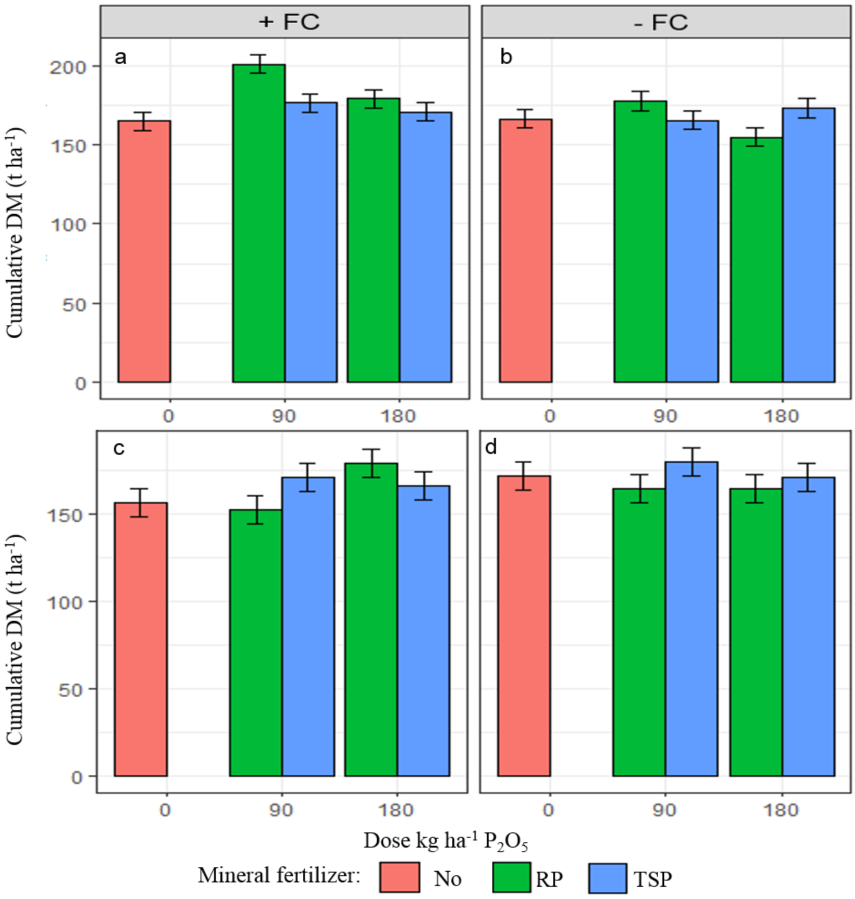

Figure 1. Cumulative dry matter (DM) (t ha^{-1}) affected by the addition of triple superphosphate (TSP) and rock phosphate (RP), at the doses of 90 and 180 kg ha^{-1} of P_2O_5, in the presence (+ FC) and absence of filter cake (- FC), at Agudos (a and b) e Macatuba sites (c and d). Treatments comparison ($p < 0.05$) can be verified through confidence intervals. Means with intersections between two intervals are not significantly different.

At Macatuba site, significant FC x mineral P source x dose ($p = 0.001$) interaction was found for accumulated DM (t ha^{-1}). The Wald-F test ($p < 0.05$) showed a significant difference between the doses tested only for RP+FC (Figure 1c). This treatment, at the dose of 180 kg ha^{-1} of P_2O_5, resulted in higher cumulative DM than half of this dose and was similar to TSP+FC at both doses. However, the addition of both mineral fertilizers in - FC did not result in cumulative DM production gains compared to the control (Figure 1d).

Significant FC x mineral P source x dose ($p = 0.003$) interaction was observed for cumulative P in the plant (kg ha^{-1}), at Agudos site. Similarly to the accumulated DM, at this site was observed a significant difference between RP doses regardless of the presence or absence of FC (Figures 2a and 2b), while when the mineral P source tested was TSP, the doses were significantly different only in + FC. For both the mineral fertilizers, the dose of 90 kg ha^{-1} of P_2O_5 resulted in the highest cumulative P (Figures 2a and b). At the end of the 5 crop cycles, all treatments resulted in a greater P uptake in + FC, except for TSP at the highest dose (Figure 2a and 2b).

