**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

**Soil phosphorus availability, dynamic and speciation in long-term high
fertilized soils**

Josimar Vieira dos Reis

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

**Piracicaba
2019**

Josimar Vieira dos Reis
Agronomist

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Advisor:
Prof. Dr. **TAKASHI MURAOKA**

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Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP

Reis, Josimar Vieira dos

Soil phosphorus availability, dynamic and speciation in long-term high
fertilized soils / Josimar Vieira dos Reis. - - Piracicaba, 2019.

98 p.

Tese (Doutorado) - - USP / Escola Superior de Agricultura "Luiz de
Queiroz".

1. Análise de solo 2. Avaliação da fertilidade do solo 3. Fracionamento
de P 4. Especiação de P 5. XANES 6. Correlação 7. Calibração I. Título

DEDICATION

To my Mother Mariza on behalf of all Teachers and Professors

To my Father João Batista on behalf of all Farmers

ACKNOWLEDGMENTS

I thank God for everything, especially for the health and for blessing me with the wonderful people in my life.

I would like to thank all the institutions and their staff who helped me to conduct this research: The University of São Paulo (USP), the “Luiz de Queiroz” College of Agriculture (ESALQ), the Department of Soil Science (LSO), and the Soils and Plant Nutrition Graduate Program for the valuable opportunity and to provide me with the doctorate education. The Center for Nuclear Energy in Agriculture (CENA) and the Soil Fertility Laboratory, where I did most of the research. The Brazilian Synchrotron Light Laboratory (LNLS) for all support with the XANES analysis, especially all the SXS Beam Line staff. The North Carolina State University (NCSU) and Crop and Soil Science Department for the excellent reception and support during my exchange scholarship. The Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES) for granting doctoral scholarship and granting sandwich doctoral scholarship.

To my advisor, Professor Takashi Muraoka for trusting in my work and giving me all the support necessary for its accomplishment. For his friendship and for all the knowledge, advice and good examples given during this period. To my advisor during my exchange scholarship, Professor Dean Hesterberg from whom I learned a lot in a short time, thank you for welcoming me so well, for the support in my research and for your friendship.

To all Professors, especially those I had the opportunity to attend classes: Thiago Osório, Carlos Cerri, Fernando Andreote, Elke Cardoso, Hudson Carvalho, Luis Alleoni. To the Professors Luis Alleoni, Rafael Otto and Carlos Cerri for the valuable suggestions on the qualifying exam. To Professor Paulo Pavinato for providing the laboratory and equipments for soil phosphorus fractionation analysis. To the Professor Cassio Abreu-Junior for the orientation in the teaching internship program (PAE). I have learned a lot from all of you.

To all friends, colleagues, and university staff that I met during the doctoral course, especially those who helped me with the hard work of the greenhouse experiments and in the lab. I would like to thank Aijânio and Cristiano for their friendship, patience, exchange of ideas and great help in all stages of this thesis development. Thalita, Arnaldo and Matheus, thank you for your help in the experiments and soil sampling. I would like to thank Mara and Sandra for their help with the analysis in the Soil Fertility Laboratory, Marina and Ruan for their help in sample preparation and data collection in LNLS, and Mayara for her help with the sequential fractionation analysis. Thanks Marcos Rodrigues and Dalton Abdala for the

support with the standards for the XANES analysis, and Luis Carlos (Guatemala) for his contribution with the XANES figures.

To all my roommates for all the good times during this period, the good friendship with you helped a lot: Allan (Cabeção), Paulo (Beijo), Fábio (Zebra), Luiz, Alexandre, and Maiquel. To my friends Lucas, José Lucas, Carol, Arnaldo, Danilo, Acácio, Thais, Bruna, Wilfrand, Luciana and so many others for their kindness and for giving me so many good times. To Clístenes, Guilherme, Luanda, Carlos Mendoza, Diana, Gabriela, Mateus and all who made the exchange scholarship period still better. To Paulo and Thays for their kindness in welcoming me into their study room and for the good times.

I especially thank Mariana for all her love and unconditional support, for giving me so many wonderful moments and for giving me the strength to go through such difficult times, for help me with the experiments, statistical analysis, for the “little maps”. Thank you for being part of my life, I really love you.

To all my family, my parents Mariza and João Batista to whom I dedicate this thesis, to my sisters Alessandra and Renata and my nephews Andressa and Victor, you are the basis of everything. To my grandparents Mário (*in memoriam*) and Erli, Antônio (*in memoriam*) and Joanira for their outstanding help in shaping my character.

I am very grateful to you all.

BIOGRAPHY

Josimar Vieira dos Reis was born in Manhuaçu, Minas Gerais State, Brazil on July 12, 1990. He finished the course of Agricultural Technician at Escola Agrotécnica Federal de Alegre (EAFA), Espírito Santo State in 2009. In the same year he started his Agronomy course at the Federal University of Viçosa (UFV), where he graduated in 2014.

In February 2016 he obtained the title of Master in Soils and Plant Nutrition from the Federal University of Viçosa (UFV). In the same month, he started his doctorate course at "Luiz de Queiroz" College of Agriculture (ESALQ) in University of São Paulo (USP), with exchange scholarship period at North Carolina State University (NCSU), United States.

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RESUMO

Disponibilidade, dinâmica e especiação de fósforo em solos altamente adubados por longo período de tempo

Análises químicas que determinam a quantidade de fósforo (P) no solo disponível para as plantas vem sendo desenvolvidas ao longo das últimas décadas. Em solos brasileiros, os extratores Mehlich-1 (M1) e Resina de Troca Iônica (IER) são os mais utilizados. Embora o extrator Mehlich-3 (M3) seja amplamente adotado como extrator oficial P em outros países, no Brasil, a maioria das pesquisas com este extrator envolvem apenas a fase de correlação. Além das análises de solo para avaliação da fertilidade, o fracionamento químico das formas de P é uma das principais análises utilizadas para obter informações a respeito da solubilidade de diferentes formas de P e sua disponibilidade para as plantas, embora não determine as espécies de P presentes no solo. O uso de técnicas de Espectroscopia de Absorção de Raios-X (XANES) tem gerado importantes informações a respeito da especiação de P em solos e pode complementar informações geradas com o fracionamento químico. Neste sentido, nosso objetivo geral neste estudo foi melhorar a recomendação e o uso de fertilizantes fosfatos em solos brasileiros altamente intemperizados para otimizar a eficiência do uso de fertilizantes e minimizar impactos ambientais causados por seu uso indiscriminado. Isso pode ser conseguido pela combinação de resultados de pesquisa que vão desde o nível básico, porém importantes para o uso racional de fertilizantes, como os trabalhos de correlação e calibração, até resultados gerados por técnicas mais refinadas como fracionamento químico e especiação por XANES. Para alcançar esses objetivos, foram coletadas 10 amostras de solo em áreas cultivadas e não cultivadas em diferentes localidades no Brasil. Foram conduzidos dois cultivos sucessivos com milho em casa de vegetação, em vasos de 2.0 dm³. Os tratamentos consistiram em um fatorial 10 x 6, sendo 10 amostras de solo e seis doses de P com quatro repetições em delineamento em blocos ao acaso. A fonte de P foi composta por uma mistura de 70 % de Superfosfato Triplo e 30 % de Bayovar. Após os cultivos, foram realizadas extrações de P com M1, M3 e IER, que foram correlacionadas com a quantidade de P absorvida e acumulada pelas plantas de milho. Os resultados do experimento de biodisponibilidade foram utilizados para os trabalhos de correlação e calibração dos extratores. Nas amostras de solos originais, foram realizados o fracionamento químico de P e a especiação por P K-edge XANES. Os maiores teores de P foram extraídos com IER. A magnitude dos coeficientes de correlação indicam que os três extratores podem ser utilizados para determinar a disponibilidade de P em solos cultivados e que o M1 pode ser substituído por M3. Os extratores M1 e M3 foram sensíveis à capacidade tampão dos solos. Assim, a utilização do M3 em solos tropicais é dependente de uma medida da capacidade tampão, preferencialmente o fósforo remanescente (P-rem). Após duas décadas de cultivo, houve um incremento considerável nas formas moderadamente lábeis e não lábeis de P nos solos, com o P adsorvido principalmente a Goethita, Hematita e Gibbsita. Estes resultados sugerem que há um grande acúmulo de P no solo, contribuindo para uma importante reserva com papel de tamponamento das frações mais lábeis no médio prazo.

Palavras-chave: Análise de solo, Avaliação da fertilidade do solo, Fracionamento de P, Especiação de P, XANES, Correlação, Calibração

ABSTRACT

Soil phosphorus availability, dynamic and speciation in long-term high fertilized soils

Soil tests that determine the amount of Phosphorus (P) in the soil that is available to crops have been developed over the past decades. In Brazilian soils, the extractants Mehlich-1 (M1) and Ion Exchange Resin (IER) are more commonly used. Although the Mehlich-3 (M3) soil test is widely adopted as official extractant for P and other nutrients in many countries, in Brazil most researches involving this extractant only addresses the correlation phase. Besides soil tests for P availability, the chemical fractionation of soil P is one of the main analyzes used to obtain information about the solubility of the different forms of P in soil and their availability to plants, but do not determine the species of P present in the soil. The use of the X-ray absorption near-edge structure (XANES) technique has generated important information about the soil P speciation and can complement the information generated with chemical fractionation. Therefore, our overarching goal is this study is to improve the use and recommendation of P fertilizers on highly weathered Brazilian soils in order to optimize P use efficiency while minimizing any adverse environmental impacts. This can be achieved by combining research results ranging from the most basic level, but extremely important for rational fertilizer use, such as correlation and calibration experiments, with results from more refined techniques such as chemical fractionation and speciation by XANES. To achieve these objectives we collected 10 soil samples from uncultivated and cultivated areas in different regions of Brazil. Besides, two successive cultivation of maize in 2.0 dm³ plastic pots were carried out under greenhouse conditions. The treatments consisted of a 10 × 6 factorial, 10 soil samples and six doses of P, with four replicates in a completely randomized design. The source of P was a mixture composed of 70 % of Triple Superphosphate and 30 % of Bayovar. After each cultivation, soil P was extracted by M1, M3, and IER and correlated with maize shoot dry matter yield, shoot P content, and shoot total amount of P. The results of the bioavailability experiment was used in extractants correlation and calibration works. In the original soil samples, we have determined the soil P pools by chemical fractionation and soil P species by P K-edge XANES. The highest soil P contents and the best correlations were obtained with IER. The magnitude of the correlation coefficients indicates that the three soil tests can be used to determine the availability of P in cultivated soils and that M1 could be replaced by M3. The M1 and M3 extractants were sensitive to soil phosphorus buffering capacity. Thus, the use of M3 in tropical soils is dependent on an interpretation table that stratifies the values based on a measure of soil phosphate buffer capacity, preferably the remaining phosphorus (P-rem). After two decades of soil cultivation, there were evident increase mainly in the moderately labile and non-labile P fractions, with P adsorbed mainly onto Goethite, Hematite, and Gibbsite. This suggests the accumulation of a large amount of P in the soil, contributing to the legacy P, which may play an important role buffering the most labile pools in the medium term.

Keywords: Soil test, Soil fertility evaluation, P fractions, Soil P speciation, XANES, Correlation, Calibration

1. GENERAL INTRODUCTION

Mainly agronomic aspects have spurred research on Phosphorus (P) in tropical soils in the past decades, due to the low availability of this nutrient to plants in highly weathered soils, which have high P sorption capacity (Fontes and Weed, 1996; Lopes and Guilherme, 2016; Motta et al., 2002; Novais and Smyth, 1999). Thus, research was carried out to understand the phenomenon of P adsorption in soil particles and to develop strategies to minimize P loss by soil fixation and increase its availability to plants after fertilization, i.e. to improve phosphate fertilizers use efficiency. In temperate soils, in addition to agronomic aspects, there is a greater environmental concern regarding the behavior of P in soil, since successive applications may lead to P leaching and eutrophication of surface and subsurface waters (Deiss et al., 2018; Pantano et al., 2016; Wang and Li, 2010).

Soil tests that determine the amount of P in the soil that is available to crops have been developed over the past decades (Cade-Menun et al., 2018; Silva and Raij, 1999). The development of soil P extractants is based on the inferred P species that are present in the soil, which leads to considerable uncertainty in the interpretation of soil P tests (Cade-Menun et al., 2018). The characteristics that determine if a soil test could be implemented in a program are: must be quick, easy to implement, inexpensive, and appropriate for a wide range of soil types and conditions (Cade-Menun et al., 2018; Jones, 1998; van Raij et al., 1986).

The main objectives of soil testing are to determine the soil nutrient availability status, to indicate the level of deficiency or excess of nutrients in the soil for crops, and to form the basis for fertilizer recommendation (Westerman et al., 1990). Besides, soil tests can indicate chemical impediments to plant growth such as acidity and presence of elements at toxic levels (Cantarutti et al., 2007). With the emphasis on soil environmental quality, soil tests are also used to determine if adequate or excess of fertilization has occurred (Westerman et al., 1990), generating indices of agronomic and ecological importance (Bortolon et al., 2016; Gatiboni et al., 2015).

In Brazilian soils the extractants Mehlich-1 (M1) and Ion Exchange Resin (IER) are more commonly used. The IER is officially used only in São Paulo State (van Raij et al., 2001). The M1 extractant was initially proposed as an official extractant for soil P, in 1954 (Nelson et al., 1953) in laboratories of the North Carolina State (USA). The facility of extraction, rapidity, low cost and clear extracts obtained, an important condition for determination by molecular absorption spectrophotometry, which eliminates the filtration process, are great advantages in the M1 use (Santos et al., 2014). The use of Resin as soil P

extractant was initially proposed by Amer et al. (1955). Subsequently, van Raij et al. (1986) and van Raij et al. (2001) adapted the use of the resin to routine laboratories of soil fertility analysis. The use of a mixture of cationic and anionic exchange resins, or Ion Exchange Resin, saturated with sodium bicarbonate, which buffers the medium and favors the P extraction, showed increasing use in laboratories (van Raij et al., 2001).

Although the Mehlich-3 (M3) soil test is widely adopted as official extractant for P and other nutrients in many countries (Jordan-Meille et al., 2012), in Brazil most researches involving this extractant only addresses the correlation phase (Bortolon et al., 2009; Brasil and Muraoka, 1997; Farias et al., 2009; Santos et al., 2014, 2015; Valadares et al., 2017). There exist a few works dealing with the calibration phase, with the interpretation of the values of P obtained by M3 (Bortolon et al., 2011; Bortolon and Gianello, 2008, 2012; Mumbach et al., 2018). Almost all works involving the calibration phase and many involving correlations were developed in soils of the southern Brazil and resulted in the creation of the strategy of transforming the P contents obtained with M3 into equivalents of M1, and using the table calibrated for M1 to interpret the values of M3 (CQFS, 2016; Mumbach et al., 2018).

Besides soil tests for P availability, the chemical fractionation of soil P is one of the main analyzes used to obtain information about the solubility of the different forms of P in and their availability to plants (Condon et al., 1985; Condon and Newman, 2011). Although, the fractionation methods have some limitations related to being operationally defined, complex, time-consuming, there is hipper-manipulation of samples and the methods do not determine the soil P species, several methods with different modifications have been proposed over the years (Condon and Newman, 2011; Cross and Schlesinger, 1995; Gatiboni et al., 2013). Soil chemical fractionation techniques are especially important when comparing the influence of soil management practices on P lability (Cherubin et al., 2016; Gatiboni et al., 2013; Pavinato et al., 2009; Rodrigues et al., 2016; Teles et al., 2017).

Soil P speciation refers to the specific chemical forms P in a soil sample, that include minerals, organic, adsorbed and precipitated forms (Hesterberg, 2010). The use of the X-ray absorption near-edge structure (XANES) technique has generated important information about the P adsorption in oxides and silicated clays in relation to the structure, bond geometry, presence of multinuclear complexes and precipitated phases, although detection sensitivity is a problem (Hesterberg, 2010; Kruse et al., 2015). In this case, P species are determined by comparing spectral characteristics of a sample with standard spectra, which present unique spectral fingerprints, with the advantage of requiring minimal or no samples pretreatments (Hesterberg et al., 1999; Kelly et al., 2008). Therefore, only by using P K-edge

XANES we can ensure that the P adsorbed in soil minerals will not change its form as can occur in chemical extractions (Cade-Menun et al., 2018; Condon and Newman, 2011).

Therefore, our overarching goal is this study is to improve the use and recommendation of P fertilizers on highly weathered Brazilian soils in order to optimize P use efficiency while minimizing any adverse environmental impacts. This can be achieved by combining research results ranging from the most basic level, but extremely important for rational fertilizer use, such as correlation and calibration experiments, with results from more refined techniques such as chemical fractionation and speciation by XANES.

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2. SOIL PHOSPHORUS AVAILABILITY TO MAIZE PLANTS DETERMINED BY DIFFERENT EXTRACTANTS IN CULTIVATED AND UNCULTIVATED SOILS

ABSTRACT

Soil chemical analysis is considered a powerful tool to transfer knowledge generated from soil fertility research to farmers, and is fundamental for the sustainable use of lime and fertilizers. Soils intensively cultivated in Brazil have different chemical characteristics, i.e., soil pH, base saturation, organic matter content, from two decades ago due to the intensive use of agricultural inputs. Due to the changes in these soil characteristics, we hypothesize that Mehlich-3 (M3) soil test has higher capability to predict phosphorus (P) availability than Mehlich-1 (M1) and Ion Exchange Resin (IER) in cultivated soils. Our objective was to assess the M1, M3 and IER soil tests capability to predict soil P availability by correlation with P taken up and accumulated by maize plants (*Zea mays* L.) cultivated in soils with contrasting P content. Two successive cultivation of maize in 2.0 dm³ plastic pots were carried out under greenhouse conditions. The treatments consisted of a 10 × 6 factorial, 10 soil samples from different regions of Brazil and six doses of P, with four replicates in a completely randomized design. The source of P was a mixture composed for 70 % of Triple Superphosphate and 30 % of Bayovar. After each cultivation, soil P was extracted by M1, M3 and IER and correlated with maize shoot dry matter yield, shoot P content, and shoot total amount of P. The highest soil P contents and the best correlations were obtained with IER. The magnitude of the correlation coefficients indicates that the three soil tests can be used to determine the availability of P in cultivated soils, and that M1 could be replaced by M3.

Keywords: Soil fertility evaluation; soil test; phosphorus fertilizers

2.1. INTRODUCTION

Brazil is expected to play an important role in food production in a scenario where the world population is estimated to reach around 9.0 billion by 2050 and food demand can double during this period (Fink et al., 2016; Pantano et al., 2016). Brazil's leading role is not only due to its large area that can potentially be explored in agriculture, but also due to its current food production that demands high soil fertilization, especially with phosphorus (P) (Roy et al., 2016; Withers et al., 2018). The P is a critical nutrient for plant production in the highly weathered and acidic soils commonly found in the tropics due to its low natural content and availability, the latter induced by strong adsorption of P onto clay minerals (Fink

et al., 2016). Thus, the efficient use of phosphate fertilizers in tropical soils is essential, especially considering that phosphate rock sources are globally limited, and there are concerns about their long-term availability (Sharpley et al., 2013).

Soil chemical analysis is a powerful tool for transferring knowledge generated from soil fertility research to farmers (Silva and Raij, 1999). Through soil analysis, doses of nutrients to be applied to soil based on crop requirements are defined. It is important to note that the soil nutrient content determined by a soil test is only an index of availability that becomes useful for estimating fertilizer application rate only after the empirical work of correlation and calibration (Cade-Menun et al., 2018; Valadares et al., 2017).