There was also a significant FC x mineral P source x dose (p = 0.045) interaction for cumulative P in the plant at Macatuba site (kg ha^{-1}). The dose effect was observed only for RP+FC, with the dose of 180 kg ha^{-1} resulting in P accumulation similar to that obtained by TSP+FC 90 kg ha^{-1}, and higher than TSP+FC 180 kg ha^{-1} (Figure 2c). However, only RP+FC 180 kg ha^{-1} differed from pure FC (Figure 2c).

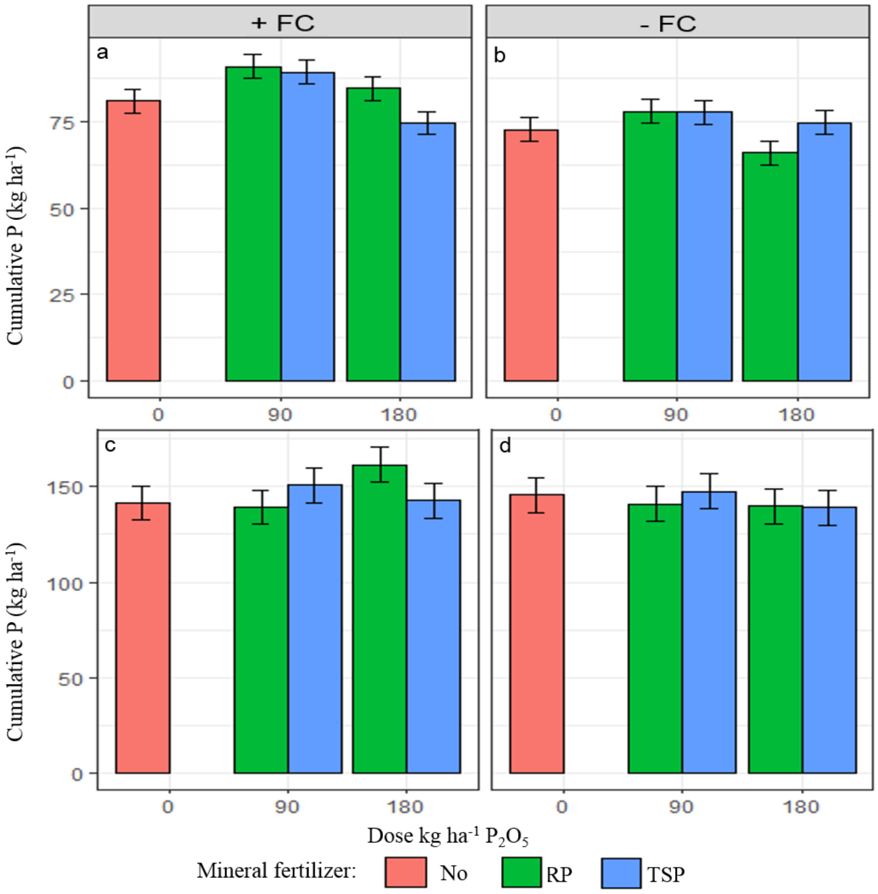

Figure 2. Cumulative phosphorus in the plant (kg ha^{-1}) affected by the addition of triple superphosphate (TSP) and rock phosphate (RP), at the doses of 90 and 180 kg ha^{-1} of P_2O_5, in the presence (+ FC) and absence of filter cake (- FC), at Agudos (a and b) e Macatuba sites (c and d). Treatments comparison (p< 0.05) can be verified through the confidence intervals. Means with intersections between two intervals are not significantly different.

6.3.2. Stalk yield

At the end of the 5-year experiment, a significant interaction was found between mineral P sources x dose ($p< 0.001$) and FC x mineral P source ($p< 0.001$) for cumulative yield (ton ha^{-1}) at Agudos site. The association of the mineral P fertilizers with FC did not result in yield gains compared to the treatment using pure FC (Figure 3a). In this sense, investigating each harvest individually, it was observed that in the first 2 crop cycles the use of pure FC performed similarly to its combination with the mineral P sources regardless of the dose (Figure 3b). Diverging from the result of cumulative DM, and P uptake, both the mineral P sources performed better when applied in the dose of 180 kg ha^{-1} of P_2O_5, regardless of the use of FC (Figure 3a). The comparison of the treatments in the presence or absence of FC showed that the byproduct addition resulted in gains in the cumulative stalk yield only when there was no P input (Control), or when the mineral fertilizer used was TSP at the dose of 180 kg ha^{-1} of P_2O_5 (Figure 3a).

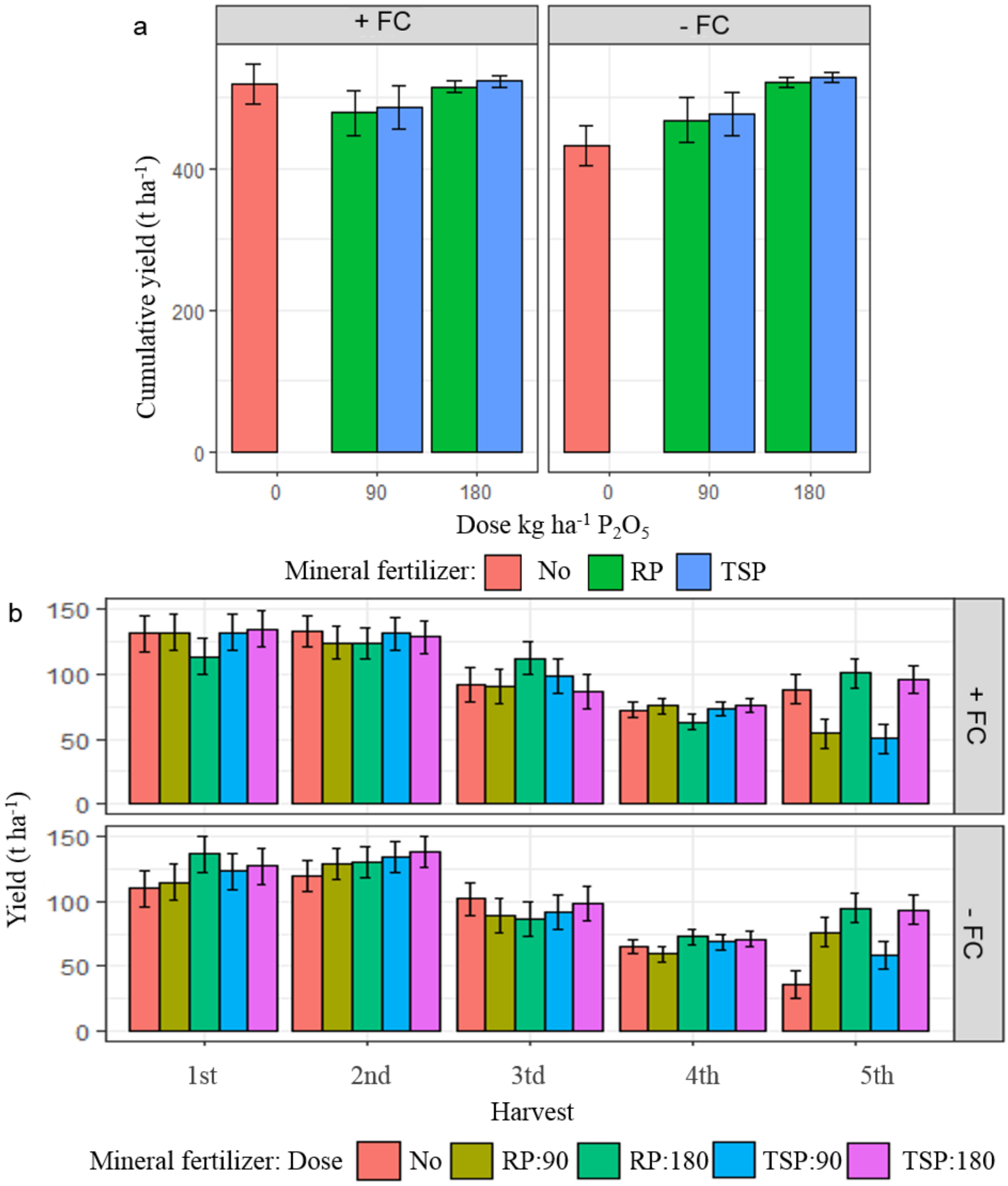

Figure 3. Cumulative stalk yield (a), and stalk yield in each harvest (b) affected by the addition of triple super phosphate (TSP) and rock phosphate (RP), at the doses of 90 and 180 kg ha^{-1} of P_2O_5, in the presence (+ FC) and absence of filter cake (- FC), at Agudos site. Treatments comparison ($p< 0.05$) can be verified through confidence intervals. Means with intersections between two intervals are not significantly different.