The concept of nutrient availability in the soil establishes that the amount of nutrient determined by a given soil test must be correlated with some variable related to plant growth or yield (Alvarez V., 1996; Bray, 1947; Cantarutti et al., 2007). In this context, the P content in the plant tissue corresponds to the standard method of determining soil P availability. The determination of soil nutrient availability for plants through chemical soil analysis is one of the main objectives of soil fertility evaluation researches. Due to the development of new cultivars and agricultural practices, as well as annual fertilizer inputs, studies that focus on assessing the efficacy of soil tests on estimating nutrient availability in soils should be frequent, rationalizing lime and fertilizers use through the standardization of soil tests.

Mehlich-1 (M1) (Nelson et al., 1953), and Ion Exchange Resin (IER) (van Raij et al., 1986) are the two official soil P extractants employed in Brazil despite the wide variety of soil classes and crops cultivated in the country. While IER is the official method for extracting P from soils in Sao Paulo State, with increasing use also in laboratories in other regions (van Raij et al., 2001), M1 is adopted by Embrapa (Brazilian Agricultural Research Corporation) and widely employed in all over the country. There is an overall correlation and calibration studies regarding M1, although in many cases, this soil test is adopted in certain regions and crops without proper calibration (Simões Neto et al., 2009). This also occurs in the cases when IER is employed in soils located outside the State of São Paulo.

Mehlich-1 is sensitive to soil P buffer capacity (PBC), and therefore the interpretation of its results is dependent on a measure of this factor, usually the soil clay content or the remaining P (P-rem). Mehlich-1 extracts, preferably the forms of P-Ca (Mehlich, 1984; Rogeri et al., 2016), overestimating, in some cases, the availability of P in soils recently fertilized with phosphate rocks (Bortolon et al., 2011). Thus, there may be an overestimation of P content determined by this soil test in soils cultivated with high doses of limestone, and with different sources of phosphate fertilizers. The long-term cultivation

creates soil conditions different from those when M1 was adopted in Brazil, when acidic soils with low P and Ca contents were predominant (Cherubin et al., 2016; Lopes and Guilherme, 2016).

The use of IER instead of Anionic Exchange Resin, initially used in the State of São Paulo (van Raij et al., 1986), aimed to increase the efficiency in laboratories, determining beyond P, also Ca^{2+} , Mg^{2+} , and K^+ (van Raij et al., 2001). However, because IER acts as a Ca^{2+} drain, it can, as well as M1, overestimate in some conditions the P availability in soils rich in forms of P bonded to Ca (P-Ca) due to liming and phosphate fertilizers application (Freitas et al., 2013; Novais et al., 2015).

Due to the high extraction of P by the M1 in soils rich in Ca, and its low correlation with plant responses, the Mehlich-2 (M2) soil test was developed and proposed to be used instead of M1 (Mehlich, 1978). However, the M2 extractant has corrosive properties to glassware, due to the use of NH_4Cl and HCl , and did not show good results for Cu^{2+} extraction (Mehlich, 1984). Thus, the Mehlich-3 (M3) soil test has been proposed to improve P extraction in soils rich in P-Ca, as well as in acidic soils, and to be a multi-element extractant, being suitable for K^+ , Ca^{2+} , Mg^{2+} , Cu^{2+} , Fe^{2+} , Mn^{2+} and Zn^{2+} extraction. The replacement of chlorides from NH_4NO_3 and HNO_3 , and the addition of de EDTA, eliminated corrosive properties and improved micronutrient extraction (Mehlich, 1984). Regarding to P, the presence of CH_3COOH buffered the pH at 2.5, avoiding excessive dissolution of P-Ca, and the NH_4F is specific to P-Al, which is the main source of P to plants in most tropical soils (Bortolon et al., 2009; Novais and Smyth, 1999).

Among the advantages of using M3 in routine soil analysis laboratories is that it is a multi-element extractant solution, which decreases analysis' costs (Milagres et al., 2007), and produces clear extracts, an essential condition for molecular absorption spectrophotometry determination of P, without the need to filter the extract (Bortolon et al., 2011; Mallarino, 2003; Milagres et al., 2007). There are some correlation and calibration works with M3 in Brazilian soils with good results, but most of them were carried out in soils of southern region (Bortolon et al., 2011, 2009; Bortolon and Gianello, 2008), including the recommendation to use M3 and tables for interpretation of its results (Bortolon and Gianello, 2012; Mumbach et al., 2018). Thus, there is a need for complementary studies in soils from other regions, and with a wide variation of characteristics related to soil PBC and P availability.

We hypothesize that Mehlich-3 soil test has a higher capability to predict soil P availability than M1 and IER in both conditions, low and high soil P availability. Our objective was to evaluate the capacity of M1, M3 and IER soil tests to predict soil P

availability by correlations with the P taken up and accumulated by maize plants (*Zea mays* L.) cultivated in soils with contrasting P content under greenhouse conditions.

2.2. MATERIAL AND METHODS

2.2.1. Soil sampling and characterization

Four soil samples were collected in areas that have been intensively cultivated for, at least, 20 years with crops that demand high P fertilization and lime (GU-C, TA-C, RP-C, and PD-C). For each of the four soil samples, in the same condition of soil class and topography, were collected soil samples in areas that have never been cultivated (GU-UC, TA-UC, RP-UC, and PD-UC). In addition, two soil samples were collected with contrasting characteristics related to soil P buffering capacity, as clay content and organic matter (PR-CS and PR-SS) (Table 1 and Table 2). Thus, 10 soil samples were collected at a depth of 0.0 - 0.2 m, air-dried, passed through a 4 mm sieve, and stored. After homogenization, sub-samples were collected and passed through a 2 mm sieve (air-dried soil sample - ADSS), for the chemical and physical characterization (Table 2).

Table 1. Soil sample identification, World Reference Bases Classification System (WRB), collection sites, type of soil use, vegetal cover, and/or crop production

Soil Sample	WRB	Collection site	Use	Vegetal cover/crop production ¹
GU-UC ²	FR	Guarapuava	Native vegetation	Atlantic forest
GU-C ³	FR		Agriculture	Potato/Wheat/Bean/Maize/Soybean
TA-UC	FR	Taquarituba	Native vegetation	Atlantic forest
TA-C	FR		Agriculture	Potato/Wheat/Bean/Maize/Soybean
RP-UC	FR	Rio Paranaíba	Native vegetation	Cerrado forest
RP-C	FR		Agriculture	Garlic/Onion/Carrot/Wheat/Maize
PD-UC	AC	Piedade	Native vegetation	Atlantic forest
PD-C	AC		Agriculture	Beet/Cabbage/Tomato/Maize
PR-CS ⁴	FR	Piracicaba	Fallow	Brachiaria grass
PR-SS ⁵	FR		Fallow	Eucalyptus

¹Areas cultivated for more than 20 years. ²UC: Uncultivated; ³C: Cultivated; ⁴CS: clayey soil; ⁵SS: sandy soil. FR: Ferralsol. AC: Acrisol.

The chemical characterization of soil samples was performed as described in van Raij et al. (2001), which adopts the Ion Exchange Resin (IER) as the official soil test for P in the State of São Paulo (Table 2). The soil pH was determined in CaCl₂ 10 mmol L⁻¹ (1:2.5 soil : solution ratio), soil organic matter (SOM) was determined by Walkley-Black method (Walkley and Black, 1934). The K⁺, Ca²⁺, and Mg²⁺ were extracted with IER. K⁺ was

determined by flame emission spectrophotometry, and Ca^{2+} and Mg^{2+} by atomic absorption spectrophotometry. Cation exchange capacity (CEC) was calculated by the sum of K^+ , Ca^{2+} , and Mg^{2+} , and total acidity ($\text{H} + \text{Al}$) was determined by SMP buffer solution (van Raij et al., 2001). The granulometric analysis was performed by the pipet method, with chemical dispersion in NaOH 0.1 mol L^{-1} (Teixeira et al., 2017).

The soil available P content was determined by IER (van Raij et al., 2001), Mehlich-1 (Nelson et al., 1953), and Mehlich-3 (Mehlich, 1984). For extraction with IER we used a mixture of anion exchange resin (Amberlite IRA-400), and cation exchange resin (Amberlite IR-120). The steps of preconditioning and treatment of the resin for use, as well as the extraction process, are described in van Raij et al. (2001). The extraction with Mehlich-1 (HCl 50.0 mmol L^{-1} + H_2SO_4 12.5 mmol L^{-1}), and Mehlich-3 (NH_4F 15 mmol L^{-1} + CH_3COOH 200 mmol L^{-1} + NH_4NO_3 250 mmol L^{-1} + HNO_3 13 mmol L^{-1} + EDTA 1 mmol L^{-1}) was performed in 1:10 soil : solution ratio (5 cm^3 : 50 mL). The samples were shaken with the extractant solution for 5 min, and then the filtration was carried out with slow quantitative filter paper. After obtaining the extracts, the P was determined by molecular absorption spectrophotometry (Murphy and Riley, 1962) for all extractants. The remaining P (P-rem) was obtained after shaking 5 cm^3 of ADSS for 1 h with 50 mL of CaCl_2 10 mmol L^{-1} , containing 60 mg L^{-1} of P (Alvarez V et al., 2000).

Table 2. Selected soil chemical and physical characteristics

Characteristics	Soil sample									
	GU-UC ^{1/}	GU-C ^{2/}	TA-UC	TA-C	RP-UC	RP-C	PD-UC	PD-C	PR-CS ^{3/}	PR-SS ^{4/}
pH- CaCl_2	4.0	4.9	5.0	5.9	5.3	5.3	4.1	5.4	4.2	4.7
SOM (g dm^{-3}) ^{5/}	69	50	27	32	41	37	50	27	32	37
P-rem (mg L^{-1}) ^{6/}	2.2	3.4	40.1	35.9	4.6	10.5	27.0	26.1	19.8	42.8
P-M1 (mg dm^{-3}) ^{7/}	4	22	25	73	4	114	3	60	2	6
P-IER (mg dm^{-3}) ^{8/}	6	30	39	123	24	141	18	123	11	13
P-M3 (mg dm^{-3}) ^{9/}	1	12	38	84	6	110	6	78	2	7
K^+ ($\text{mmol}_c \text{ dm}^{-3}$)	2.8	3.5	2.1	3.7	2.5	3.0	2.3	3.5	2.0	1.6
Ca^{2+} ($\text{mmol}_c \text{ dm}^{-3}$)	23	44	26	39	26	32	21	30	12	41
Mg^{2+} ($\text{mmol}_c \text{ dm}^{-3}$)	17	14	13	20	10	8	5	14	6	11
Al^{3+} ($\text{mmol}_c \text{ dm}^{-3}$)	28	4	2	0	1	1	13	1	12	3
CEC ($\text{mmol}_c \text{ dm}^{-3}$) ^{10/}	228	134	83	81	86	85	126	82	84	76
BS (%) ^{11/}	19	46	49	78	45	51	22	58	24	44
Clay (g kg^{-1})	487	599	270	330	485	469	340	339	489	247
Sand (g kg^{-1})	500	70	680	640	340	370	600	530	330	730

^{1/}UC: soil samples collected in uncultivated areas. ^{2/}C: soil samples collected in cultivated areas. ^{3/}CS clayey soil. ^{4/}SS sandy soil. ^{5/}Soil organic matter (Walkley and Black, 1934) ($\text{C. Org} \times 1.724$). ^{6/}Remaining P (Alvarez V et al., 2000). Soil P content by ^{7/}Mehlich-1, ^{8/}Ion Exchange Resin and ^{9/}Mehlich-3. ^{10/}Cation exchange capacity at pH 7.0 [$\text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+} + (\text{H} + \text{Al})$]. ^{11/}Base saturation [$(\text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+})/\text{CEC} \times 100$].

2.2.2. Greenhouse experiment design

The experiment was carried out under greenhouse conditions. The treatments consisted of a 10×6 factorial, 10 soil samples and six doses of P, with four replicates in a randomized block design. The experimental units consisted of plastic pots with 2.0 dm^3 of soil where two maize plants (*Zea mays* L.) were cultivated. Two consecutive cultivations of maize were carried out for 45 days each. The second cultivation was performed by sowing the maize seeds without removing the root system from the previous crop.

From the soil samples passed through a 4.0 mm sieve, the mass equivalent to the volume of 2.0 dm^3 was weighed and packed in transparent plastic bags. The dose of limestone to be applied to each soil was calculated according to the base saturation method (van Raij et al., 2001) to raise soil base saturation up to 70 %. The limestone used was composed of a mixture of CaCO_3 (p.a.) and MgCO_3 (p.a.) in the proportion of 4:1 (mol of Ca : mol of Mg), with PRNT of 103 %. The amount of the mixture applied in each soil was: GU-UC: 6.0 g pot^{-1} ; GU-UC: 0.52 g pot^{-1} ; TA-UC: 1.69 g pot^{-1} ; RP-UC: 2.09 g pot^{-1} ; PR-C: 1.57 g pot^{-1} ; PD-UC: 3.42 g pot^{-1} ; PD-C: 0.95 g pot^{-1} ; PR-CS: 3.75 g pot^{-1} ; PR-SS: 1.92 g pot^{-1} ; there was no need liming requirement for the soil sample TA-C. The doses of limestone were applied individually to the 2.0 dm^3 of soil corresponding to each experimental unit. After the limestone application, the samples were moistened up to 80 % of the maximum water retention capacity and incubated for 15 days. After incubation, the soil samples were air-dried and, again passed through a 4.0 mm sieve.

The doses of P applied to each soil sample was determined according to P-rem (Alvarez V et al., 2000). The sources of P were Triple Superphosphate (20 % of P) and Bayovar reactive phosphate rock (13 % of P). The mixture was composed by 70 % of Triple Superphosphate, and 30 % of Bayovar, which were mixed to the soil sample at the moment of application. The doses of P were applied in the total volume of soil, corresponding to the following levels: 0.0, 0.5, 1.0, 2.0, 3.0, and 5.0. The level 5.0 corresponds to the highest dose in the range. Thus, the doses of P applied were: 0, 65, 130, 260, 390, and 650 mg dm^{-3} for the soils GU-C and GU-UC; 0, 35, 70, 140, 210, and 350 mg dm^{-3} for the soils TA-C and TA-UC; 0, 56, 112, 224, 336, and 560 mg dm^{-3} for the soils RP-C and RP-UC; 0, 41, 82, 164, 246, and 410 mg dm^{-3} for the soils PD-C, PD-UC, and PR-CS; 0, 30, 60, 120, 180, and 300 mg dm^{-3} for the soil PR-SS.

The fertilization with other nutrients was carried out by applications in the form of nutrient solution, according to some modifications made in the recommendation of Novais et

al. (1991). The applications were made in three times, the first one 5 days after seeding, second 16 days after seeding, and third 31 days after seeding. In each application, we used 1/3 of the following total dose in the first cultivation. For the second cultivation, we applied only 1/3 of the total dose 10 days after the seeding. The doses and sources have corresponded to 300 mg dm⁻³ of N [(NH₂)₂CO]; 160 mg dm⁻³ of K (KCl); 80 mg dm⁻³ of S [(NH₄)₂SO₄]; 0.81 mg dm⁻³ of B (H₃BO₃); 1.33 mg dm⁻³ of Cu (CuSO₄.5H₂O); 1.55 mg dm⁻³ of Fe (FeCl₃.6H₂O); 3.66 mg dm⁻³ of Mn (MnCl₂.4H₂O); 0.15 mg dm⁻³ of Mo (NaMoO₄.2H₂O), and 4.0 mg dm⁻³ of Zn (ZnSO₄.7H₂O).

After each cultivation, the maize shoot dry matter was collected, dried in a forced air circulation oven at 65 °C for 72 h, after which the shoot dry matter yield was determined. The P content in the plant tissue was determined after nitric-perchloric digestion (3:1), by molecular absorption spectrophotometry (Murphy and Riley, 1962). After the determination of P content in the plant tissue from the two plants cultivated in each pot, the maize shoot total amount of P was calculated.

After the first and second cultivations, 100 cm³ of soil samples were taken from all experimental units and passed through a 2 mm sieve. After the first cultivation, the soil was passed through a 4 mm sieve and returned to the pots in order to perform the second cultivation. The samples collected after each cultivation were air-dried and passed through a 2.0 mm sieve for analysis of available P by M1(Nelson et al., 1953), M3 (Mehlich, 1984), and IER (van Raij et al., 1986) as described above.

2.2.3. Statistical analyzes

The data were subjected to analysis of variance, using the program SISVAR 5.6 (Ferreira, 2011), for significant differences among the factors soils and doses of P, and their interactions using the *t*-test (*p* < 0.05). Then, regression analysis were carried out by unfolding the effects of doses of P applied in each soil on maize shoot dry matter yield, shoot P content, and total amount of P.

The soil tests' predictive capacity was evaluated by Pearson's linear correlation between the soil P content and maize shoot dry matter yield, shoot P content, and shoot total amount of P. For this purpose, we used the data from the control, where there was no P application. The relation between the soil tests was also performed by Pearson's linear correlation.

2.3. RESULTS AND DISCUSSION

2.3.1. Uptake and accumulation of phosphorus by plants

Different dose-response curves of shoot dry matter yield and P accumulation by maize plants were obtained. The amplitude of the applied doses of P allowed to reach a stabilization trend in the shoot dry matter yield, as shown by the quadratic and quadratic root model adjustments, except for the samples GU-C and TA-C, where the linear model was adjusted (Figure 1). The only sample in which there was no response to P application was RP-C, where there was no response to shoot dry matter yield (Figure 1), shoot P content, and shoot total amount of P (Table 4) due to its high soil P content (Table 2). As a result, the highest values of shoot dry matter yield ($19.5 \text{ g plant}^{-1}$), shoot P content (1.0 g kg^{-1}), and shoot total amount of P ($20.6 \text{ mg plant}^{-1}$) were obtained in this soil. The largest increase in shoot dry matter yield in response to application of P occurred in sample GU-UC, where the yield increased from 1.0 g plant^{-1} when P was not applied, to $30.3 \text{ g plant}^{-1}$ with the maximum dose of P. The lowest average of shoot dry matter yield ($1.72 \text{ g plant}^{-1}$), shoot P content (0.5 g kg^{-1}), and shoot total amount of P ($0.9 \text{ mg plant}^{-1}$) were obtained in the sample GU-C.

Unlike observed in samples RP-C and PD-C, there was no plant growth compatible with the doses of P applied in the samples GU-C and TA-C, where plant growth was higher in soil samples collected in uncultivated areas, even in the treatments with application of P (Figure 1, a, b). This fact explains the linear response obtained for shoot dry matter yield in these soils, while the expected would be a root quadratic model or no response, as observed in the sample RP-C, due their high P availability compared to their counterparts sampled in uncultivated areas (Table 2). The micronutrient availability in all soil samples was classified as high (van Raij et al., 1997) before liming (Table 3). As liming was performed prior to cultivation, a decrease in available micronutrient contents was expected, mainly for Fe and Mn. However, there was no decrease in Mn contents after liming (Table 3). This fact, associated with the application of Mn via nutrient solution during the experiment could explain the low plant growth in soils GU-C and TA-C. Nevertheless, micronutrient content, including Mn, was higher in other soils where there was good plant growth (Table 3). Another hypothesis is that there may have been a remnant of herbicide in the soil, which was sampled in the field shortly after the maize crop be harvested in these two areas.

Table 3. Soil micronutrient content before and after liming

Soil sample	----- Before liming -----				----- After liming -----			
	Cu	Fe	Mn	Zn	Cu	Fe	Mn	Zn
	mg dm ⁻³							
GU-UC ¹	10.7	288	17.2	4.0	6.9	77	42.7	1.9
GU-C ²	8.2	89	6.4	2.2	5.8	31	10.1	1.2
TA-UC	1.3	130	8.0	2.6	0.9	28	14.9	2.0
TA-C	1.0	30	5.0	2.2	0.8	11	33.5	1.5
RP-C	3.8	16	4.2	5.5	2.9	25	19.1	4.85
RP-UC	7.2	46	9.6	8.8	6.4	29	9.6	14.9
PD-C	0.9	300	19.2	3.1	0.4	98	42.4	1.8
PD-UC	4.0	78	4.4	8.6	3.6	47	5.6	5.7
PR-CS ³	1.4	336	35.2	1.8	0.8	25	42.9	0.7
PR-SS ⁴	0.8	104	11.2	2.9	0.4	35	27.8	2.0

¹UC: soil samples collected in uncultivated areas. ²C: soil samples collected in cultivated areas. ³CS clayey soil.

⁴SS sandy soil. The micronutrients were extracted by DTPA solution at pH 7.3 according to (van Raij et al., 2001), and determined by atomic absorption spectrophotometry (ICP-AES).

There was a predominance of linear increase in the shoot P content in response to the addition of P, and linear and quadratic increments for P accumulation (Table 4). The low values of the linear coefficients in the equations of the shoot P content, and the adjustment of quadratic and quadratic root equations for shoot dry matter yield and shoot total amount of P proves the efficiency of determining the doses of P to be applied in dose-response experiments according to P-rem (Alvarez V et al., 2000). This approach allows the stratification of the doses of P according to soil P buffering capacity (PBC), which does not occur when the same dose ranges are used for soil with different PBC (Bortolon et al., 2009; Simonete et al., 2015).

Figure 1. Equations of maize shoot dry matter yield ($\hat{y}$, g plant⁻¹) as function of doses of P (x , mg dm⁻³) applied in 10 soil samples collected in cultivated and uncultivated areas (a, b, c, and d), and in two complementary soil samples (e and f) collected in fallow areas. UC: soil samples collected in uncultivated areas. C: soil samples collected in cultivated areas. CS: clayey soil. SS: sandy soil. ^{ns} $p \geq 0.10$; [°] $0.10 > p \geq 0.05$; ^{*} $0.05 > p \geq 0.01$; ^{**} $0.01 > p \geq 0.001$; ^{***} $p < 0.001$.

Table 4. Equations of maize shoot P content ($\hat{y}$, g kg⁻¹), and maize shoot total amount of P ($\hat{y}$, mg plant⁻¹) as function of doses of P (x , mg dm⁻³) applied in ten soil samples

Soil sample	Equation	R ²
	Shoot P content	
GU-UC ^{1/}	$\hat{y} = 0.47 + 0.0008^{***} x$	0.99
GU-C ^{2/}	$\hat{y} = 0.46 + 0.0002^{*} x$	0.75
TA-UC	$\hat{y} = 0.54 + 0.0020^{***} x$	0.99
TA-C	$\hat{y} = 0.89 - 0.0301^{*} \sqrt{x} + 0.0020^{*} x$	0.91
RP-UC	$\hat{y} = 0.47 + 0.0003^{o} x$	0.59
RP-C	$\hat{y} = \bar{y} = 1.06$	-
PD-UC	$\hat{y} = 0.44 + 0.0018^{***} x$	0.99
PD-C	$\hat{y} = 0.74 + 0.0006^{**} x$	0.86
PR-CS ^{3/}	$\hat{y} = 0.45 + 0.0015^{***} x$	0.96
PR-SS ^{4/}	$\hat{y} = 0.49 + 0.0024^{***} x$	0.96
Shoot total amount of P		
GU-UC	$\hat{y} = 0.93 + 0.0738^{***} x - 0.000048^{**} x^2$	0.99
GU-C	$\hat{y} = 0.47 + 0.0018^{**} x$	0.96
TA-UC	$\hat{y} = 4.36 + 0.0456^{***} x$	0.98
TA-C	$\hat{y} = 3.93 + 0.0200^{**} x$	0.92
RP-UC	$\hat{y} = 1.16 + 0.0320^{*} x - 0.000029^{o} x^2$	0.96
RP-C	$\hat{y} = \bar{y} = 20.60$	-
PD-UC	$\hat{y} = 1.33 + 0.0809^{***} x - 0.000065^{*} x^2$	0.99
PD-C	$\hat{y} = 10.58 + 0.0137^{**} x$	0.85
PR-CS	$\hat{y} = 1.16 + 0.0711^{**} x - 0.000063^{o} x^2$	0.98
PR-SS	$\hat{y} = 2.22 + 0.0600^{***} x$	0.98

^{1/}UC: soil samples collected in uncultivated areas. ^{2/}C: soil samples collected in cultivated areas. ^{3/}CS: clayey soil. ^{4/}SS: sandy soil. ^o 0.10 > p ≥ 0.05; * 0.05 > p ≥ 0.01; ** 0.01 > p ≥ 0.001; *** p < 0.001.

2.3.2. Soil phosphorus content extracted by soil tests

The soil P content varied mainly due to the characteristics of each soil test, and due to soil P buffer capacity. Although the initial soil P content had a great interference in the soil tests' recovery rates, with more extraction in soils with higher soil P availability (Table 5). The highest soil P contents were determined by IER in both cultivations in treatments without P application, and when all applied doses were considered. The lowest P contents were obtained with the M3, with values similar to those obtained by the M1. In the first cultivation the average of soil P content, considering all doses, were 61.0 mg dm⁻³ for M1, 62.2 mg dm⁻³ for M3, and 110.3 mg dm⁻³ for IER. In the second cultivation, the averages were 62.4 mg dm⁻³ for M1, 58.9 mg dm⁻³ for M3 and 99.9 mg dm⁻³ for IER.

The highest extraction of P by IER and M3 in relation to M1 is a result corroborated by several studies (Bortolon and Gianello, 2012, 2008; Camêlo et al., 2015; Mumbach et al., 2018), although in some cases M1 could extract more P than IER (Freitas et al., 2013). The absolute amount of P extracted by a soil test does not represent the real amount of P available in the soil, but is an index of availability that needs to be correlated with the total amount of P taken up and accumulated by plants during their life cycle (Alvarez V., 1996; Cantarutti et al.,

2007; Valadares et al., 2017). Thus, the amount of P extracted depends, among other factors, on the principles of the methods extraction, soil characteristics and properties, and soil management.

The principle of soil P extraction by M1 is the solubilization of phosphates by H_3O^+ ions, the exchange of SO_4^- by the PO_4^- that are adsorbed with lower binding energy, and the occupation of the PO_4^- adsorption sites by SO_4^- , what keeps the desorbed PO_4^- in solution (Bortolon et al., 2009; Nelson et al., 1953). The M1 loss of extraction capacity occurs in less acidic soils by increasing the pH of the extraction solution that is not buffered, and in clayey soils by the consumption of SO_4^- ions in adsorption sites not occupied by PO_4^- (Novais et al., 2015). The M3 also works with the acidic solubilization of soil phosphates, but at a buffered pH of 2.5, due to the presence of acetic acid (Bortolon et al., 2009; Mehlich, 1984), which results in less solubilization of P-Ca forms than M1. Besides, the NH_4F extracts specifically forms of P-Al (Mehlich, 1984), which is considered the main source of P availability in tropical soils (Bortolon et al., 2009; Novais and Kamprath, 1978). One factor that leads to a higher extraction of P by M3 in relation to M1 is the presence of EDTA in M3, which may be responsible for extracting some organic forms of P (Gatiboni et al., 2005; Mumbach et al., 2018).

The extraction with IER is based on the process of adsorption of orthophosphate ions in soil solution by the anionic resin and, consequently, the desorption of the P adsorbed in soil particles that are in equilibrium with soil solution during the agitation with soil : water (1:10 v/v) for 16 h. During this process, there is no loss of extraction capacity likewise M1 (Novais et al., 2015), what is the main reason for the greater extraction by IER. However, other important factor that determines the amount of P extracted by each soil test is the source of P. When the main source of P is a rock phosphate, there is a tendency for higher P extraction by M1, due its acidity (pH of the extractant solution is about 1.2), that solubilize predominantly P-Ca and, in some cases, there is an overestimation of available P. This was the case of more soil P content by M1 than IER found by Freitas et al. (2013) in the treatments with rock phosphates, and more soil P content by IER in the treatment with Triple Superphosphate.

In this study, we used a proportion of 70 % of Triple Superphosphate and 30 % of phosphate rock (Bayóvar), that was not sufficient to induce a higher extraction of P by M1 in relation to IER, even in the second cultivation, where there was more time for the reaction and release of P from rock phosphate. The average of soil P extracted in the treatment with no P application (level 0.0) was 24.9 mg dm^{-3} by M1, 38.3 mg dm^{-3} by IER, and 62.8 mg dm^{-3} by IER, what can be explained by the loss of extraction capacity by M1 and M3, which did not

occur with IER. In level 5.0, with a high dose of soluble phosphate and rock phosphate, the trend was the same, average of 127.0 mg dm^{-3} by M1, 102.9 mg dm^{-3} by M3, and 177.9 mg dm^{-3} by IER. Two factors may have contributed to these results. The first one was the elevation of soil pH after liming, what decreases the dissolution of rock phosphate (Valadares et al., 2017), and increases the loss of extraction capacity by M1 (Camêlo et al., 2015; Novais et al., 2015), and M3 (Valadares et al., 2017), due to their sensitivity to soil PBC (Bortolon and Gianello, 2012). The second one is the fact that the IER also works at a Ca^{2+} sink, what does not occur when only the Anionic Exchange Resin is used (Freitas et al., 2013; Novais et al., 2015). The Anionic Exchange Resin was the first method used in laboratories in the State of São Paulo (van Raij et al., 1986), however, with the use of mixed resin (anionic + cationic), this soil test also extracts forms of P-Ca, but in a smaller proportion than M1 (Freitas et al., 2013; Novais et al., 2015).

The quantification of soil available P extracted by IER is made after the desorption of P from resin in a solution of NH_4Cl 0.8 mol L^{-1} and HCl 0.2 mol L^{-1} with determination by spectrophotometry, using the molybdenum blue method (Murphy and Riley, 1962). For determination, it is necessary to add gelatin to the reagent, in order to avoid precipitation of the phosphomolybdic complex due to the high concentration of ammonium in the solution (van Raij et al., 2001). So, it is noteworthy that the method used to determine P in the extract plays an important role in the result (Nagul et al., 2015). For example, when the molybdenum blue method of Murphy and Riley (1962) is used without the addition of gelatin, the amount of P extracted by the IER is underestimated.

Table 5. Equations of soil available P content by Mehlich-1, Mehlich-3, and Ion Exchange Resin ($\hat{y}$, mg dm⁻³) as function of doses of P (x , mg dm⁻³) applied in ten soil samples

Soil sample	Equation	R ²
	Mehlich-1	
GU-UC ^{1/}	$\hat{y} = -1.68 + 0.1241^{***} x$	0.97
GU-C ^{2/}	$\hat{y} = 15.11 + 0.1756^{***} x$	0.99
TA-UC	$\hat{y} = 19.01 + 0.2928^{***} x$	0.99
TA-C	$\hat{y} = 52.79 + 0.5337^{***} x$	0.95
RP-UC	$\hat{y} = -3.44 + 0.1310^{**} x$	0.94
RP-C	$\hat{y} = 81.83 + 0.1828^{**} x$	0.94
PD-UC	$\hat{y} = -4.36 + 0.2356^{**} x$	0.94
PD-C	$\hat{y} = 57.84 + 0.1910^{*} x$	0.71
PR-CS ^{3/}	$\hat{y} = -2.07 + 0.2044^{***} x$	0.98
PR-SS ^{4/}	$\hat{y} = 13.97 - 0.0024^{ns} x + 0.0013^{*} x^2$	0.98
Mehlich-3		
GU-UC	$\hat{y} = 4.53 + 0.0201^{ns} x + 0.0001^{*} x^2$	0.99
GU-C	$\hat{y} = 17.33 + 0.0705^{***} x$	0.97
TA-UC	$\hat{y} = 45.50 + 0.2104^{**} x$	0.92
TA-C	$\hat{y} = 84.47 + 0.3277^{***} x$	0.98
RP-UC	$\hat{y} = 7.02 + 0.1093^{***} x$	0.99
RP-C	$\hat{y} = 109.30 + 0.1012^{**} x$	0.85
PD-UC	$\hat{y} = 1.26 + 0.1861^{**} x$	0.94
PD-C	$\hat{y} = 89.24 + 0.0984^{*} x$	0.81
PR-CS	$\hat{y} = 5.53 + 0.1444^{***} x$	0.99
PR-SS	$\hat{y} = 15.03 + 0.2016^{***} x$	0.98
Ion Exchange Resin		
GU-UC	$\hat{y} = 9.59 + 0.0810^{*} x + 0.0002^{*} x^2$	0.99
GU-C	$\hat{y} = 48.35 + 0.2678^{***} x$	0.99
TA-UC	$\hat{y} = 38.60 + 0.2948^{*} x$	0.76
TA-C	$\hat{y} = 98.96 + 0.3857^{***} x$	0.99
RP-UC	$\hat{y} = 27.08 + 0.3515^{***} x$	0.97
RP-C	$\hat{y} = 300.84 + 0.1887^{*} x$	0.76
PD-UC	$\hat{y} = 7.23 + 0.2048^{***} x$	0.97
PD-C	$\hat{y} = 104.25 + 0.2508^{*} x$	0.80
PR-CS	$\hat{y} = 13.39 + 0.0269^{ns} x + 0.0006^{*} x^2$	0.99
PR-SS	$\hat{y} = 13.26 + 0.3430^{**} x - 0.0005^{*} x^2$	0.99

^{1/}UC: soil samples collected in uncultivated areas. ^{2/}C: soil samples collected in cultivated areas. ^{3/}CS: clayey soil. ^{4/}SS: sandy soil. ° 0.10 > p ≥ 0.05; * 0.05 > p ≥ 0.01; ** 0.01 > p ≥ 0.001; *** p < 0.001.

2.3.3. Correlation between soil tests and plant responses

To evaluate the efficiency of soil tests to predict the availability of soil P to plants, Pearson's linear correlation was established between the soil available P by the soil tests in the controls (level 0.0 of P) and plant responses (shoot dry matter yield, shoot P content and shoot total amount of P). Close correlation coefficients ($r > 0.70$) were obtained between soil available P by soil tests and plant responses in both cultivations. The highest correlation coefficients were obtained with the shoot P content (Table 6). The correlation coefficients of M1 and M3 were similar for all measurements in both crops. The M1 and M3 soil tests were only superior to the IER in the correlations established with the shoot P content (Table 6).

Although the highest correlation coefficients were obtained with shoot P content, this is a measure subject to the effects of dilution and concentration, due to the higher or lower plant growth. Therefore, conclusions should be taken based on the results of the correlations with dry matter yield or, preferably, with total amount of the nutrient in plant tissues (Alvarez V., 1996). Thus, the IER was the soil test that best correlated with the standard method, which is the amount of P absorbed and accumulated by plant. For both cultivation, the highest correlation coefficients between the soil tests and the shoot dry matter yield and shoot total amount of P were obtained with IER, followed by M3 (Table 6).

Table 6. Correlation coefficients between soil P test and shoot dry matter yield, shoot P content, and shoot total amount of P of maize plants cultivated in 10 soils, in two consecutive cultivation under greenhouse conditions

	Shoot dry matter	Shoot P content	Shoot total amount of P
	First cultivation		
Mehlich-1	0.83**	0.97***	0.87***
Mehlich-3	0.85**	0.98***	0.87**
Ion Exchange Resin	0.88***	0.82**	0.94***
	Second cultivation		
Mehlich-1	0.84**	0.90***	0.84**
Mehlich-3	0.87**	0.92***	0.86**
Ion Exchange Resin	0.95***	0.87**	0.95***

** $0.01 > p \geq 0.001$; *** $p < 0.001$.

An important factor responsible for the best correlation obtained with IER where the high values of P extracted in the soil RP-C by this extractant. The low shoot dry matter yield and, consequently, low accumulation of P by plants in the samples GU-C and TA-C, even with high P content in these soils, impaired fit of linear model for correlation for all soil tests, as shown for the points corresponding to the sample TA-C in figure 2. However, due to the high levels of P extracted by IER in sample RP-C, the effect of low plant growth was less evident in the correlation established with IER. The P content By IER in soil RP-C was far above the levels extracted by M1 and M3 (approximately 300 mg dm^{-3} with IER against, about 100 mg dm^{-3} with M1 and M3) (Figure 3). Another influence of the higher P contents extracted by IER on the correlation coefficients is the smaller error in the determination of P by molecular absorption spectrophotometry, especially in soils with lower P availability. Thus, a soil test that, for the same soil or set of soils, extracts more P has an important advantage over the others.

There was a slight superiority of M3 over M1 in the correlation coefficients. This can be explained due to the presence of acetic acid in M3, which buffers the pH of the extract, making M3 not strongly acidic as M1, and thus, less sensitive to the form of P-Ca (Brasil and

Muraoka, 1997; Valadares et al., 2017) that may be in forms not available to most cultivated crops in soils of tropical regions. In this way, the M3 soil test can advantageously replace the M1 in soils fertilized with rock phosphates, and in soils with high limestone applications, which had their characteristics modified over the cultivation time (Cherubin et al., 2016).

As good correlations ($r > 0.70$) were obtained with the three soil tests, it can be stated that they can be used to determine soil P availability. This result also found by other authors, however, in most cases using soils with low P availability (Bortolon et al., 2009; Brasil and Muraoka, 1997; Simonete et al., 2015). However, the decision to use a soil test should take into account other factors such as simplicity of use in routine laboratories and low cost, for example. In this sense, the M3 has advantages because it is a multi-element soil test, and therefore reduces the analysis costs, besides facilitating the analysis in routine laboratories (Milagres et al., 2007; Mumbach et al., 2018). However, extensive work on field calibration of M3 with the crops of interest is required, as already done on a small scale for southern Brazil (Bortolon and Gianello, 2012; Mumbach et al., 2018; Rogeri et al., 2016).

Figure 2. Correlation coefficients between soil P content by Mehlich-1, Mehlich-3, and Ion Exchange Resin soil tests and maize shoot total amount of P in two consecutive cultivation in ten soil samples under greenhouse conditions ($n = 10$). * $p < 0.05$. ○ points related to sample TA-C. ● points related to sample RP-C.

Linear regressions were established to assess the degree of association between the P content extracted by the soil tests in all treatments and soils (Figure 3). There was a high degree of association between M1 and M3 in both cultivations ($R^2 = 0.85$ and 0.83), and a lower degree of association between M1 and M3 with IER (Figure 3, B, C, E, and F). The high degree of association between the M1 and M3 is due to their similar extraction principles as previously discussed. This allows the equations established between the M1 and M3 to be used for interpretation of M3 soil P content using calibration tables established for the M1 extractant as proposed by Mumbach et al. (2018) and suggested in the States of Rio Grande do Sul and Santa Catarina (CQFS, 2016). However, it does not supplant the need for correlation and calibration researches for the M3 if it is used to determine P availability in Brazilian soils. The same approach cannot be employed for IER since its principle of extraction is different from M1 and M3, and there is a large dispersion of points, generating two distinct population of points and, consequently, decreasing the prediction capacity of the models, as can be observed in figure 3.

Figure 3. Correlation between soil P content determined by Mehlich-1, Mehlich-3 and Ion Exchange Resin soil tests in two consecutive cultivations of maize under greenhouse conditions for 45 days each ($n = 60$). First cultivation (A, B, C); second cultivation (E, F, G).

2.4. CONCLUSIONS

The Ion Exchange Resin and Mehlich-3 presented higher soil P extraction capacity than Mehlich-1, even with the application of phosphate rock.