At Macatuba site, the use of FC did not affect the cumulative stalk yield, and only the interaction fertilizer x dose ($p= 0.002$) was found. The treatments comparison in each harvest revealed that in the first 2 sugarcane cycles, all the fertilizers and doses tested resulted in the same yield, although there was a sharp drop in the stalk production from the first to the second year (Figure 4b). In the fourth cycle, the use of TSP in the dose of 90 kg ha^{-1} of P_2O_5 resulted in the highest yield and it was equally efficient than the double of this dose in the fifth year when TSP was the only treatment that performed better than the control.

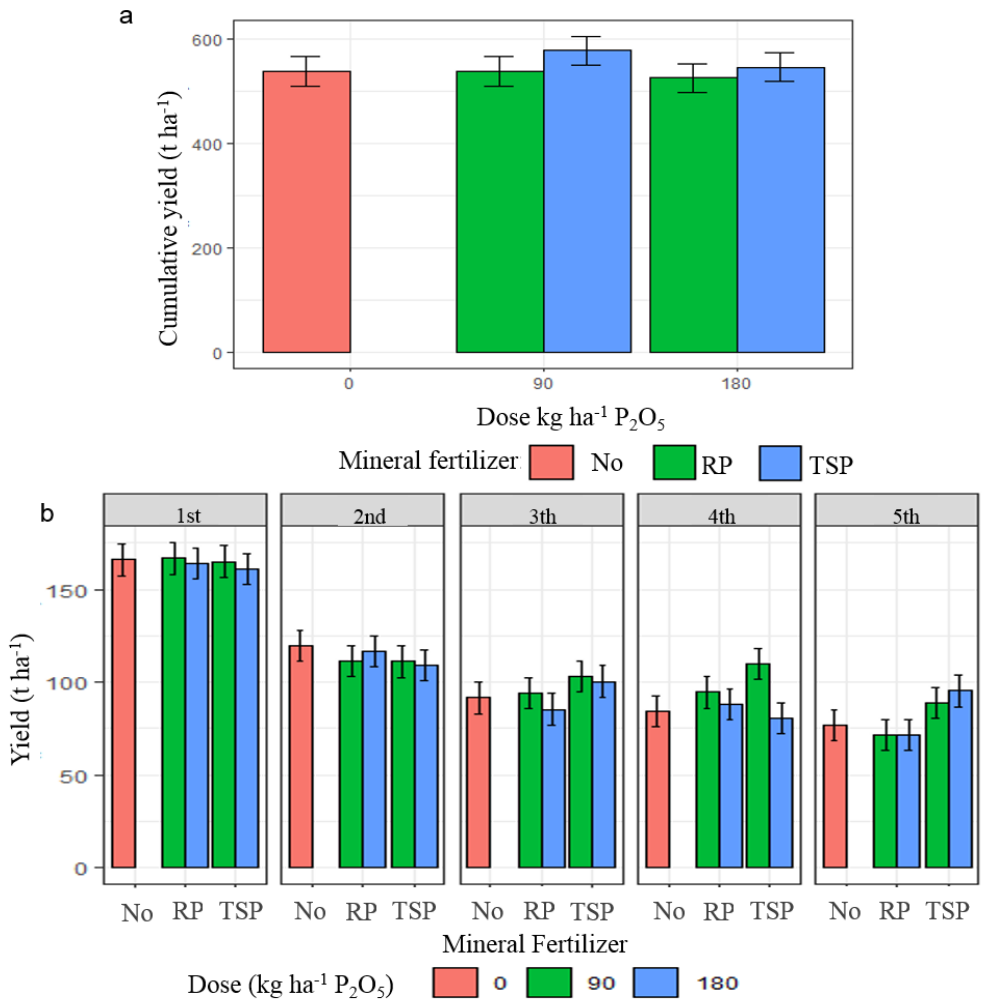

Figure 4. Cumulative stalk yield (a), and stalk yield in each harvest (b) affected by the addition of triple super phosphate (TSP) and rock phosphate (RP), at the doses of 90 and 180 kg ha^{-1} of P_2O_5, in the presence (+ FC) and absence of filter cake (-FC), at Macatuba site. Treatments comparison ($p < 0.05$) can be verified through confidence intervals. Means with intersections between two intervals are not significantly different.

6.4. Discussion

6.4.1. Cumulative dry matter and P uptake

The association of FC with the mineral P sources tested resulted in higher DM (ton ha^{-1}) and P accumulation (kg ha^{-1}) in the plants at the end of 5-yrs of sugarcane cultivation, in both the experiment locations (Figures 1a and 1b; 2a and 2b). These results show a positive effect of the FC addition on increasing the P availability/

uptake by the crop, as previously stated in other studies (Almeida Júnior et al. 2011; Caione et al. 2015; Lima Vasconcelos et al. 2017; Borges et al. 2019).

The use of pure FC (~125 kg ha^{-1} of P_2O_5) in the soil resulted in a P uptake by the plants higher than the obtained by the addition of 180 kg ha^{-1} of P_2O_5 as RP and TSP (- FC) ($p < 0.001$ and 0.014, for FC x RP and FC x TSP respectively). In other words, plants grown in soil fertilized only with FC received less P_2O_5 than those grown in soil fertilized with the mineral P sources but were able to acquire more P. Thus, the positive effect of FC on the cumulative DM and P concentration in the plant does not seem to be related only to its P content (1.3 % of P_2O_5). A possible explanation for this result could be the potential of FC to increase the P availability in the soil by the release of low molecular weight organic acids (LMWOAs), which can complex metallic cations, and compete for P adsorption sites in the soil (Menezes-Blackburn et al. 2016b; Richardson and Simpson 2011; Qin et al. 2013; Wang et al. 2021). However, this greater availability was not observed in the labile P pool in the soil - as discussed in chapter 5 – indicating that this effect can occur in the rhizosphere region, with a low effect in the bulk soil. Other potential benefits of FC such as the increase in the OM content, changes in the community and diversity of the microbial community, amelioration of physical properties, and increased root system development in the soil (Arruda et al., 2019; Soltangheisi et al., 2019) may have contributed to the increased P acquisition by the plant.

In the clayey soil of Macatuba, the increase in RP dose positively affected the DM production and P uptake when it was associated with FC, which was not observed in Agudos. Also, the positive effect of FC was observed only at the dose of 180 kg ha^{-1} of RP. It indicates a small or no response to FC used individually or associated with TSP in this location, while at Agudos, the mineral P sources performed better if associated with FC. Although both experimental areas were located in soils with low initial available P content, the soil texture is quite distinct, changing the maximum P retention capacity (P max) and the P availability. In the clayey soil of Macatuba, the P max is ~4.5 folders higher than in the sandy soil of Agudos (1557 mg kg^{-1} and 342 mg kg^{-1}, respectively), leading to low fertilizer efficiency, especially for the water-soluble source. This is a piece of important information for P fertilization management because the P input in sugarcane cultivation is predominantly carried out with the use of high doses of water-soluble mineral fertilizers, which our results showed is not an advantageous practice.

In addition to distinct soil parameters, the history of land use possibly contributed to the low response of organic and mineral phosphate fertilization in the clayey soil of Macatuba. As an area cultivated for approximately fifty years with sugarcane, and frequent P input, the initial total P stock (legacy P) in the soil before the experiment was 1472.3 mg kg^{-1} (in the 0-20 cm layer), corresponding to ~ 4 folders higher than the sandy soil of Agudos.

6.4.2. Stalk yield

At Agudos, the use of pure FC resulted in cumulative stalk yield similar to the treatments in which it was associated with mineral fertilizers, and higher than the control, demonstrating that FC had special importance in the absence of mineral P input (Figure 3a). The evaluation of its effect in each harvest explains this result since among the 5-yrs, only on the third, this material resulted in stalk yield lower than when was combined with a mineral P source (RP at a dose of 180 kg ha^{-1} of P_2O_5) (Figure 3b).

Both RP and FC have a low solubilization rate compared to TSP, as revealed by the column perfusion test presented in Chapter 4. However, each of these products has its own P-release kinetics and mechanisms. RP is basically constituted of calcium phosphates, with a high residual effect due to the low dissolution rate in soils with pH above 5.5 and high Ca content (Gatiboni et al. 2007; Silveira et al. 2018; Oliveira et al. 2019). As an organic material, the total P released from FC depends on its C and N content and is well known that materials with low C: N, and C: P ratios, such as FC (Table 2), may have rapid mineralization (Umrit and Friesen 1994; Silver and Miya 2001; Alamgir et al. 2012). Given that, at Agudos, the P release from RP was slower than FC, explaining why the positive effect of RP+FC in the dose of 180 kg ha^{-1} of P_2O_5, was observed only after 3-yrs from the application. Meanwhile, FC kept the same yield in the first two years, but it decreased in the third.