Although the highest correlation coefficients were obtained with Ion Exchange Resin, the magnitude of the coefficients indicates that the three soil tests can be used to determine the availability of P in soils with contrasting soil availability and P buffer capacity.

The Mhlich-3 soil test can substitute Mehlich-1 by using the regression equations between the soil P content determined by the extractants and Mehlich-3 has the important advantage of do not excessively solubilize phosphates rock. However, researches of calibration at field level need to be done.

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3. INFLUENCE OF SOIL PHOSPHORUS BUFFERING CAPACITY ON SOIL PHOSPHORUS CRITICAL LEVELS AND RECOVERY RATES DETERMINED BY MEHLICH-1, MEHLICH-3 AND ION EXCHANGE RESIN EXTRACTANTS

ABSTRACT

Due to the complex reactions involving phosphorus (P) in soil, several soil tests are used worldwide to access its availability to plants. Changes in the chemical attributes of Brazilian soils after decades of cultivation, especially the increase of P availability in highly fertilized soils, require further evaluation of the soil tests currently in use. Our objectives were: (i) to determine soil phosphorus recovery rates and critical levels by Mehlich-1, Mehlich-3 and Ion Exchange Resin extractants in soils with high and low P availability, and (ii) to investigate the effect of soil attributes related to phosphate buffering capacity on extractants' recovery rates. The experiment was carried out under greenhouse conditions, the treatments were generated by the combination of a 10×6 factorial (10 soil samples and six doses of P), with four replicates in a randomized block design. The experimental units consisted of plastic pots with 2.0 dm³ of soil in which two maize plants (*Zea mays* L.) were cultivated for 45 days. After cultivation, plant yield indexes were determined and soil P recovery rates (*Prr*) and critical levels (*Pcl*) were calculated by each extractant. The influence of soil P buffering capacity on the *Prr* was also investigated. The IER presented higher *Prr*, due to the fact that it was not sensitive to soil P buffering capacity, as occurred with M1 and M3. Remaining P (P-rem) was the estimate of soil P buffering capacity that best correlated with *Prr*-M3. Thus, the use of the Mehlich-3 extractant in tropical soils is dependent on an interpretation table that stratifies the values based on a measure of soil P buffering capacity, preferably the P-rem.

Keywords: Soil test; soil fertility; calibration; correlation

3.1. INTRODUCTION

Phosphorus (P) is the nutrient that most limits primary production in tropical agroecosystems (Elser, 2012; Novais and Smyth, 1999; Roy et al., 2016) and therefore plays a key role in the agricultural and social development of the countries in this region. Among the various factors of agricultural production, soil fertility researches and scientific innovations in this area are considered one of the most important components for the development of Brazilian agriculture (Lopes and Guilherme, 2016). Phosphate fertilization combined with liming were fundamental techniques for the constant increase of food production in Brazilian

high weathered soils during the last decades (Guarçoni and Sobreira, 2017; Lopes and Guilherme, 2016).

The study of the P cycle in natural ecosystems reveals a well-closed cycle with low losses, which is due to the absence of gaseous phases, the low solubility of Ca phosphates forms, high P adsorption energy to Fe and Al oxides in the soil, as well as the low rate of erosion occurrence (Tiessen et al., 2011). However, in agroecosystems, significant losses of P occur, mainly due to the great adsorption and fixation of P to Fe and Al oxides in tropical soils (Fink et al., 2016; Roy et al., 2016; Withers et al., 2018). Thus, the strong interaction of P with the mineral particles of tropical soils is an important factor that governs its availability to plants (Fink et al., 2016).

Due to the complex reactions involving soil P availability, several soil tests are used worldwide to access soil P availability (Jones, 1998; Silva and Raij, 1999; Valadares et al., 2017). The soil tests that seeks to determine P availability varies in its chemical composition, extraction forms, soil : solution ratio (Silva and Raij, 1999), and extract P quantities ranging from near the soil solution concentrations to values near total P (Novais and Smyth, 1999). Thus, the soil available P content determined by these extractants are only indexes of availability that correlates with plant growth and yield (Alvarez V., 1996; Cantarutti et al., 2007; Valadares et al., 2017) and needs to be calibrated in order to be interpreted and used for phosphate fertilizer recommendation (Cade-Menun et al., 2018; Westerman et al., 1990).

In Brazil, Mehlich-1 (M1) and Ion Exchange Resin (IER) are used as extractants of available P (Teixeira et al., 2017; van Raij et al., 2001). The IER is officially used in the state of São Paulo, while M1 is widely used in the country (Valadares et al., 2017). The correlation and calibration researches for M1 and IER in Brazil were performed mainly using acidic soils with low P availability, a common characteristic of uncultivated tropical soils, mainly in the Cerrado region in Brazil (Lopes and Guilherme, 2016). However, due to decades of cultivation, with applications of phosphate fertilizers, liming and various management practices, the soil chemical characteristics are no longer the same as when these soil tests were implemented (Cherubin et al., 2016; Valadares et al., 2017). Recent correlation studies show that the Mehlich-3 (M3) extractant has good results for P extraction in Brazilian soils, with the main advantage of being a multi-element extractant (Bortolon and Gianello, 2012; Mumbach et al., 2018; Rogeri et al., 2016; Simões Neto et al., 2009; Valadares et al., 2017). However, few studies address the calibration phase, with the definitions of P recovery rates (P_{rr}) and critical P levels (P_{cl}) determined by these extractants in soils with high P availability. Most of the research in this regard was carried out in the southern region of the

country (Bortolon and Gianello, 2012; Mumbach et al., 2018; Rogeri et al., 2016), requiring further research involving soils from other regions.

The success of soil testing is dependent upon the amount of researches, and the quality of data generated (Westerman et al., 1990) thus, research on soil fertility evaluation involving correlation and calibration of extractants should be constant, aiming to optimize the use of phosphate fertilizers, a finite natural resource (Pantano et al., 2016; Van Vuuren et al., 2010). One of the multiple factors that influence the quality of phosphate fertilizer recommendation is the sensitivity of the extractant used to the soil phosphate buffering capacity. If the extractant has a sensitivity to this factor, it is necessary to stratify the table of interpretation of values in function of this feature as occurs with M1 (Mumbach et al., 2018; Ribeiro et al., 1999).

We hypothesize that the Mehlich-3 extractant is sensitive to soil phosphate buffering capacity even in soils with high phosphorus availability. Our objectives were: (i) to determine soil phosphorus recovery rates and critical levels by Mehlich-1, Mehlich-3 and Ion Exchange Resin extractants in soils with high and low P availability, and (ii) to investigate the effect of soil attributes related to phosphate buffering capacity on extractants' recovery rates.

3.2. MATERIAL AND METHODS

3.2.1. Soil samples collection and characterization

Ten soil samples were collected at a depth of 0.0 – 0.2 m in cultivated and uncultivated areas, from three states and with contrasting characteristics related to P buffering capacity as shown in figure 1 and table 1. Four soil samples were collected in long-term cultivated areas with high soil P availability, and for reference, for each of the four soil samples, one soil sample in the same condition of soil class and topography were collected in uncultivated areas. Two more soil samples were collected in fallow areas with low P availability but with contrasting soil texture (Table 1). Except for the soils RP-UC and RP-C, which are located in the Cerrado biome, all other soils are located in the Atlantic Forest biome. The soil class according to World Reference Bases Classification System (IUSS, 2015) was Ferralsol for soils from GU, TA, RP, and PR and Acrisol for soils from PD. The main crop grown in the soils GU-C, TA-C, and RP-C is potato, in succession with soybean or corn and with wheat and oats as winter crops. In the soil PD-C, the main crop is maize and

tomato, without winter crop cultivation. In all cultivated areas, cultivation has been going on for over 20 years with high applications of P and liming, mainly for potato crop requirements.

After sampling, the soil samples were air-dried, passed through a 4 mm sieve for cultivation in plastic pots, and sub-samples were collected and passed through a 2 mm sieve (air-dried soil sample - ADSS), for the chemical and physical characterization (Table 1). The chemical characterization of soil samples was performed as described in van Raij et al. (2001) which adopts IER as extractant, additionally the soil P availability was determined by M1 (Nelson et al., 1953) and M3 (Mehlich, 1984). The remaining P (P-rem) was determined according to Alvarez V et al. (2000) by shaking 5 cm³ of ADSS for 1 h with 50 mL of CaCl₂ 10 mmol L⁻¹, containing 60 mg L⁻¹ of P. The clay and sand soil content were determined by the pipet method, with chemical dispersion in NaOH 0.1 mol L⁻¹ (Teixeira et al., 2017).

Figure 1. Soil sampling sites showing the municipalities of sampling (GU: Guarapuava; TA: Taquaritiba; RP: Rio Paranaíba; PD: Piedade; PR: Piracicaba).

Table 1. Selected soil sample characteristics

Soil	WRB	pH CaCl ₂	SOM ⁵	P-rem ⁶	P M1	P M3	P IER	Ca ²⁺	Al ³⁺	CEC ⁷	BS ⁸	Clay	Sand
			g dm ⁻³	mg L ⁻¹	----	mg dm ⁻³	----	mmol _c dm ⁻³	----	mmol _c dm ⁻³	%	g kg ⁻¹	g kg ⁻¹
GU-UC ¹	FR	4.0	69	2.2	4	1	6	23	28	228	19	487	500
GU-C ²	FR	4.9	50	3.4	22	12	30	44	4	134	46	599	70
TA-UC	FR	5.0	27	40.1	25	38	39	26	2	83	49	270	680
TA-C	FR	5.9	32	35.9	73	84	123	39	0	81	78	330	640
RP-UC	FR	5.3	41	4.6	4	6	24	26	1	86	45	485	340
RP-C	FR	5.3	37	10.5	114	110	141	32	1	85	51	469	370
PD-UC	AC	4.1	50	27.0	3	6	18	21	13	126	22	340	600
PD-C	AC	5.4	27	26.1	60	78	123	30	1	82	58	339	530
PR-CS ³	FR	4.2	32	19.8	2	2	11	12	12	84	24	489	330
PR-SS ⁴	FR	4.7	37	42.8	6	7	13	41	3	76	44	247	730

¹UC: soil samples collected in uncultivated areas. ²C: soil samples collected in cultivated areas. ³CS clayey soil. ⁴SS sandy soil. ⁵Soil organic matter (Walkley and Black, 1934) (C. Org $\times$ 1.724). ⁶Remaining P (Alvarez V et al., 2000). M1= Mehlich-1, M3 = Mehlich-3; IER = Ion Exchange Resin. ⁷Cation exchange capacity [K^+ + Ca^{2+} + Mg^{2+} + (H + Al)]. ⁸BS = Base saturation [$(K^+ + Ca^{2+} + Mg^{2+})/CEC \times 100$]. Clay and sand: pipet method, with chemical dispersion in NaOH 0.1 mol L⁻¹ (Teixeira et al., 2017).

For the determination of Maximum P Adsorption Capacity (MPAC), 2.5 g of ADSS were shaken in a horizontal agitator end-over-end (30 rpm) at 25 °C for 24 h with 25 mL of CaCl₂ 0.01 mol L⁻¹ containing 11 doses of P, with three replicates. The source of P was KH₂PO₄ p.a. and the doses were determined according to the value of P-rem and have corresponded to the following levels: 0.00; 0.05; 0.10; 0.15; 0.25; 0.35; 0.45; 0.55; 0.70; 0.85, and 1.00 (Alvarez V et al., 2000). The dose of P corresponding to level 5.0, the maximum dose of the range, was 260 mg L⁻¹ for soils GU-UC and GU-C; 80 mg L⁻¹ for soils TA-UC, TA-C and PRSS; 200 mg L⁻¹ for RP-C and RP-UC; and 110 mg L⁻¹ for soils PD-UC, PD-C and PR-CS. After shaking, the samples were centrifuged at 3,000 rpm for 5 min and filtered. The concentration of P in the equilibrium solution was determined by molecular absorption spectroscopy (Murphy and Riley, 1962). The amount of P adsorbed to the soil was calculated by the difference between the amount of P added and the concentration of P in the equilibrium solution. The data were fitted to the Langmuir's Isotherm equation: $q = a \times b \times c / 1 + (a \times c)$, in which c is the P concentration in the equilibrium solution (mg L⁻¹), q is the amount of P adsorbed to the soil (mg kg⁻¹), a is a constant related to the P binding energy to soil (L mg⁻¹) and b is the soil MPAC (mg kg⁻¹).

3.2.2. Greenhouse experiment set up

For the experiment under greenhouse conditions, the treatments were generated by the combination of a 10 $\times$ 6 factorial (10 soil samples and six doses of P), with four replicates

in a randomized block design. The experimental units consisted of plastic pots with 2.0 dm³ of soil in which two maize plants (*Zea mays* L.) were cultivated for 45 days.

Before cultivation, the volume corresponding to 2.0 dm³ of each soil sample was weighed and packed in plastic bags in order to apply the recommended doses of lime, calculated by the method of soil base saturation according to van Raij et al. (2001). The lime was composed of a mixture of CaCO₃ (p.a.) and MgCO₃ (p.a.) in the proportion of 4:1 (mol of Ca : mol of Mg). Thereafter, the samples were moistened up to 80 % of the maximum water retention capacity and incubated for 15 days. After incubation, the soil samples were air-dried and, again passed through a 4.0 mm sieve. The doses of P were determined according to P-rem (Alvarez V et al., 2000). The sources of P were Triple Superphosphate (20 % of P) and Bayovar rock phosphate (13 % of P). The mixture was composed of 70 % of Triple Superphosphate, and 30 % of Bayovar, which were mixed to the soil sample at the moment of application.

The doses of P were applied in the total volume of the 2.0 dm³ of soil, corresponding to the following levels: 0.0, 0.5, 1.0, 2.0, 3.0, and 5.0. Level 5.0 corresponds to the highest dose in the range. Thus, the doses of P applied were: 0, 65, 130, 260, 390, and 650 mg dm⁻³ for the soils GU-C and GU-UC; 0, 35, 70, 140, 210, and 350 mg dm⁻³ for the soils TA-C and TA-UC; 0, 56, 112, 224, 336, and 560 mg dm⁻³ for the soils RP-C and RP-UC; 0, 41, 82, 164, 246, and 410 mg dm⁻³ for the soils PD-C, PD-UC, and PR-CS; 0, 30, 60, 120, 180, and 300 mg dm⁻³ for the soil PR-SS. On the same day of P application, five corn seeds were sown in each pot, and five days after seedling emergence thinning was done leaving two plants in each pot for cultivation.

The other nutrients were applied as nutrient solution, according to modifications made in the recommendation of Novais et al. (1991). The doses and sources have corresponded to: 300 mg dm⁻³ of N [(NH₂)₂CO]; 160 mg dm⁻³ of K (KCl); 80 mg dm⁻³ of S [(NH₄)₂SO₄]; 0.81 mg dm⁻³ of B (H₃BO₃); 1.33 mg dm⁻³ of Cu (CuSO₄.5H₂O); 1.55 mg dm⁻³ of Fe (FeCl₃.6H₂O); 3.66 mg dm⁻³ of Mn (MnCl₂.4H₂O); 0.15 mg dm⁻³ of Mo (NaMoO₄.2H₂O), and 4.0 mg dm⁻³ of Zn (ZnSO₄.7H₂O).

At the end of the cultivation, the maize shoot dry matter was collected, dried in a forced air circulation oven at 65 °C for 72 h, after which the shoot dry matter yield was determined. The P content in the plant tissue was determined after nitric-perchloric digestion (3:1), by molecular absorption spectrophotometry (Murphy and Riley, 1962). After the determination of P content in the plant tissue from the two plants cultivated in each pot, the maize shoot total amount of P was calculated. 100 cm³ of soil samples were taken from all

experimental units, air-dried and passed through a 2 mm sieve for analysis of soil available P by M1(Nelson et al., 1953), M3 (Mehlich, 1984), and IER (van Raij et al., 1986).

3.2.3. Data analysis

3.2.3.1. Soil phosphorus recovery rates and critical levels

The data were subjected to analysis of variance, using the program SISVAR 5.6 (Ferreira, 2011). The regression equations of the maize shoot dry matter yield were adjusted as a function of the doses of P applied to each soil. With the equations of maize shoot dry matter yield as a function of P doses, the recommended doses (RD) were calculated to reach 90 % of the maximum yield ($0.9 \hat{y}_{\max}$) (Table 3). In order to determine the RD, the first derivative was equaled to zero, and then obtaining the dose corresponding to the maximum yield. Once the value of $0.9 \hat{y}_{\max}$ was obtained, it was replaced in the same equation in order to obtain the dose corresponding to $0.9 \hat{y}_{\max}$. For the soils where the dose corresponding to the maximum yield was outside the experimental space of the doses of P applied, and for those whose quadratic root model best fitted the data, but that there was no maximum point, the highest dose of P applied in each soil was taken as the dose corresponding to the maximum yield.

The soil P recovery rates P (P_{rr}) by the soil tests corresponds to the angular coefficient of the first degree equations that relates the soil P availability as a function of the doses of P applied to the soils. When the models adjusted were equations of the second degree, we calculated the differential P_{rr} between the interval of the dose zero and the dose that corresponds to $0.9 \hat{y}_{\max}$. The calculation was performed by the following equation: $P_{rr} = (P_k - P_0)/dP_k$, where P_k is soil P content by the soil test in the dose of P that corresponds to $0.9 \hat{y}_{\max}$, P_0 is the soil P content by the soil test in the control (dose zero), and dP_k is the amount of P applied in the dose P_k . The soil P critical levels (P_{cl}) were calculated by replacing the doses of P corresponding to $0.9 \hat{y}_{\max}$ in the equations of soil P content by each soil test. With the P_{rr} and P_{cl} values, we made correlations and adjusted predictive models of P_{rr} and P_{cl} with soil characteristics related to soil P buffer capacity.

3.2.3.2. Principal component analysis

Multivariate analysis was performed using the principal component analysis method (PCA), in order to check for multiple and linear relations between soil P content by extractants, P taken up by plants, and soil P characteristics related to PBC. The PCA analysis generated a biplot plot with the functions of through FactoMineR package (Lê et al., 2008), factoextra (Kassambara and Mundt, 2017), and tidyverse (Wickham, 2017). Initially, to perform the PCA analysis the data were scaled through the base package (R Core Team, 2018). To improve the visualization of the management effect in relation to soil type, the marginal effect of the soil type was partially eliminated, so that from each variable, the overall average was subtracted (independent of the treatment in each soil) for each soil prior to PCA analysis, resulting in a partial PCA as described in Legendre and Legendre (2012).

3.3. RESULTS AND DISCUSSION

3.3.1. Soil P recovery rates

The soil P recovery rates (*Prr*) varied according to soil and its chemical and physical characteristics. On average, lowest recovery rates were obtained with M3 (*Prr*-M3 = 15 %) and the highest with IER (*Prr*-IER = 25 %), a value similar to that obtained with M1 (*Prr*-M1 = 23 %) (Table 2). In general, the relationship between the doses of P added to soil and the P content recovered by the extractants is expressed by a linear equation (Alvarez V., 1996; Westerman et al., 1990). However, for the three extractants, polynomial equations of the second degree were adjusted for some soils, mainly for IER, where this model fitted for the data of three soils. The soils in which the model of the second degree has been adjusted are characterized by low P availability (GU-UC, PR-CS, and PR-SS). This suggests that with the addition of increasing doses of P to soils with low P content, there is a sudden increase in P availability, which for the set of soils in this study, was better identified by IER.