Interestingly, although the association of FC with mineral fertilizers (TSP 90, RP 90, and 180 kg ha^{-1} P_2O_5) had increased the P accumulation in the plant it was not converted into stalk yield in the sandy soil. Our results are in accordance with those reported by Borges et al. (2020) using biochar produced from sugarcane residue, which observed low sugarcane yield even with increased P availability in the soil. Other factors may have affected the crop yield, from the nutritional status of other macro elements to the climate conditions. Also, in some cases, P can be absorbed and accumulated, but not metabolized by the plant (Marschner 2012).

The evaluation of the sugarcane harvests in Macatuba revealed that the mineral phosphate fertilization became important for crop yield only from the third harvest onwards (Figure 4b), which is probably related to the soil characteristics as OM content, water hold capacity, total C, and P max. In the fourth crop cycle, the use of TSP resulted in the greatest yield, and in the fifth year, only this fertilizer (in both doses) was efficient in maintaining the yield higher than the control. The comparison of sugarcane yield over time within each treatment confirms that under TSP there was a better uniformity of the yield than for RP (Figure S1), indicating a residual effect that was not expected for this fertilizer. The TSP releases P into the soil solution at rates higher than the crop's needs, favoring reactions with other soil constituents that can convert P into less available forms (Menezes-Blackburn et al. 2016a; Menezes-Blackburn et al. 2016b). However, with time the nutrient can be converted into available forms depending on the bound stability and the P content in the soil. This dynamic was confirmed by the investigation of the P stocks in the soil sampled after the last harvest when the P content in the moderately labile pool decreased and the labile pool increased in TSP+FC 180 kg^{-1} P_2O_5 (data shown in chapter 5).

6.5. Conclusions

Collectively, the results showed that the P fertilization with FC and mineral sources had different effects on the sugarcane DM production, P uptake, and yield depending on the soil attributes and previous land use. In Agudos the use of RP+FC at the dose of 90 kg ha^{-1} P_2O_5 was enough to increase the cumulative DM and P uptake at the end of 5 crop cycles. On the other hand, in Macatuba the same result was achieved only with the dose of 180 kg ha^{-1} P_2O_5. Based on these sugarcane parameters the use of RP+FC was a good strategy to increase the P uptake by the plants and reduce the use of TSP, in both soils. However, from a practical point of view, the yield is the most important parameter to be evaluated. In this sense, in the sandy soil from Agudos, the association of FC with the mineral fertilizers was effective in increasing the cumulative yield only if associated with TSP in the highest dose tested. In this area regardless of the FC addition, the same yield was obtained by TSP and RP at the dose of 180 kg ha^{-1}, showing that RP can replace TSP. In the clayey soil from Macatuba, the yield was less responsive to the FC addition, and in the last harvest only TSP, in both the doses tested, performed better than the control.

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Supplementary material

Table S1. Phosphorus agronomic efficiency (kg ha^{-1} P) affected by the addition of triple superphosphate (TSP) and rock phosphate (RP), at the doses of 90 and 180 kg ha^{-1} of P_2O_5, in the presence (+ FC) and absence of filter cake (- FC), at Agudos and Macatuba sites, in each harvest.

Treatment	Dose (kg ha^{-1} P_2O_5)	1st	2nd	3rd	4th	5th	Cumulative yield
Agudos site (sandy soil)							
FC	0	387	256	0	138	943	1548
TSP	90	326	389	0	95	574	1113
	180	220	239	0	74	738	1223
TSP + FC	90	235	127	0	86	155	568
	180	185	70	0	81	448	674
RP	90	119	246	0	0	1026	912
	180	334	146	0	97	753	1130
RP + FC	90	235	54	0	109	197	480
	180	26	33	80	0	485	611
Macatuba site (clayey soil)							
FC	0	306	172	82	86	255	903
TSP	90	97	0	564	737	525	1823
	180	5	0	219	0	324	380
TSP + FC	90	120	0	56	288	186	616
	180	54	0	38	35	193	276
RP	90	38	0	233	417	0	478
	180	83	23	16	215	0	293
RP + FC	90	181	0	5	95	113	334
	180	49	14	0	0	55	15

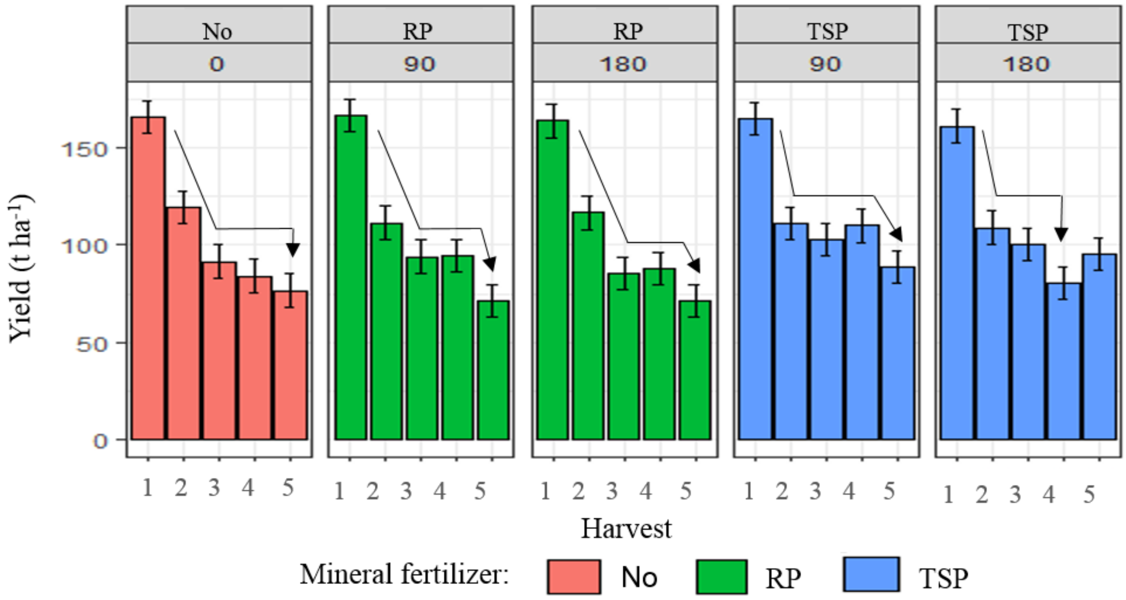

Figure S1. Stalk yield (ton ha^{-1}) in each harvest affected by the addition of triple super phosphate (TSP) and rock phosphate (RP), at the doses of 90 and 180 kg ha^{-1} of P_2O_5 at Macatuba site. Treatments comparison (*p*< 0.05) can be verified through the confidence intervals. Means with intersections between two intervals are not significantly different.

Table S2. Stalk yield (ton ha^{-1}) in each harvest, cumulative dry matter (DM), and cumulative P (kg ha^{-1}) affected by the addition of triple super phosphate (TSP) and rock phosphate (RP), at the doses of 90 and 180 kg ha^{-1} of P_2O_5, in the presence (+ FC) and absence of filter cake (- FC), at Agudos and Macatuba site (raw data).

Treatment	Dose (kg ha^{-1} P_2O_5)	Harvest						Cumulative DM	Cumulative P (kg ha^{-1})
		1st	2nd	3rd	4th	5th	Cumulative		
Agudos site (sand soil)									
Control	0	110	119	102	65	36	432	166	73
FC	0	132	134	92	73	88	519	165	81
TSP	90	123	135	91	69	58	476	165	78
	180	128	138	98	71	94	528	173	75
TSP+FC	90	133	131	98	73	51	486	176	89
	180	135	129	87	76	96	523	171	75
RP	90	115	129	89	59	76	468	177	78
	180	137	131	86	73	95	521	155	66
RP+FC	90	133	125	91	76	54	478	201	91
	180	114	124	113	63	101	514	179	85
Macatuba site (clayey soil)									
Control	0	157	115	89	82	70	513	172	146
FC	0	174	124	94	87	84	563	157	142
TSP	90	161	111	111	111	90	584	180	148
	180	158	109	106	74	95	543	171	139
TSP+FC	90	169	111	95	109	87	571	171	151
	180	165	109	94	86	96	550	166	143
RP	90	159	114	98	98	62	531	165	141
	180	164	117	90	99	66	536	165	140
RP+FC	90	175	109	90	91	80	544	153	139
	180	164	117	80	77	77	515	180	162

7. FINAL CONSIDERATIONS

In this study, five experiments were conducted in an attempt to characterize the physico-chemical properties of filter cake (FC), and investigate how its association with triple superphosphate (TSP) and rock phosphate (RP) affect the P availability and dynamics in the soil, and the agronomic performance of sugarcane.