The *Prr*-M1 and *Prr*-M3 varied according to soil PBC, as both extractant had higher recovery rates in less buffered soils (PR-SS and TA) and lower recovery rates in more buffered soils (GU and RP), regardless of P content in these soils. However, this behavior was not observed for *Prr*-IER, which suggests the low sensitivity of this extractant to soil PBC. In most studies comparing the available P content by M1 with other extractants, generally, the available P content obtained with M1 is lower, as are the *Prr*-M1 (Bortolon et al., 2009; Bortolon and Gianello, 2012; Novais et al., 2015; Valadares et al., 2017). This is owing to the

fact that in most of correlation works acidic soils with low levels of available P are used, as there is a necessity to establish response curves for P fertilization (Cantarutti et al., 2007). When M1 is used in clayey soils its extraction capacity is lower and, consequently, low P contents are obtained due to the consumption of the SO_4^- ions from the extractant, which are adsorbed on non-phosphate-occupied adsorption sites in soil, and therefore there is no anionic exchange of sulfate with phosphate (Bortolon et al., 2009; Silva and Raij, 1999). This effect can be observed due to the higher *Prr*-M1 in less buffered soils (Table 2).

The lower *Prr*-M3 in more buffered soils suggests that the same mechanism of loss of extraction capacity that occurs with M1 should also occur with M3. The M3 extractant has a more complex chemical constitution (NH_4F 15 mmol L^{-1} + CH_3COOH 200 mmol L^{-1} + NH_4NO_3 250 mmol L^{-1} + HNO_3 13 mmol L^{-1} + EDTA 1 mmol L^{-1}) than M1 (HCl 50.0 mmol L^{-1} + H_2SO_4 12.5 mmol L^{-1}). In M3, the ion F^- from NH_4F promotes anion exchange with the phosphate adsorbed to Fe and Al oxides, while NH_4^+ acts on the extraction of cations. As in M1 there is also an acid dissolution of Ca phosphates, but to a lesser extent due to pH buffering by acetic acid around 2.5 (Beegle, 2005; Bortolon et al., 2009; Mehlich, 1984). Thus, one of the factors contributing to the low *Prr*-M3 may be the adsorption of fluoride anions to soil adsorption sites that are not occupied by phosphates, similar to what happens to sulfate ions in M1. In addition, there may have been less dissolution of P-Ca forms in relation to M1 and IER since rock phosphate was added to all soils (Bayovar).

Another mechanism that promotes loss of M1 extraction capacity is the decrease of its acidity (Novais et al., 2015). It occurs when M1 is used in soils with pH around 6, and its initial pH of 1.2 (Nelson et al., 1953) is raised to pH values close to the soil pH and, thus its mechanism of extraction by acid dissolution is smaller (Freitas et al., 2013; Novais et al., 2015). However, in the set of soils of the present study, the mechanism of loss of anion exchange capacity seems to be more effective than due to the decrease in acidity, since on average, the soils have a pH above 5.5 and yet M1 had similar recovery rates as the ones obtained with IER (Table 2).

The higher *Prr*-IER can be explained by the fact that this extractant presents a higher extraction of forms of P-Ca compared to M3 and M1 (Valadares et al., 2017). The extractant initially proposed for P extraction in the State of São Paulo was composed only of anionic resin (Anionic Exchange Resin – AER) (van Raij, 1983; van Raij et al., 1986). However, in order to increase the spectrum of action and efficiency in the laboratory, the IER (cationic + anionic) was then used, and IER is also a drain for Ca^{2+} (Freitas et al., 2013; Valadares et al., 2017). However, some studies show that although there is a sensitivity of the IER to ions Ca^{2+}

in high fertilized soils or in soils amended with rock phosphate, this extractant has a good correlation with the P taken up by plants (Mumbach et al., 2018; Valadares et al., 2017). Therefore, the IER extracts only the most labile forms of P-Ca, since its extraction principle is based on P desorption to the solution with the agitation of soil : solution for 16 h and later adsorption into resin (van Raij et al., 1986).

Table 2. Equations of soil available P content by Mehlich-1 (M1), Mehlich-3 (M3), and Ion Exchange Resin (IER) ($\hat{y}$, mg dm⁻³) as function of doses of P (x , mg dm⁻³) applied in ten soil samples cultivated with maize for 45 days under greenhouse conditions, and soil P recovery rates (Prr) by each soil test

Equation		R ²	Prr-M1
Soil sample	Mehlich-1		
GU-UC ^{1/}	$\hat{y} = -1.68 + 0.1241^{***} x$	0.97	0.1241
GU-C ^{2/}	$\hat{y} = 15.11 + 0.1756^{***} x$	0.99	0.1756
TA-UC	$\hat{y} = 19.01 + 0.2928^{***} x$	0.99	0.2928
TA-C	$\hat{y} = 52.79 + 0.5337^{***} x$	0.95	0.5337
RP-UC	$\hat{y} = -3.44 + 0.1310^{**} x$	0.94	0.1310
RP-C	$\hat{y} = 81.83 + 0.1828^{**} x$	0.94	0.1828
PD-UC	$\hat{y} = -4.36 + 0.2356^{**} x$	0.94	0.2356
PD-C	$\hat{y} = 57.84 + 0.1910^{*} x$	0.71	0.1910
PR-CS ^{3/}	$\hat{y} = -2.07 + 0.2044^{***} x$	0.98	0.2044
PR-SS ^{4/}	$\hat{y} = 13.97 - 0.0024^{ns} x + 0.0013^{*} x^2$	0.98	0.2437 ^{5/}
Average			0.2315
Mehlich-3		R ²	Prr-M3
Soil sample	Mehlich-3		
GU-UC	$\hat{y} = 4.53 + 0.0201^{ns} x + 0.0001^{*} x^2$	0.99	0.0588 ^{5/}
GU-C	$\hat{y} = 17.33 + 0.0705^{***} x$	0.97	0.0705
TA-UC	$\hat{y} = 45.50 + 0.2104^{**} x$	0.92	0.2104
TA-C	$\hat{y} = 84.47 + 0.3277^{***} x$	0.98	0.3277
RP-UC	$\hat{y} = 7.02 + 0.1093^{***} x$	0.99	0.1093
RP-C	$\hat{y} = 109.30 + 0.1012^{**} x$	0.85	0.1012
PD-UC	$\hat{y} = 1.26 + 0.1861^{**} x$	0.94	0.1861
PD-C	$\hat{y} = 89.24 + 0.0984^{*} x$	0.81	0.0984
PR-CS	$\hat{y} = 5.53 + 0.1444^{***} x$	0.99	0.1444
PR-SS	$\hat{y} = 15.03 + 0.2016^{***} x$	0.98	0.2016
Average			0.1508
Ion Exchange Resin		R ²	Prr-IER
Soil sample	Ion Exchange Resin		
GU-UC	$\hat{y} = 9.59 + 0.0810^{*} x + 0.0002^{*} x^2$	0.99	0.1585 ^{5/}
GU-C	$\hat{y} = 48.35 + 0.2678^{***} x$	0.99	0.2678
TA-UC	$\hat{y} = 38.60 + 0.2948^{*} x$	0.76	0.2948
TA-C	$\hat{y} = 98.96 + 0.3857^{***} x$	0.99	0.3857
RP-UC	$\hat{y} = 27.08 + 0.3515^{***} x$	0.97	0.3515
RP-C	$\hat{y} = 300.84 + 0.1887^{*} x$	0.76	0.1887
PD-UC	$\hat{y} = 7.23 + 0.2048^{***} x$	0.97	0.2048
PD-C	$\hat{y} = 104.25 + 0.2508^{*} x$	0.80	0.2508
PR-CS	$\hat{y} = 13.39 + 0.0269^{ns} x + 0.0006^{*} x^2$	0.99	0.1402 ^{5/}
PR-SS	$\hat{y} = 13.26 + 0.3430^{**} x - 0.0005^{*} x^2$	0.99	0.2483 ^{5/}
Average			0.2491

^{1/} UC: soil samples collected in uncultivated areas. ^{2/} C: soil samples collected in cultivated areas. ^{3/} CS: clayey soil. ^{4/} SS: sandy soil. [°] 0.10 > p ≥ 0.05; ^{*} 0.05 > p ≥ 0.01; ^{**} 0.01 > p ≥ 0.001; ^{***} p < 0.001. ^{5/} Differential soil P recovery rate obtained between the dose of P = 0.0 mg/dm³ and the dose of P that corresponds to 90 % of the maximum yield (0.9 $\hat{y}_{max}$) in each soil.

The recovery rates of a nutrient in the soil determined by a certain soil test are critical variables in the calibration works, as they express the behavior of the soil test in different soil types. Equations that relate the doses of a nutrient applied to soils with the nutrient content extracted by a soil test are important because they are used in soil fertility evaluation researches to define the critical levels in the soil (Alvarez V., 1996; Cantarutti et al., 2007; Corey, 1987).

3.3.2. Soil P critical levels

The shoot dry matter yield response curves obtained as a function of P rates applied to soils varied according to initial soil P content in each soil. Except for the soils GU-C and TA-C, where the linear model was adjusted, and for RP-C in which there was no response, the quadratic and quadratic root model adjustments shows that the amplitude of the applied doses of P allowed reaching a stabilization trend in the shoot dry matter yield (Table 3). For the soils in which there was no maximum point in the adjusted curve, the highest dose of P applied was taken as the dose corresponding to the maximum yield. The point related to 90 % of maximum shoot dry matter yield ($0.9 \hat{y}_{\max}$) ranged from 2.48 g plant⁻¹ in soil GU-C to 32.50 g plant⁻¹ in GU-UC soil (Table 3). The low value obtained for DR in the soil PD-C is due to the quadratic root model adjustment, which was significant at the level of 10 % since there was almost no response to fertilization in this soil.

The lowest values of $0.9 \hat{y}_{\max}$ obtained in GU-C and TA-C soils were due to the low plant growth in this soil, even lower than plant yield obtained in their counterparts sampled in uncultivated areas (GU-UC and TA-UC) (Table 3). This fact explains the linear response obtained for shoot dry matter yield in these soils, while the expected would be a root quadratic model or no response. We raised two hypotheses for what may have occurred in these soils. The first is that micronutrient toxicity could have occurred. The micronutrient availability in all soil samples was classified as high (van Raij et al., 1997) before liming. As liming was performed prior to cultivation, a decrease in available micronutrient contents was expected, mainly for Fe and Mn. However, there was no decrease in Mn contents after liming. This fact, associated with the application of Mn via nutrient solution during the experiment, could explain the low plant growth in soils GU-C and TA-C. Nevertheless, micronutrient content, including Mn, was higher in other soils where there was good plant growth. The second hypothesis is that there may have been a remnant of herbicide in the soil, which was sampled in the field shortly after the maize crop be harvested in these two areas.

Table 3. Equations for maize shoot dry matter production ($\hat{y}$, g/plant) according to the doses of P (x , mg/dm³) applied to each soil, 90 % of the maximum shoot dry matter yield (0.9 $\hat{y}_{\max}$), and recommended doses (RD) to achieve 0.9 $\hat{y}_{\max}$

Soil	Equation	R ²	RD	0.9 $\hat{y}_{\max}$
			mg dm ⁻³	g plant ⁻¹
GU-UC	$\hat{y} = 2.76 + 0.1155^{**} x - 0.0001^{**} x^2$	0.98	387.5	32.50
GU-C	$\hat{y} = 1.06 + 0.0026^{**} x$	0.95	544.3	2.48 ^{1/}
TA-UC	$\hat{y} = 7.31 + 0.5357^{*} \sqrt{x} - 0.0034^{ns} x$	0.97	221.3	14.53 ^{1/}
TA-C	$\hat{y} = 5.39 + 0.0160^{*} x$	0.82	286.6	9.89 ^{1/}
RP-UC	$\hat{y} = 2.36 + 0.0590^{**} x - 0.000062^{*} x^2$	0.98	313.2	14.76
RP-C	$\hat{y} = \bar{y} = 19.54$	-	560.0	19.54 ^{1/}
PD-UC	$\hat{y} = 1.36 + 1.7463^{**} \sqrt{x} - 0.0395^{*} x$	0.99	214.6	18.47 ^{1/}
PD-C	$\hat{y} = 12.53 + 0.6432^{o} \sqrt{x} - 0.0255^{o} x$	0.76	20.7	14.93
PR-CS	$\hat{y} = 2.50 + 0.1143^{**} x - 0.0002^{*} x^2$	0.97	188.9	16.95
PR-SS	$\hat{y} = 1.82 + 1.3171^{**} \sqrt{x} - 0.0276^{o} x$	0.99	189.3	14.72 ^{1/}

^{ns} $p \geq 0.10$; ^o $0.10 > p \geq 0.05$; ^{*} $0.05 > p \geq 0.01$; ^{**} $0.01 > p \geq 0.001$. ^{1/} 90 % of the maximum yield considered as the highest dose of P applied to the soil.

After obtaining the RD for each soil, they were replaced in the equations for soil P recovered by each extractant (Table 2) in order to obtain the soil P critical levels (*Pcl*) (Table 4). The large variations obtained in *Pcl* values are primarily due to the different response curves obtained for plant growth and due to the variation in *Prr* of each extractant. However, *Pcl* values follow the trend of recovery rates, with higher values obtained with IER, followed by M1 and M3. The high *Pcl* obtained is due to the fact that the plants were cultivated in pots, with the application of the doses of P on the whole soil volume (2.0 dm³). In this case, there is a tendency for higher *Pcl* values than in field experiments or when fertilizer is applied in pot experiments simulating the application of P in the pit in the field (Leite et al., 2009, 2006).

Interpretation tables of soil nutrient availability are defined based on the establishment of the critical level of the nutrient in soil determined by a soil test. Based on the soil critical level value, which is defined as the soil nutrient content that provides 90 % of the maximum yield for experiments conducted under greenhouse conditions, or that provides the maximum efficient economic yield for field works, fertility classes are defined (Alvarez V., 1996). Thus, the soil nutrient content that defines the classes of availability are values that depend on the sensitivity of the extractants to the soil buffer capacity. It is also noted worthy that the definition of the classes is based on the relative yield of the crops (usually in the ranges of 50, 70 and 90 % of the maximum yield) and that these values are arbitrarily defined as there is no inflection in the fertilization response curve that justify such divisions (Cantarutti et al., 2007).

Table 4. Soil P critical level by Mehlich-1 (Pcl-M1), Mehlich-3 (Pcl-M3), and Ion Exchange Resin (Pcl-RTI) determined in ten soil samples cultivated with maize for 45 days under greenhouse conditions. Average, standard deviation, and coefficient of variation (CV) of P critical levels obtained by each soil test

Soil	Pcl-M1	Pcl-M3	Pcl-RTI
	----- mg/dm ³ -----		
GU-UC ¹	46.4	27.3	74.2
GU-C ²	110.7	55.7	194.1
TA-UC	83.8	92.1	103.8
TA-C	205.7	178.4	209.5
RP-UC	37.6	41.3	137.2
RP-C	184.2	166.0	406.5
PD-UC	46.2	41.2	51.2
PD-C	61.8	91.3	109.5
PR-CS ^{3/}	36.5	32.8	39.9
PR-SS ^{4/}	60.1	53.2	70.3
Average	87.3	77.9	139.6
standard deviation	61.3	54.4	109.6
CV (%)	70.2	69.8	78.5

^{1/} UC: soil samples collected in uncultivated areas. ^{2/} C: soil samples collected in cultivated areas. ^{3/} CS: clayey. ^{4/} SS: sandy soil.

3.3.3. Soil phosphorus maximum adsorption capacity

The determination of soil maximum phosphorus adsorption capacity (MPAC) is important because it provides an estimate of the magnitude of the soil sorption complex and assists in the decision making of important management practices such as phosphate fertilizer (Barrow, 2015; Oliveira et al., 2014). The non-linear model of the Langmuir isotherm is the most common used as it enables to estimate the MPAC of soils and to get an idea of the soil P binding energy (Novais and Smyth, 1999). There was a good fit for the non-linear model of Langmuir isotherm ($R^2 > 0.90$) for all soils with the addition of 11 P levels, as recommended by Alvarez V et al. (2000) (Figure 2). The estimated MPAC values were consistent with the soil PBC, estimated by clay content and P-rem. The MPAC ranged from 158.2 mg kg⁻¹ in the soil PR-SS to 1972.3 mg kg⁻¹ in GU-C, which are, respectively, the least and most buffered soils.

Although there was a good fit of the non-linear model, it is evident that for some soils another model could fit better, as in the case of the soil TA-UC (Figure 2, c). This is one of the problems of using the non-linear model of Langmuir Isotherm and it is associated with the fact that the mathematical model must have its origin at zero and must reach a maximum adsorption point (Barrow, 2008; Oliveira et al., 2014). However, several researches show the effectiveness of estimating the MPAC of soils using the non-linear model of Langmuir isotherm in soils with different physical, chemical, and mineralogical characteristics (Barrow, 2015; Novais and Smyth, 1999; Oliveira et al., 2014; Valladares et al., 2003).

Figure 1. Maximum P adsorption capacity (MPAC) of soil samples collected in cultivated (B, D, F, H), uncultivated (A, C, E, G), and fallow areas (I, J). UC = uncultivated, C = cultivated, CS = clayey soil, SS = sandy soil.

Unlike MPAC, the values of the constant related to the soil P binding energy were not consistent with the PBC of the soils. The soil P binding energy values ranged from 0.1480 in the soil RP-C to 0.4040 in the soil RP-UC (Figure 2). There was a tendency to obtain lower binding energy values in cultivated soils, which was expected since the high doses of P applied over the years occupy a large amount of adsorption sites in these soils.

The mineralogical constitution of soils is formed by Kaolinite, Gibbsite, Goethite, and Hematite. The only particularities correspond to the presence of Interlayered Aluminum Hydroxyl in the soils from GU and TA and a prominent peak that corresponds to Gibbsite in the soils from RP, which may account for the higher binding energy found in this soil. The adjustment of the Langmuir isotherm in its non-linear form is based on the basic principles that adsorption occurs in a single layer, and that the adsorption energy is constant and does not vary with the amount adsorbed (Barrow, 2008; Novais and Smyth, 1999). However, some studies show that the soil P binding energy is not constant but varies during the adsorption process (Oliveira et al., 2014). Thus, some authors suggest that the estimation of the binding energy should be obtained from linearized Langmuir isotherm models, in which two or more adsorption regions are obtained (Alvarez and Fonseca, 1990; Wang and Li, 2010). Therefore, the non-linear model is suitable for estimating soil MPAC, but the estimation of the binding energy is more accurate when using linear models as suggested by Oliveira et al. (2014).

3.3.4. Influence of soil phosphate buffering capacity on phosphorus availability

Principal component analysis (PCA) was used to simultaneously examine relationships among variables related to soil tests behavior and soil attributes related to PBC. To improve the visualization of the management effect, cultivated vs uncultivated soils, in relation to soil type, the marginal effect of the soil type was partially eliminated, resulting in a partial PCA (Legendre and Legendre, 2012). The PCA analysis allowed to verify the trend of multivariate relationship between the soils and the considered attributes. The soil attributes investigated explained 68.9 % of the total variability of the data (Figure). Data were separated into four groups, in which there was a clear separation between the soils collected in cultivated areas and the soils collected in uncultivated areas. Regarding the soil chemical attributes, as expected, uncultivated soils were correlated with higher levels of SOM, CEC and Al^{3+} , while cultivated soils correlate with higher Ca^{2+} contents, higher pH and BS values.

The *Prr*-M1 and *Prr*-M3 were grouped with PR-SS, less buffered, and cultivated soils, where soil P content is higher. The grouping of P-rem together with *Prr*-M1 and *Prr*-M3

corroborates that these extractants have greater extraction capacity in soils less buffered, with higher P-rem values. The Prr-IER did not show any association with the soil attributes related to soil PBC. The variable a , related to the soil P binding energy, and MPAC are highly associated since they are generated from the same equation and were grouped together with the soil RP-C, in which the highest binding energy value was obtained. As discussed earlier, this must be due to the higher gibbsite content in this soil, which explains the segregation of these soils from the others.