In the first experiment (Chapter 2), a method to quantify P in sugarcane leaves *in vivo* and *in situ* using portable X-ray fluorescence (pXRF) spectroscopy, was developed. However, the differences between the physical-chemical composition of the samples and the laboratory standards caused a little underestimation of the P concentration in the leaves. Producing standards chemically and physically similar to the leaf can be challenging and this issue must be addressed in further studies.

In the second experiment (Chapter 3), carried out under controlled conditions for 120 days, the pXRF technique was used to quantify the P content in sugarcane leaves over time. Also, the P concentration in the soil solution and the P content in the soil pools were determined to evaluate the FC effect on the P release and availability from TSP and RP. The reduction in the amount of P applied as TSP when it was associated with FC did not affect the P availability and uptake by sugarcane, indicating the possibility of decreasing the P supplied via TSP. The results provided evidence that FC affects the RP solubilization in the soil, which we hypothesized, was related to low molecular weight organic acids (LMWOAs) action. To validate this hypothesis would be necessary to quantify the LMWOAs in the soil amended with FC, however, these acids are usually found in a very low concentration in the soil and are fast degraded, making its quantification in this kind of experiment very difficult.

To confirm if the greater P release in the soil solution observed for RP+FC compared to pure RP was related to a direct effect of FC on RP dissolution, the Kinect of P release was evaluated in a column perfusion test (Chapter 4). In this experiment, FC and its combination with TSP and RP were directly flushed with deionized water, meaning that there was no influence of the soil and plants on the P release from fertilizers. The results showed that FC increases the soil pH and most of its total P content is released within 12 hours. As in the soil solution, the P concentration in the eluent was lower in TSP+FC than in TSP, because in this treatment FC replace 35% of the total P applied. However, in this experiment, FC did not affect the P release from RP as observed in the previous one. This divergence can be due to the sources of RP used, which were Bayóvar in the greenhouse experiment, and hydroxylapatite for analysis in the column perfusion test. There is also the possibility that the positive effect of FC on the P release from RP is dependent on soil and plant effects. In this sense, in the fourth experiment (Chapter 4) we concluded that FC positively affected the P availability from RP in the non-limed acidic soil because of the FC's ability to decrease the soil P sorption capacity. It was also concluded that the lower P released by TSP+FC did not affect the P availability in the acidic limed soil, and is important to highlight that this result was obtained using the same soil as in experiment two, which was also limed. Thus, we can state that in this soil condition, the association of FC with TSP does not compromise the P availability and P uptake.

In the last experiment (Chapters 5 and 6) the long-term effect of the association of FC with TSP and RP (tested in the dose of 90 and 180 kg ha^{-1} P_2O_5) in the P dynamics in the soil and the agronomic performance of sugarcane was tested in two Oxisols (sandy and clayey). In both soils, FC associated with RP reduced the P conversion into low available forms. In the sandy soil, the mixture RP+FC at the dose of 180 kg ha^{-1} of P_2O_5 was as efficient as TSP (in the same dose and regardless of the presence of FC) in increasing the labile pool of P and the cumulative sugarcane yield, showing that when associated to FC and applied at the dose of 180 kg ha^{-1} of P_2O_5 the RP can replace TSP. As observed in Chapters 3 and 4, the byproduct increased the P availability from RP, and there was a reduction in the contribution of P-Ca in the soil, which strongly indicates a higher dissolution of RP when applied in the presence of FC. On the other hand, in the clayey soil, after 5 sugarcane crop cycles, only TSP+FC at the dose of 180 kg ha^{-1} of P_2O_5 increased the available P in the soil and the yield. Thus, the association of FC with TSP and RP had different effects on the P release and dynamics in the soil and on the sugarcane performance, depending on the soil condition. However, is difficult to state to what extent the results were related to the soil characteristics because of the different land use histories of each site.

By the combination of results, it is concluded that the use of FC can alter the rate of P release from mineral fertilizers (TSP and RP), increasing its availability in the soil, and leading to enhanced P acquisition by sugarcane plants, as hypothesized. It is also possible to conclude that the FC effect is dependable on the soil condition. This research showed the great potential of FC in partially replacing mineral P fertilizers. However, further analysis is still needed to better comprehend the chemical and physical role of FC in the dissolution and availability of P from TSP and RP.