Figure 3. Principal component analysis between soil characteristics related to soil phosphate buffering capacity, soil P availability, soil P recovery rates (Prr), and P critical level (Pcl) in ten soil samples collected in cultivated (C) and uncultivated (UC) areas. Soil P availability was determined by Mehlich-1 (P-M1), Mehlich-3 (P-M3), Ion Exchange Resin (P-IER) extractants, and by shoot dry matter yield (SDM), shoot P content (SPC), and shoot total amount of P (STA of P) of maize plants cultivated for 45 days under greenhouse conditions. Soil characteristics related to soil phosphate buffering capacity: pH-H₂O, Al³⁺, Ca²⁺, BS = Base saturation BS, SOM = soil organic matter, CEC = cation exchange capacity, clay content, MPAC = soil maximum phosphorus adsorption capacity, a = soil phosphorus binding energy. The soil samples were identified according to the municipalities of collection in Brazil: GU = Guarapuava; TA = Taquaritiba; RP = Rio Paranaíba; PD = Piedade; PR = Piracicaba).

The soil *Pcl* values of the three extractants were very associated, due to the magnitude of the values (Table 4) and were associated with all plant yield indices, since they are variables dependent on the adjustment of the plant yield equations. Plant yield indices were associated with the soil RP-C because this soil had the highest plant production, (Table 3), as well as the highest soil P contents by the extractants (Table 2).

Correlation between soil P recovery rates by the extractants and soil attributes related to soil PBC were established. Mehlich-3 was the most sensitive to soil PBC, correlating with clay content, P-rem, and MPAC. The M1 extractant correlated only with the clay content, and there was no correlation between IER and the selected attributes (Table 5). Negative correlations show that there was a sensitivity of M3 to soil PBC, that is, in more buffered soils the *Prr*-M3 is lower. Positive correlation coefficients with P-rem are due to the fact that in more buffered, the P-rem is lower (Alvarez V et al., 2000).

Table 5. Correlation coefficients between soil phosphorus recovery rates (P-rr) by Mehlich-1 (*Prr*-M1), Mehlich-3 (*Prr*-M3), and Ion Exchange Resin (*Prr*-IER) and soil attributes related with soil phosphorus buffering capacity

	Clay	P-rem	SOM	H + Al	MPAC
<i>Prr</i> -M1	- 0.52 ^{ns}	0.67*	- 0.44 ^{ns}	- 0.49 ^{ns}	- 0.58 ^{ns}
<i>Prr</i> -M3	- 0.69*	0.80*	- 0.48 ^{ns}	- 0.50 ^{ns}	- 0.75*
<i>Prr</i> -IER	- 0.25 ^{ns}	0.24 ^{ns}	- 0.29 ^{ns}	- 0.10 ^{ns}	- 0.10 ^{ns}

P-rem: remaining phosphorus; SOM: soil organic matter; H + Al: potential acidity (determined at pH 7.0); MPAC: soil maximum phosphorus adsorption capacity. ^{ns} $p \geq 0.5$; * $p \leq 0.05$.

The higher correlation coefficients obtained between *Prr*-M3 and P-rem and MPAC than with soil clay content is because P-rem and MPAC are two measures which, besides reflecting soil clay content, also indicate, indirectly, the quality of the clay, if it has more or less phosphate adsorption sites (Alvarez and Fonseca, 1990; Alvarez V et al., 2000; Rogeri et al., 2016). Since P-rem and clay content are routinely performed in the laboratories of soil analysis, equations that relate *Prr*-M3 with these two attributes were established (Figure 4). The low coefficients of determination are due to the high *Prr* obtained in the soil TA-C (~ 32 %) (Table 2), which is less buffered and has high P availability (Table 1).

The tables of interpretation of soil P availability by the M1 take into account a measure of soil buffering capacity, clay content or P-rem (Ribeiro et al., 1999; Teixeira et al., 2017), what is not necessary for the interpretation of the results obtained with IER (van Raij et al., 2001, 1997). Due to the sensitivity of the M3 to soil PBC, for its routinely use in soil analysis laboratory in Brazil will be necessary that the interpretation tables of its results take into consideration a stratification of the soil P availability according to soil PBC, preferably using P-rem.

Figure 4. Correlation between soil phosphorus recovery rates by Mehlich-3 extractant (*Prr*-M3) and soil clay content (A) and remaining phosphorus (P-rem) (B) in ten soil samples with contrasting soil P content and buffering capacity.

The sensitivity of the M3 extractant to soil PBC has been proven to greater or lesser intensity under different soil conditions (Bortolon and Gianello, 2012, 2008; Mumbach et al., 2018; Simões Neto et al., 2009). In experiments under field conditions in soils cultivated with

sugarcane in Pernambuco State, the *Prr*-M3 were more correlated with MPAC (0.86*) than with P-rem (0.83*), and there was no correlation between *Prr*-IER and the soil attributes studied (clay, P-rem, and MPAC) (Simões Neto et al., 2009). Similar results were also obtained from P extraction in 301 soil samples from Santa Catarina State, where M3 extraction capacity was higher than M1, but both were sensitive to soil clay content (Mumbach et al., 2018). The use of P-rem instead of soil clay content as a measure of soil PBC is also recommended for the interpretation of P availability in soils of Rio Grande do Sul State if M1 and M3 extractants are used (Rogeri et al., 2016). These results are common in tropical soils, however, the use of soil PBC indices for the interpretation of P availability by M3 in temperate soils is not necessary (Herlihy and McCarthy, 2006). Thus the higher or lower sensitivity of M3 to PBC is dependent on the chemical, physical and mineralogical characteristics of the studied soils, but for Brazilian soils conditions it is evident the need to take into account the PBC for interpretation of soil P availability by M3.

3.4. CONCLUSIONS

Ion Exchange Resin presented higher phosphorus extraction capacity than Mehlich-1 and Mehlich-3 extractants, regardless of soil chemical characteristics.

Soil samples collected in cultivated areas showed lower phosphorus binding energy to soil and this binding energy is more related to mineralogy than to clay content.

The extractants Mehlich-1 and Mehlich-3 were sensitive to soil phosphorus buffering capacity. Thus, the use of the Mehlich-3 extractant in tropical soils is dependent on an interpretation table that stratifies the values based on a measure of soil phosphate buffer capacity, preferably the remaining phosphorus (P-rem).

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4. FATE OF PHOSPHORUS DETERMINED BY CHEMICAL FRACTIONATION AND P K-EDGE XANES IN LONG-TERM CULTIVATED BRAZILIAN SOILS

ABSTRACT

The chemical fractionation of soil P is one of the main analyzes used to obtain information about the solubility of forms of P and their availability to plants. However, the fractionation methods have some limitations, including do not determine the soil P species. The use of the X-ray absorption near-edge structure (XANES) technique has generated important information about the P speciation in soil and environmental samples. Thus, Our objectives were to evaluate how P speciation determined by P K-edge XANES relates to pools of P determined by sequential chemical fractionation in long-term high fertilized tropical soils and how these pieces of informations helps to understand the fate of P applied in soil over decades. Ten soil samples were collected at the 0.0 – 0.20 m layer, from cultivated and uncultivated areas. In addition to chemical, physical and mineralogical characterization of soil samples, a bioavailability experiment was carried out under greenhouse conditions. The experimental units consisted of plastic pots with 2.0 dm³ of soil where two maize plants (*Zea mays* L.) were cultivated for 45 days. After cultivation, the shoot dry matter yield, P accumulation by plants, and soil P contents by Mehlich-1, Mehlich-3 and Ion Exchange Resin extractants were determined. In the original soil samples, we have determined the soil P pools by chemical fractionation and soil P species by P K-edge XANES. After two decades of soil cultivation, there was evident increase mainly in the moderately labile and non-labile P fractions. This suggests the accumulation of a large amount of P in the soil, contributing to the legacy P, which may play an important role in buffering the most labile pools in the medium term. There was no increase in P-Ca forms due to successive applications of liming and phosphate fertilizers in cultivated soils. Thus, the labile and moderately labile forms of P are mainly adsorbed onto P Fe and Al oxides (Hematite, Goethite, and Gibbsite).

Keywords: Soil P speciation, Hedley's fractionation, sequential chemical fractionation

4.1. INTRODUCTION

The highly weathered and acidic soils in Brazil are inherently infertile but respond well to fertilization and liming, supporting high agricultural production for decades (Lopes and Guilherme, 2016). Much of the development of Brazilian agriculture production is based on research on the dynamics and availability of P in tropical soils, since this nutrient is the

most limiting for agricultural production in the tropics (Liu et al., 2018; Novais and Smyth, 1999). Modification in soil management practices and land-use is expected to change soil P dynamics and alter soil physical, chemical and biological properties, and thus the chemical nature soil P species that governs their bioavailability (Abdala et al., 2018; Boitt et al., 2018; Liu et al., 2018).

The chemical fractionation of soil P is one of the main analyzes used to obtain information about the solubility of the different forms of P in and their availability to plants (Condrón et al., 1985; Condrón and Newman, 2011). Although, the fractionation methods have some limitations related to be operationally defined, complex, time consuming, there is hipper-manipulation of samples and the methods do not determine the soil P species, several methods with different modifications have been proposed over the years (Condrón and Newman, 2011; Cross and Schlesinger, 1995; Gatiboni et al., 2013). Soil chemical fractionation techniques are especially important when comparing the influence of soil management practices on P lability (Cherubin et al., 2016; Gatiboni et al., 2013; Pavinato et al., 2009; Rodrigues et al., 2016; Teles et al., 2017).

In order to investigate the dynamics and bioavailability of soil P, it is necessary to proceed the separation and identification of the different forms of soil P. It occurs because soil phosphorus dynamics is determined by the complex interactions between inorganic, organic and microbial forms of P (Condrón et al., 1985; Hesterberg, 2010; Oberson and Joner, 2005). One of the ways to overcome the operational definitions of the fractionation methods is to validating links between extraction and reactivity of each fraction (Condrón and Newman, 2011; Liu et al., 2013). It can be done by the knowledge of which P species are present in the sample.

Soil P speciation refers to the specific chemical forms P in a soil sample, that include minerals, organic, adsorbed and precipitated forms (Hesterberg, 2010). The use of the X-ray absorption near-edge structure (XANES) technique has generated important information about the P adsorption in oxides and silicated clays in relation to the structure, bond geometry, presence of multinuclear complexes and precipitated phases, although detection sensitivity is a problem (Hesterberg, 2010; Kruse et al., 2015). In this case, P species are determined by comparing spectral characteristics of a sample with standard spectra, which present unique spectral fingerprints, with the advantage of requiring minimal or no sample pretreatments (Hesterberg et al., 1999; Kelly et al., 2008). Therefore, only by using P K-edge XANES we can ensure that the P adsorbed in soil minerals will not change its form as can occurred in chemical extractions (Cade-Menun et al., 2018; Condrón and Newman, 2011).

Our hypothesis is that successive applications of liming and phosphate fertilizers in tropical soils increase the proportion of P-Ca forms, and thus increase the labile fractions of P in these soils. Our objectives were to evaluate how P speciation determined by P K-edge XANES spectroscopy relates to pools of P determined by sequential chemical fractionation in long-term high fertilized tropical soils and how these pieces of information helps to understand the fate of P applied in soil over decades.

4.2. MATERIAL AND METHODS

4.2.1. Collection and characterization of soil samples

Ten soil samples were collected at the 0.0 – 0.20 m layer, from cultivated and uncultivated areas in November 2016 at the beginning of the raining season in all study areas. We selected four areas cultivated by at least 20 years with agricultural crops that demand high doses of phosphate fertilizers. Next to each cultivated area, in the same condition of topography and soil class, more four soil samples were collected from native uncultivated areas, as reference (Figure 1). The soil samples RP-UC and RP-C were collected in the Cerrado biome and all the other soil samples were collected in the Atlantic Forest biome. The main crop grown in the soils GU-C, TA-C, and RP-C is potato, in succession with soybean or corn, and with wheat and oats as winter crops. In the soil PD-C, the main crop is maize and tomato, without winter crop cultivation. In all cultivated areas, cultivation has been going on for over 20 years with high applications of P and liming, mainly for potato and tomato crop requirements.

After sampling, the soil samples were air-dried, passed through a 2.0 mm sieve (Air-Dried Soil Sample - ADSS), and stored for chemical and physical characterization (Table 1). The analysis for chemical characterization was performed according to van Raij et al. (2001). The soil available P content was extracted with Mehlich-1 (Nelson et al., 1953), Mehlich-3 (Mehlich, 1984), and Ion Exchange Resin (van Raij et al., 1986) soil tests. The remaining P (P-rem) was determined according to Alvarez V. et al. (2000). The granulometric analysis was performed by chemical dispersion in NaOH 0.1 mol L⁻¹, and the clay content determined by the pipette method (Donagemma et al., 2011). To determine the maximum P adsorption capacity, 2.5 g of ADSS were shaken with 25 mL of CaCl₂·2H₂O 0.01 mol.L⁻¹ containing 11 rates of P, as KH₂PO₄ (p.a.) during 24 h. Then, the samples were centrifuged for 15 min at 3,000 rpm and filtered. The P concentration in the filtered was determined by colorimetry

(Murphy and Riley, 1962). The rates of P varied according to the rem-P of each soil and corresponded to the levels 0.00; 0.05; 0.10; 0.15; 0.25; 0.35; 0.45; 0.55; 0.70; 0.85, and 1.00 (Alvarez V et al., 2000). The MPAC was estimated by fitting the data of the rates of P and P concentration in the filtered to the Langmuir equation, described as: $q = a \times b \times c / 1 + (a \times c)$; in which c is the P concentration in the equilibrium solution (mg L^{-1}), q is the amount of P adsorbed to the soil (mg kg^{-1}), a is a constant related to the P binding energy to soil (L mg^{-1}), and b is the soil MPAC (mg kg^{-1}).

Table 1. Selected soil chemical and physical characteristics of soil samples collected in cultivated and uncultivated areas

Characteristics	Soil sample									
	GU-UC ¹	GU-C ²	TA-UC	TA-C	RP-UC	RP-C	PD-UC	PD-C	PR-CS ³	PR-SS ⁴
pH-H ₂ O	4.5	5.5	5.5	7.0	5.8	5.9	4.6	6.0	4.9	5.5
SOM (g dm^{-3}) ⁵	69	50	27	32	41	37	50	27	32	37
P-rem (mg L^{-1}) ⁶	2.2	3.4	40.1	35.9	4.6	10.5	27.0	26.1	19.8	42.8
P-M1 (mg dm^{-3}) ⁷	4	22	25	73	4	114	3	60	2	6
P-IER (mg dm^{-3}) ⁸	6	30	39	123	24	141	18	123	11	13
P-M3 (mg dm^{-3}) ⁹	1	12	38	84	6	110	6	78	2	7
Total P (mg kg^{-1}) ¹⁰	1899	2396	506	940	2185	3927	601	1466	933	466
K ⁺ ($\text{mmol}_c \text{ dm}^{-3}$)	2.8	3.5	2.1	3.7	2.5	3.0	2.3	3.5	2.0	1.6
Ca ²⁺ ($\text{mmol}_c \text{ dm}^{-3}$)	23	44	26	39	26	32	21	30	12	41
Mg ²⁺ ($\text{mmol}_c \text{ dm}^{-3}$)	17	14	13	20	10	8	5	14	6	11
Al ³⁺ ($\text{mmol}_c \text{ dm}^{-3}$)	28	4	2	0	1	1	13	1	12	3
CEC ($\text{mmol}_c \text{ dm}^{-3}$) ¹¹	228	134	83	81	86	85	126	82	84	76
BS (%) ¹²	19	46	49	78	45	51	22	58	24	44
MPAC (mg kg^{-1}) ¹³	1820	1972	186	311	1404	1156	325	467	572	158
a (L mg^{-1}) ¹⁴	0.19	0.18	0.24	0.16	0.40	0.15	0.30	0.19	0.18	0.38
Clay (g kg^{-1})	487	599	270	330	485	469	340	339	489	247
Sand (g kg^{-1})	500	70	680	640	340	370	600	530	330	730

¹/UC: soil samples collected in uncultivated areas. ²/C: soil samples collected in cultivated areas. ³/CS clayey soil. ⁴/SS sandy soil. ⁵/Soil organic matter (Walkley and Black, 1934) ($\text{C. Org} \times 1.724$). ⁶/Remaining P (Alvarez V et al., 2000). Soil P content by ⁷/Mehlich-1, ⁸/Ion Exchange Resin, and ⁹/Mehlich-3. ¹⁰/Digestion with HNO₃ conc. + HF conc. + HClO₄ conc. ¹¹/Cation exchange capacity [$\text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+} + (\text{H} + \text{Al})$]. ¹²/Base saturation [$(\text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+})/\text{CEC} \times 100$]. ¹³/Soil Maximum P adsorption Capacity (Langmuir Isoterm). ¹⁴/constant related to the P binding energy to soil.

The mineralogical analysis was performed in the clay fraction separated by sedimentation, according to Teixeira et al. (2017). Oriented slides were prepared with natural clay. The Mineralogical analysis was performed by X-ray diffraction using Co K α as a source of X-ray in the interval between 4 – 50 °2 θ , at 0.02 °2 θ intervals at 1 step s⁻¹, with 40 kV voltage and 30 mA current. For peak identification we used the program "Raios-X simplificado" available for download at (http://www.dps.ufv.br/?page_id=742) (Figure 2).

Figure 1. Soil sampling sites detailing the location of soil collected in cultivated and uncultivated areas. In the soil samples code the first two letters represents the municipalities of sampling (GU: Guarapuava; TA: Taquaritiba; RP: Rio Paranaíba; PD: Piedade; PR: Piracicaba), and the other letters represents the samples characteristics (UC: soil sample collected in uncultivated areas; C: soil samples collected in cultivated areas; CS clayey soil; SS sandy soil).

Figure 2. X-ray diffractograms of oriented clay fraction of soil samples collected in uncultivated (UC) and cultivated (C) areas. VIH: Vermiculite with Interlayered Aluminum Hydroxyl. Kt: Kaolinite. Gb: Gibbsite. Gt: Goethite. Qz: Quartz. Hm: Hematite. At: Anatase. The soil samples were identified according to the municipalities collection in Brazil (GU = Guarapuava; TA = Taquarituba; RP = Rio Paranaíba; PD = Piedade; PR = Piracicaba).

4.2.2. Bioavailability experiment

The bioavailability experiment was carried out under greenhouse conditions. The experimental units consisted of plastic pots with 2.0 dm³ of soil where two maize plants (*Zea mays* L.) were cultivated for 45 days. The soil samples were air-dried, passed through a 4.0 mm sieve, and the volume corresponding to 2.0 dm³ of each soil sample was weighed and packed in plastic bags in order to apply the recommended doses of lime, based on Soil Base Saturation Method (van Raij et al., 2001). The lime was composed of a mixture of CaCO₃ (p.a.) and MgCO₃ (p.a.) in the proportion of 4:1 (mol of Ca : mol of Mg). The amount of the mixture applied in each soil was: GU-UC: 6.0 g pot⁻¹; GU-UC: 0.52 g pot⁻¹; TA-UC: 1.69 g pot⁻¹; RP-UC: 2.09 g pot⁻¹; PR-C: 1.57 g pot⁻¹; PD-UC: 3.42 g pot⁻¹; PD-C: 0.95 g pot⁻¹; PR-CS: 3.75 g pot⁻¹; PR-SS: 1.92 g pot⁻¹; there was no need to apply lime in the soil sample TA-C. Thereafter, the samples were moistened up to 80 % of the maximum water retention capacity and incubated for 15 days. After incubation, the soil samples were air-dried and, again passed through a 4.0 mm sieve. Thereafter, soil P content was determined by Mehlich-1 (Nelson et al., 1953), Mehlich-3 (Mehlich, 1984), and Ion Exchange Resin (van Raij et al., 1986) extractants.

There was no P application and the other nutrients were applied as nutrient solution, according to the recommendation of Novais et al. (1991) with modifications. The doses and sources have corresponded to: 300 mg dm⁻³ of N [(NH₂)₂CO]; 160 mg dm⁻³ of K (KCl); 80 mg dm⁻³ of S [(NH₄)₂SO₄]; 0.81 mg dm⁻³ of B (H₃BO₃); 1.33 mg dm⁻³ of Cu (CuSO₄.5H₂O); 1.55 mg dm⁻³ of Fe (FeCl₃.6H₂O); 3.66 mg dm⁻³ of Mn (MnCl₂.4H₂O); 0.15 mg dm⁻³ of Mo (NaMoO₄.2H₂O), and 4.0 mg dm⁻³ of Zn (ZnSO₄.7H₂O).

At the end of cultivation, the maize shoot dry matter was collected, dried in a forced air circulation oven at 65 °C for 72 h, after which the shoot dry matter yield was determined. The P content in the plant tissue was determined by molecular absorption spectrophotometry (Murphy and Riley, 1962) after digestion in nitric-perchloric acid (3:1). After the determination of P content in the plant tissue from the two plants cultivated in each pot, the maize shoot total amount of P was calculated.

4.2.3. Fractionation of soil phosphorus

The sequential soil P fractionation was performed according to the methodology proposed by Hedley et al. (1982), modified by Condron et al. (1985), which is the most

commonly used methodology in tropical soils (Gatiboni et al., 2013; Rocha et al., 2019; Rodrigues et al., 2016; Teles et al., 2017). Sequentially, the following extractions were performed: (i) Anionic Exchange Resin (P_{AER}), (ii) $0.5 \text{ mol L}^{-1} \text{ NaHCO}_3$ at pH 8.5 (P_{BIC}), (iii) $0.1 \text{ mol L}^{-1} \text{ NaOH}$ ($P_{NaOH \text{ I}}$), (iv) $1.0 \text{ mol L}^{-1} \text{ HCl}$ (P_{HCl}), $0.5 \text{ mol L}^{-1} \text{ NaOH}$ ($P_{NaOH \text{ II}}$), and residual P ($P_{residual}$). The extractions were performed in a 15-mL centrifuge tube containing 0.5 g of ADSS. To determine P_{AER} , 10 mL of water and an anionic exchange resin membrane with 2 cm^2 saturated with NaHCO_3 0.5 mol L^{-1} were added to the centrifuge tube. Then, the tubes were shaken in a vertical agitator end-over-end (33 rpm) for 16 h at 25°C . After this period, the resin was removed from the tube and transferred to another 15 mL centrifuge tube containing HCl 0.5 mol L^{-1} solution (extractant), allowed to stand for 90 min, and then shaken for 30 min for P extraction. The P content (P_{AER}) was determined in the supernatant by molecular absorption spectroscopy according to Murphy and Riley (1962), after being centrifuged at 4,000 rpm for 20 min.

After P_{AER} extraction, the subsequent extractions were performed by adding 10 mL of each extractant solution, then shaken in a vertical agitator end-over-end (33 rpm) for 16 h at 25°C , and thereafter the suspensions were centrifuged at 4,000 rpm for 20 min, and the clear supernatants were collected for P determination. An aliquot of the alkali extracts were digested with H_2SO_4 and $(\text{NH}_4)_2\text{S}_2\text{O}_8$ in an autoclave at 121°C for total P determination. To determine the $P_{residual}$, the remaining soil in the centrifuge tube was dried at 50°C , milled and digested with H_2SO_4 conc. + H_2O_2 conc. in the presence of saturated MgCl_2 (Olsen and Sommers, 1982). After each extraction, the soil remnant was washed with NaCl 0.5 mol L^{-1} in order to remove debris from the anterior extractant. Inorganic P in alkali extracts (P_{iBIC} , $P_{iNaOH \text{ I}}$ and $P_{iNaOH \text{ II}}$) was determined by molecular absorption spectrophotometry according to Dick and Tabatabai (1977). Inorganic P obtained in acid extracts (P_{AER} and P_{iHCl}) and obtained after digestion of the alkali extracts were determined by molecular absorption spectrophotometry according to Murphy and Riley (1962). The organic P fractions (P_{oBIC} , $P_{oNaOH \text{ I}}$, and $P_{oNaOH \text{ II}}$) were calculated by the difference between total P and inorganic P in each extract. Thus, nine P fractions were obtained: P_{AER} , P_{iBIC} , P_{oBIC} , $P_{iNaOH \text{ I}}$, $P_{oNaOH \text{ I}}$, P_{iHCl} , $P_{iNaOH \text{ II}}$, $P_{oNaOH \text{ II}}$, and $P_{residual}$.

4.2.4. Phosphorus K-edge XANES

The XANES spectra for standards and soil samples were acquired at Beamline SXS (Soft X-ray Spectroscopy) of the Brazilian Synchrotron Light Laboratory (LNLS), Campinas-

SP, Brazil. The ADSS and reference standards were macerated in agate mortar and passed through a 125 mesh sieve. Thereafter, some samples were diluted in boron nitride to yield a P concentration of $\sim 0.10\%$ in order to decrease the self-absorption effect. The samples were uniformly spread onto a double-side carbon P-free tape in thin layer, which was mounted to a sample holder that was inserted into the experimental chamber under vacuum. The sample holder was placed in a 45° angle in relation to incident monochromatic beam, so the P K-edge XANES data were collected in fluorescence mode using a Si (111) double crystal monochromator. During the data acquisition, the experimental chamber was manually moved in and out in relation to the incident X-ray beam in order to maintain the detector dead time below 8% .

The storage ring operating during the data acquisition had an energy of 1.37 GeV and a current range of 110 - 250 mA. A reference energy (E^0) of 2150.7 eV was used for beamline's energy calibration, with a calcium phosphate standard (Bayóvar phosphate rock), and scans were collected in energy ranging from 2120 to 2300 eV. For soil samples and standards, P K-edge XANES data were collected with accumulation time of 1.0 s per data point, with varying step sizes of 1.0 eV from 2120.0 to 2145.0 eV, 0.2 eV from 2145.2 to 2180.0 eV, 1.0 eV from 2181.0 to 2220.0 eV, and 3.0 eV from 2223.0 eV to 2350.0 eV. Each spectrum was obtained by merging between 13 to 18 individual scans for soil samples, and merging five individual scans for standards.

The reference standards analyzed were Hydroxyapatite, Phytic acid, Strengite, Brushite, Monetite, Tricalcium Phosphate and Bayóvar obtained from commercial suppliers. The sorbed P standards were P sorbed onto Goethite (P-Goethite), Hematite (P-Hematite), Caulinite (P-Caulinite), Gibbsite (P-Gibbsite), and Calcite (P-Calcite), that was obtained by sorbing K_2HPO_4 salt to mineral analogs of commonly found clay minerals and Al and Fe oxides in soils as describes in Abdala et al (2015) and Hesterberg et al. (1999).

4.2.5. Data analysis

Data analysis of the bioavailability experiment, with plant growth and P absorbed and accumulated by plants, and the sequential soil P fractionation were performed using the R software (R Core Team, 2018). Comparisons made between data from the same soil collected in cultivated or uncultivated areas for each fraction analyzed were compared by the *t*-test ($p < 0.05$). Initially, the variances of the treatments were compared for their homogeneity by the *F*-test ($p < 0.05$). According to the presence of homogeneity or heteroscedasticity, the *F*-test was

performed. For the above comparison, the stats package of R program was used (R Core Team, 2018).

The analysis of the spectra obtained of P K-edge XANES was performed by linear combination fitting (LCF) using the program Athena in Demeter software package (Ravel and Newville, 2005). The normalization was performed after the data calibration by finding the zero crossings in the first deviation. For normalization we used a variable interval of energy range of $E^0 - 10$ to $E^0 + 30$ eV. LCF was performed by using possible binary, ternary, and quaternary combinations of the selected standards after iteratively use all standards and eliminated those that yielded fit as negative values (Werner and Prietzel, 2015). The E^0 was allowed to vary and the weights of the selected standards in use during the iterative methods were forced to sum one. The standards were defined according to R values.

4.3. RESULTS AND DISCUSSION

4.3.1. Soil attributes and P availability

There were pronounced differences between the chemical attributes of soils collected in cultivated areas in relation to their counterparts collected in uncultivated areas (Table 1). The average pH of soils collected in cultivated areas was 5.6, while the pH of soils collected in uncultivated areas was 6.1, which is the result of successive applications of limestone for crop production over the years. This also reflected in higher nutrient content, higher base saturation, and lower Al^{3+} content in cultivated soils. Except for the soil TA, in which there was a small increase in soil organic matter (SOM) content with cultivation, for the other soils, the cultivation promoted a decrease in SOM. Although cultivation practices caused an increase in soil pH and base saturation, there was a tendency to decrease soil CEC with cultivation, which indicates that SOM is an important component for charge generation in these soils and play an important role on soil P availability (Fink et al., 2016) (Table 1). The soils PR-CS and PR-SS collected in fallow areas have chemical properties typical from tropical soil with low nutrient content and high acidity (Cherubin et al., 2016; Lopes and Guilherme, 2016), although PR-SS soil has a higher base saturation.

Regarding the attributes that reflect the status of P in soils, cultivation increased soil P contents, both total P and available P determined by Mehlich-1, Melhlich-3, and Ion Exchange Resin extractants. Very high values of total and available P were obtained in the soil RP-C. The soils GU and TA presented the highest clay content and, consequently, the

highest maximum phosphate adsorption capacity (MPAC) (Table 1). Although there was a tendency to decrease the constant related to P binding energy (a) in soil with cultivation, this value was not very consistent with soil clay content and MPAC. This may be due to the fact that the non-linear fit of the Langmuir Isotherm can generate values of a which correspond to an average of the values that would be obtained when the isotherm is linearly adjusted, when regions with different binding energies are obtained (Barrow, 2008; Novais and Smyth, 1999; Oliveira et al., 2014). Therefore, it is necessary to take this into account when interpreting soil P binding energy from data obtained by isotherm adjustments.

In addition to the soil clay content, the type of clay is crucial for determining soil P adsorption (Fontes and Weed, 1996). Except for the soil RP, which has the most expressive peaks of gibbsite (Gb), kaolinite (kt), is the predominant clay mineral presented in the soils (Figure 2). The mineralogy of soils GU and TA are similar and, besides the presence of Kt, Gb, Goethite (Gt), and Hematite (Hm + Gt), these soils still have Vermiculite with Interlayered Aluminum Hydroxyl (HIV). The mineralogical constitution of the clay fraction of soil from PD is the simplest, consisting of Kt, Gb, and Gt. The lability and speciation models for P in tropical soils show that P adsorbed onto or coprecipitated with Al and Fe oxides are the forms less labile whereas the Ca phosphate (monenite and octacalcium phosphates) are more labile (Hesterberg, 2010). Therefore, due to the predominance of Fe and Al oxides in soils, it is possible to expect low P content in labile fractions, unless liming promotes precipitation of more soluble calcium phosphates in cultivated soils.

There was no plant growth compatible with soil P availability in the soils GU-C and TA-C (Table 2). The micronutrient availability in all soil samples was classified as high (van Raij et al., 1997) before liming. As liming was performed prior to cultivation, a decrease in available micronutrient contents was expected, mainly for Fe and Mn. However, there was no decrease in Mn contents after liming. This fact, associated with the application of Mn via nutrient solution during the experiment, could explain the low plant growth in soils GU-C and TA-C. Nevertheless, micronutrient content, including Mn, was higher in other soils where there was good plant growth. Other hypothesis is that there may have been a remnant of herbicide in the soil, which was sampled in the field shortly after the maize crop be harvested in these two areas. For the other soils there was higher shoot dry matter yield, shoot P content and shoot total amount of P in samples collected in cultivated areas.

Table 2. Maize shoot dry matter yield, shoot P content, and shoot total amount of P after 45 days of cultivation under greenhouse conditions

Soil	Shoot dry matter	Shoot P content	Shoot total amount of P
	g plant ⁻¹	g kg ⁻¹	mg plant ⁻¹
GU-UC	1.08 ± 0.14 a	0.45 ± 0.09 a	0.48 ± 0.09 a
GU-C	1.07 ± 0.05 a	0.44 ± 0.03 a	0.46 ± 0.03 a
TA-UC	7.16 ± 0.97 b	0.54 ± 0.04 a	3.88 ± 0.56 a
TA-C	4.02 ± 1.66 a	0.88 ± 0.11 b	3.67 ± 1.93 a
RP-UC	2.42 ± 1.74 a	0.40 ± 0.07 a	1.01 ± 0.77 a
RP-C	17.95 ± 2.02 b	0.95 ± 0.15 b	16.99 ± 3.40 b
PD-UC	1.73 ± 0.22 a	0.48 ± 0.04 a	0.84 ± 0.18 a
PD-C	12.53 ± 3.40 b	0.80 ± 0.12 b	9.88 ± 2.22 b
PR-CS	1.19 ± 0.16 a	0.39 ± 0.05 a	0.47 ± 0.10 a
PR-SS	2.07 ± 0.23 b	0.52 ± 0.07 b	1.08 ± 0.26 b

UC: soil samples collected in uncultivated areas. C: soil samples collected in cultivated areas. CS: clayey soil. SS: sandy soil. The soil samples were identified according to the municipalities of collection in Brazil (GU = Guarapuava; TA = Taquarituba; RP = Rio Paranaíba; PD = Piedade; PR = Piracicaba). The letters a and b compare the results between uncultivated and cultivated soils, and between clayey and sandy soil sampled in the same municipality. Mean values followed by the same letter in the same sampled municipality did not differ by t test ($p < 0.05$). $\pm$ = standard deviation of the mean.

High values of available P were obtained in cultivated soils, mainly in RP-C soil. On average, the IER extractant had higher extraction power, followed by M3 and M1, with IER extracting more than twice the P contents extracted by M1 (Table 3). Similar result found by other authors in Brazilian soils, but considering only soils with low P availability (Bortolon et al., 2009; Bortolon and Gianello, 2012; Mumbach et al., 2018). This result is associated with the loss of M1 extraction capacity in clayey soils and in soils with high pH (Silva and Raij, 1999). Although high P contents were extracted in cultivated soils, on average these values represented about 2.3, 2.8, and 4.4 % of the soil total P for M1, M3 and IER, respectively. Thus, the P content in plants cultivated for 45 days represented about 0.6% of the total soil P. These values indicate that the P available by a given soil test is nothing more than an availability index that must correlate with the P absorbed and accumulated by the plants (Alvarez V., 1996; Cade-Menun et al., 2018; Valadares et al., 2017).

Table 3. Soil P content by Mehlich-1, Mehlich-3, and Ion Exchange Resin extractants after liming

Soil	Mehlich-1	Mehlich-3	Ion Exchange Resin
	----- mg dm ⁻³ -----		
GU-UC	2.8 ± 1.9	3.8 ± 0.5	9.1 ± 0.2
GU-C	15.2 ± 1.3	13.1 ± 1.9	32.4 ± 1.5
TA-UC	22.7 ± 1.5	43.0 ± 1.0	26.0 ± 0.8
TA-C	73.8 ± 2.2	99.4 ± 5.6	89.8 ± 0.8
RP-UC	4.0 ± 0.6	6.6 ± 1.3	26.3 ± 0.4
RP-C	96.8 ± 1.0	120.6 ± 33.3	298.3 ± 16.9
PD-UC	3.6 ± 0.8	8.8 ± 0.4	10.2 ± 0.4
PD-C	57.3 ± 2.4	87.6 ± 10.6	87.1 ± 3.9
PR-CS	3.2 ± 0.7	4.8 ± 0.8	9.6 ± 0.8
PR-SS	7.1 ± 0.8	7.6 ± 4.3	10.6 ± 0.6
Average	28.6	39.5	59.9

UC: soil samples collected in uncultivated areas. C: soil samples collected in cultivated areas. CS: clayey soil. SS: sandy soil. The soil samples were identified according to the municipalities of collection in Brazil (GU = Guarapuava; TA = Taquaritiba; RP = Rio Paranaíba; PD = Piedade; PR = Piracicaba).

4.3.2. Soil P pools determined by sequential fractionation

Before to interpret the results of each of the nine fractions provided by the sequential fractionation, it is important to have an overview of the total soil P content and its lability (Figure 3). The total soil P content, given by the sum of the fractions, was higher in cultivated than in uncultivated soils, and higher in the clayey than in the sandy soil. Except for the soils TA-C, PD-C, and PR-C in which the difference between the total P determined by digestion (Table 1) and the total P obtained with the sum of the fractions was - 33 %, - 25 %, and - 29 %, respectively, for the other soils this difference did not exceed 10 %. Most of the soil P is in recalcitrant form, given by the sum between the non-labile and residual fractions. The lowest fraction in all soils was the labile fraction, followed by the moderately labile fraction.

Regarding the modification of soil P pools after cultivation during two decades, the moderately labile and non-labile fractions increased in all soils with the cultivation, however the labile fraction increased only in soils RP and PD (Table 4 and Figure 3). The P-residual fraction also changed only in two of the cultivated soils, GU and TA. Thus, the main changes occurred in moderately labile and non-labile fractions, regardless of the chemical and mineralogical characteristics of the soils. For soils with contrasting textures, the labile was the only pool that did not differ for both soils. The clay soil presented lower amount of P in the moderately labile fraction in relation to the sandy soil, but higher amount in recalcitrant form, in the non-labile and residual fractions (Figure 3).

The highest amount of P in moderately labile and non-labile fractions is commonly found in studies investigating P dynamics in Brazilian soils due to different soil managements practices (Pavinato et al., 2009; Soltangheisi et al., 2018; Teles et al., 2017). Our results

corroborate that much of the P added to soil, even in highly fertilized soils such as TA and RP, is in moderately available forms in the medium and short term, constituting an important P reservoir in our soils, called legacy P (Rodrigues et al., 2016; Withers et al., 2018).

Table 4. Soil P fraction of samples collected in long-term cultivated (C) and uncultivated (UC) areas, and a reference clayey (CS) and sandy (SS) soil collected in fallow areas. Average of four repetitions $\pm$ standard deviation

Soil sample	Soil P fraction			
	Labile	Moderately labile	Non-labile	Residual
	mg kg ⁻¹			
GU-UC	98.43 $\pm$ 10.26	424.77 $\pm$ 42.14	158.59 $\pm$ 5.06	1119.03 $\pm$ 115.49
GU-C	96.27 $\pm$ 11.93	596.34 $\pm$ 101.74	227.78 $\pm$ 9.06	1589.73 $\pm$ 122.31
TA-UC	101.41 $\pm$ 31.91	187.42 $\pm$ 17.68	57.01 $\pm$ 4.47	140.56 $\pm$ 46.49
TA-C	125.75 $\pm$ 6.66	259.71 $\pm$ 16.38	118.73 $\pm$ 6.79	221.27 $\pm$ 5.13
RP-UC	79.79 $\pm$ 3.89	386.15 $\pm$ 46.69	238.85 $\pm$ 33.01	1674.92 $\pm$ 270.97
RP-C	306.12 $\pm$ 12.46	1072.56 $\pm$ 107.50	609.99 $\pm$ 21.49	1639.56 $\pm$ 289.78
PD-UC	77.41 $\pm$ 3.54	144.47 $\pm$ 18.17	43.18 $\pm$ 6.46	375.43 $\pm$ 83.27
PD-C	158.17 $\pm$ 4.90	460.81 $\pm$ 26.21	164.12 $\pm$ 27.15	313.76 $\pm$ 50.74
PR-CS	70.16 $\pm$ 8.62	168.41 $\pm$ 8.26	73.90 $\pm$ 9.45	346.41 $\pm$ 67.18
PR-SS	66.01 $\pm$ 2.83	180.56 $\pm$ 63.84	43.73 $\pm$ 4.65	136.38 $\pm$ 31.39

The soil samples were identified according to the municipalities of collection in Brazil (GU = Guarapuava; TA = Taquarituba; RP = Rio Paranaíba; PD = Piedade; PR = Piracicaba).

Figure 3. Sequential P fractionation of soil samples collected in long-term cultivated (C) and uncultivated (UC) areas, and a reference clayey (CS) and sandy (SS) soil collected in fallow areas. The soil samples were identified according to the municipalities of collection in Brazil (GU = Guarapuava; TA = Taquarituba; RP = Rio Paranaíba; PD = Piedade; PR = Piracicaba). The symbols compare the fractions between uncultivated and cultivated areas, and between clayey and sandy soil. Mean values followed by the same symbol did not differ by t test ($p < 0.05$). Error bars = standard deviation of the mean.

The labile pool represented a small proportion in relation to the total soil P (Table 4) and was mainly composed by fractions Pi_{BIC} and Po_{BIC} (Figure 4). The largest Pi_{AER} fraction was obtained in the soil TA-C, in which the fraction Po_{BIC} was practically absent and in the soil RP-C, which presented the largest labile pool. Cultivation has increased Pi forms in all soils except for the fraction Pi_{BIC} in the soil GU. The labile Po (Po_{BIC}) decreased with cultivation in soils TA and PD, however there was no difference in soils GU and RP. The soils collected in fallow areas with contrasting texture had a large proportion of Po constituting the labile pool and a small amount present in the Pi_{AER} fraction, mainly in clay soil (Figure 4).

Figure 4. Labile P fractions determined by sequential P fractionation of soil samples collected in long-term cultivated (C) and uncultivated (UC) areas, and a reference clayey (CS) and sandy (SS) soil collected in fallow areas. The soil samples were identified according to the municipalities of collection in Brazil (GU = Guarapuava; TA = Taquarituba; RP = Rio Paranaíba; PD = Piedade; PR = Piracicaba). The symbols compare the fractions between uncultivated and cultivated areas, and between clayey and sandy soil. Mean values followed by the same symbol did not differ by t test ($p < 0.05$). Error bars = standard deviation of the mean.

Although the labile pool represented only about 3 to 21 % of the total soil P (Figure 3), this fraction is of great ecological and agronomic relevance, since it represents the readily available P for plants and therefore will soon enter in the food chain (Weihrach and Opp, 2018). The low levels of Pi_{AER} in relation to the total P corroborate with the data in table 3,

showing the importance of correlation works for the use of extractants (Beegle, 2005; Westerman et al., 1990).

The moderately labile P pool represented 16 to 42 % of the total soil P of the soil set (Table 4). The Po, represented by the fraction $Po_{NaOH\ I}$ was the main constituent of this moderately P pool and with the exception of the soil TA, cultivation increased the moderately labile Po content in the soils (Figure 5). The fraction $Pi_{NaOH\ I}$ increased in all soils with cultivation. Although present in much smaller amount than the other two fractions, the fraction Pi_{HCl} also had an increase in soils due to cultivation except for the soil GU in which the cultivation did not modified this fraction. The soils PR with contrasting textures had similar P content in the fraction $Po_{NaOH\ I}$, but the sandy soil (PR-SS) presented higher P content in fractions $Pi_{NaOH\ I}$ and Pi_{HCl} (Figure 5). The Po of this fraction represents the P associated with fulvic and humic acids adsorbed to Fe and Al oxides and to SOM (Gatiboni et al., 2013; Rodrigues et al., 2016). The large amount of Po found mainly in cultivated soils indicates that the addition of P promotes a higher biomass production that when decomposing keeps P in less labile forms (Oberson and Joner, 2005), forming an important component of legacy P in tropical soils.

According to the sequential chemical fractionation assumptions (Hedley et al., 1982), the largest P contents found in the moderately labile fraction indicate that much of the P in uncultivated soils is strongly adsorbed to Fe and Al oxides ($Pi_{NaOH\ I}$), and that this is the same fate for P added via fertilizer in the short term (Menezes-Blackburn et al., 2018; Rodrigues et al., 2016). Virtually no forms of P-Ca were found in the soil, which would be extracted in the Pi_{HCl} fraction (Gatiboni et al., 2013). This may be due to the low use of reactive phosphate rocks as a source of P in the areas and the predominance of the use of highly soluble sources. Moreover, the pH range of the studied soils, even in cultivated soils, favors the dissolution of P-Ca forms (Hesterberg, 2010). Thus, the small amount of P-Ca found should be related to liming application over cultivation cycles in cultivates areas (Pavinato et al., 2009).

Figure 5. Moderately Labile P fractions determined by sequential P fractionation of soil samples collected in long-term cultivated (C) and uncultivated (UC) areas, and a reference clayey (CS) and sandy (SS) soil collected in fallow areas. The soil samples were identified according to the municipalities of collection in Brazil (GU = Guarapuava; TA = Taquarituba; RP = Rio Paranaíba; PD = Piedade; PR = Piracicaba). The symbols compare the fractions between uncultivated and cultivated areas, and between clayey and sandy soil. Mean values followed by the same symbol did not differ by t test ($p < 0.05$). Error bars = standard deviation of the mean.

The most recalcitrant fraction of P in the soil, formed by non-labile P plus residual P, was the largest pool in relation to total soil P. Together, these fractions represented 41 to 80 % of the total soil P of the soil set (Table 4). The non-labile pool was mainly formed by Po, while the Pi represented a smaller fraction. However, both organic and inorganic fractions of non-labile P ($Pi_{NaOH II}$ and $Po_{NaOH II}$) increased with cultivation in all soils (Figure 6). High P contents were obtained in the residual fraction, and the cultivation did not promote alteration in the contents of this fraction in the soils RP and PD. The soils PR-CS and PR-SS, collected in fallow areas presented similar contents in labile and moderately labile P fractions (Figure 4 and Figure 5). However, there was a significant difference in the recalcitrant pool of P (Figure 6), with more organic and residual forms of P in the clay soil. These results are consistent with the high P adsorption capacity of the soils and in accordance with several works (Gatiboni et al., 2013; Rodrigues et al., 2016; Soltangheisi et al., 2018; Teles et al., 2017) in tropical soils.

Figure 6. Non-labile P fractions determined by sequential P fractionation of soil samples collected in long-term cultivated (C) and uncultivated (UC) areas, and a reference clayey (CS) and sandy (SS) soil collected in fallow areas. The soil samples were identified according to the municipalities of collection in Brazil (GU = Guarapuava; TA = Taquarituba; RP = Rio Paranaíba; PD = Piedade; PR = Piracicaba). The symbols compare the fractions between uncultivated and cultivated areas, and between clayey and sandy soil. Mean values followed by the same symbol did not differ by t test ($p < 0.05$). Error bars = standard deviation of the mean.

Regardless of whether cultivated or not cultivated, most of the soil P is in inorganic or geochemical form, but this fraction is higher in cultivated soils than its counterparts (Figure 7). It is important to note that the geochemical P includes the residual P (Cross and Schlesinger, 1995; Rodrigues et al., 2016), which can include some Po not determined in previous extractions. Except for the soil TA, the biological P fraction also increased with cultivation in all other soils. The PR-CS had higher P content in geochemical forms than PR-SS soil, but both had similar P content in biological forms. The contribution of biological P to soil total P ranged from 24 % in the soil RP-UC to 47 % in the soil PR-SS. These results confirm the great capacity of P adsorption in clay soils of tropical regions, mainly associated with Fe and Al oxides (Fontes and Weed, 1996), even after decades of liming, which is important for the future evolution of management practices aiming increased sustainable use of P (Roy et al., 2016; Withers et al., 2018).

Figure 7. Geochemical and biological P pools determined by the sum of inorganic and organic fractions, respectively, obtained by sequential P fractionation of soil samples collected in long-term cultivated (C) and uncultivated (UC) areas, and a reference clayey (CS) and sandy (SS) soil collected in fallow areas. The soil samples were identified according to the municipalities of collection in Brazil (GU = Guarapuava; TA = Taquarituba; RP = Rio Paranaíba; PD = Piedade; PR = Piracicaba). The symbols compare the fractions between uncultivated and cultivated areas, and between clayey and sandy soil. Mean values followed by the same symbol did not differ by t test ($p < 0.05$). Error bars = standard deviation of the mean.

4.3.3. Soil Phosphorus Speciation by X-ray Absorption Near-Edge Structure Spectroscopy

The XANES fitting analyses indicated that all soil samples contained at least two species of P, and that adsorbed phases predominated in relation to mineral forms of P in soils (Table 5 and Figure 8). Most of soil total P (Pt), about 60 % to 89 % is adsorbed on Fe-oxides and Al-oxides (P-Goethite and P-Hematite). Except for soils PR-SC and PR-SS in which most of P is adsorbed to Hematite, in all other soils P-Goethite is the main P adsorbed specie found. The adsorption of P onto Al-oxide (P-Gibbsite) was found in the soils TA, RP and PD-C, not constituting more than 25% of the soil Pt. Organic P in soils, represented by the standard phytic acid represented, on average, 15 % of Pt and was present in all soils except for soil PD-C. Of all the mineral forms of P added as standards in the linear combination fit analysis, only Bayovar (Peruvian reactive rock phosphate) and Strengite were found in PD-C and PR-CS, respectively (Table 5 and Figure 8).

Table 5. Phosphorus K-edge XANES fitting results with the relative proportions (percentage normalized to sum 100) of P chemical species (standards) determined by linear combination fitting that yielded the best fit to the soil samples

Sample	R factor	Sum ⁵	Phytic acid	P-Goethite	P-Hematite	P-Gibbsite	Strengite	Bayovar
----- % of total P $\pm$ uncertainty of fit -----								
GU-UC ¹	0.0019	1.027	11.0 ($\pm$ 1)	89.0 ($\pm$ 1)	-	-	-	-
GU-C ²	0.0030	1.005	12.6 ($\pm$ 1)	87.4 ($\pm$ 1)	-	-	-	-
TA-UC	0.0019	1.045	15.0 ($\pm$ 1)	40.2 ($\pm$ 3)	19.5 ($\pm$ 3)	25.3 ($\pm$ 1)	-	-
TA-C	0.0015	1.009	16.3 ($\pm$ 1)	45.2 ($\pm$ 2)	17.1 ($\pm$ 3)	21.4 ($\pm$ 1)	-	-
RP-UC	0.0033	1.158	11.4 ($\pm$ 1)	73.1 ($\pm$ 2)	-	15.5 ($\pm$ 1)	-	-
RP-C	0.0012	1.047	11.7 ($\pm$ 1)	42.2 ($\pm$ 2)	32.1 ($\pm$ 3)	14.0 ($\pm$ 1)	-	-
PD-UC	0.0030	1.044	22.3 ($\pm$ 1)	50.7 ($\pm$ 3)	27.0 ($\pm$ 3)	-	-	-
PD-C	0.0090	1.066	-	47.7 ($\pm$ 2)	18.2 ($\pm$ 3)	23.7 ($\pm$ 1)	-	10.4 ($\pm$ 1)
PR-CS ³	0.0060	0.975	11.6 ($\pm$ 1)	-	80.0 ($\pm$ 1)	-	8.4 ($\pm$ 2)	-
PR-SS ⁴	0.0010	1.002	26.6 ($\pm$ 1)	10.8 ($\pm$ 3)	62.6 ($\pm$ 3)	-	-	-

^{1/}UC: soil samples collected in uncultivated areas. ^{2/}C: soil samples collected in cultivated areas. ^{3/}CS clayey soil.

^{4/}SS sandy soil. ^{5/}Sum before normalization. P- = Phosphorus sorbed to respective mineral.

The cultivation did not promote many differences in relation to the species of P present in soils, mainly in the soils of GU and TA, in which the species and the proportions found were similar in cultivated and uncultivated soils (Figure 8). In the soil RP-UC, 73 % of Pt was adsorbed to Goethite, while in RP-C soil 32 % of Pt was adsorbed to Hematite and 42 % to Goethite. The soils from PD presented the greatest difference between the species in relation to cultivation. In the soil PD-C, the XANES-fitting did not detect the presence of Phytic acid, that was found the soil PD-UC, and in addition to P-Hematite and P-Goethite, 24% of Pt was adsorbed to Gibbsite and 10 % has been identified as Bayovar, the only form of Ca-phosphate found in the studied soils. Regarding to the soils collected in fallow areas, PR-SS showed higher amount of organic P (Phytic acid) than PR-CS, and about 63 % P-Hematite and 10 % P-Goethite. Most of the P in the soil PR-CS is adsorbed to Hematite (80 %) and 8 % to Strengite.

A)

B)

Figure 8. Phosphorus speciation in soils determined from phosphorus K-edge XANES spectroscopy. A) Relative proportions of fitting standards in the overall fits for XANES spectra from soils collected in long-term cultivated (C) and uncultivated areas (UC), and reference clay (CS) and sand (SS) soil collected in fallow areas. B) Proportion of soil species in relation to total P. The soil samples were identified according to the municipalities of collection in Brazil (GU = Guarapuava; TA = Taquaritiba; RP = Rio Paranaíba; PD = Piedade; PR = Piracicaba).

The practically absence of P-Ca in the LCF analysis is consistent with chemical fractionation data and also with other studies conducted in tropical soils due to the conditions for high solubility of calcium phosphates (Hesterberg, 2010). Our hypothesis of increasing forms of P-Ca due to the high doses of P allied to liming in cultivated soils was not supported by fractionation or speciation techniques. However, P-Ca accumulation in soils with low pH values are reported (Beauchemin et al., 2003; Sato et al., 2005), but in these cases the presence of P-Ca is more associated with soil mineralogy.

The absence of P-kaolinite standard in the LCF analysis shows that kaolinite has low P adsorption capacity, especially when present in soils with high Fe and Al oxide contents (Fontes and Weed, 1996). The amount of organic P, represented by phytic acid is consistent with the amounts found in P speciation works (Abdala et al., 2018), although the ^{31}P NMR technique is more sensitive in identifying organic forms of P in soils than XANES (Cade-Menun et al., 2018; Kruse et al., 2015; Liu et al., 2013).

Phosphorus adsorbed onto Fe oxides (Goethite and Hematite) and Al oxide (Gibbsite) are the main constituents of soils that contribute to the adsorption of phosphates with higher or lower binding energy (Abdala et al., 2015; Fontes and Weed, 1996). Thus, almost all of the P_i found in moderately labile fractions in chemical fractionation must be adsorbed onto Gibbsite and Hematite, and a small portion of P_o adsorbed to these minerals in soils, although this was not supported by XANES analysis. Anyway, the combined use of soil P chemical fractionation and XANES are important to provide unique and complementary information regarding the fate of P in high fertilized soils (Abdala et al., 2018; Eriksson et al., 2016; Liu et al., 2013).

4.4. CONCLUSIONS

Although cultivation practices caused an increase in soil pH and base saturation, there was a tendency to decrease soil cation exchange capacity with cultivation, which indicates that soil organic matter is an important component for the charge generation in these soils and play an important role in soil P availability.

After two decades of soil cultivation, there were evident increase mainly in the moderately labile and non-labile P fractions. This suggests the accumulation of a large amount of P in the soil, contributing to the legacy P, which may play an important role buffering the most labile pools in the medium term.

There was no increase in P-Ca forms due to successive applications of liming and phosphate fertilizers in cultivated soils. Thus, the labile and moderately labile forms of P are mainly adsorbed onto P Fe and Al oxides (Hematite, Goethite, and Gibbsite).

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APPENDIX

APPENDIX A. Annexes related to Chapter 2

Table 1. Shoot dry matter production, shoot P content, and shoot total amount of P of maize plants in response to five levels of P applied in 10 soil samples (first cultivation)

Soil	Level of applied P						Average
	0.0	0.5	1.0	2.0	3.0	5.0 ^{5/}	
Shoot dry matter							
	g plant ⁻¹						
GU-UC ^{1/}	1.08	10.62	17.57	25.79	28.33	30.27	18.94
GU-C ^{2/}	1.07	1.20	1.60	1.65	1.90	2.88	1.72
TA-UC	7.16	10.58	11.75	13.48	13.42	16.53	12.15
TA-C	4.02	7.34	6.38	8.33	8.28	10.88	7.54
RP-UC	2.42	5.10	8.15	13.62	14.14	16.03	9.91
RP-C	17.95	17.95	18.99	23.11	19.38	19.88	19.54
PD-UC	1.73	10.10	13.48	18.48	19.09	20.19	13.84
PD-C	12.53	14.98	17.21	17.17	15.03	15.55	15.41
PR-CS ^{3/}	1.19	7.46	12.14	16.44	17.83	18.37	12.24
PR-SS ^{4/}	2.07	7.15	11.17	12.91	14.75	16.18	10.70
Average	5.12	9.25	11.84	15.09	15.21	16.68	12.20
Shoot P content							
	g kg ⁻¹						
GU-UC	0.449	0.542	0.582	0.664	0.776	0.970	0.664
GU-C	0.435	0.455	0.535	0.527	0.561	0.577	0.515
TA-UC	0.543	0.611	0.699	0.815	0.930	1.251	0.808
TA-C	0.883	0.774	0.825	0.813	0.834	1.059	0.865
RP-UC	0.400	0.524	0.557	0.573	0.532	0.629	0.536
RP-C	0.947	1.100	0.992	1.143	1.150	1.013	1.058
PD-UC	0.481	0.509	0.569	0.691	0.899	1.172	0.720
PD-C	0.804	0.721	0.766	0.826	0.906	1.009	0.839
PR-CS	0.387	0.554	0.624	0.698	0.760	1.086	0.685
PR-SS	0.516	0.590	0.640	0.672	0.933	1.224	0.763
Average	0.584	0.638	0.682	0.742	0.834	0.999	0.745
Shoot total amount of P							
	mg plant ⁻¹						
GU-UC	0.48	5.72	10.22	17.04	21.81	28.73	14.00
GU-C	0.46	0.54	0.84	0.87	1.08	1.67	0.91
TA-UC	3.88	6.45	8.10	11.09	12.50	20.92	10.49
TA-C	3.67	5.55	5.24	6.77	6.89	11.59	6.62
RP-UC	1.01	2.68	4.54	7.85	7.55	10.07	5.62
RP-C	16.99	19.46	18.83	26.39	21.87	20.04	20.60
PD-UC	0.84	5.12	7.67	12.78	17.04	23.62	11.18
PD-C	9.88	10.50	12.97	13.73	13.29	16.04	12.73
PR-CS	0.47	4.12	7.51	11.53	13.70	19.92	9.54
PR-SS	1.08	4.23	7.15	8.62	13.91	19.70	9.12
Average	3.88	6.44	8.31	11.67	12.96	17.23	10.08

^{1/}UC: soil samples collected in uncultivated areas. ^{2/}C: soil samples collected in cultivated areas. ^{3/}CS: clayey soil. ^{4/}SS: sandy soil ^{5/}The dose of P corresponding to level 5.0 was 650 mg.dm⁻³ to the soils GU-UC and GU-C; 350 mg.dm⁻³ to the soils TA-UC and TA-C; 560 mg.dm⁻³ to the soils RP-UC and RP-C; 410 mg.dm⁻³ to the soils PD-UC, PD-C and PR-CS, and 300 mg.dm⁻³ to the soil PR-SS.

Table 2. Analysis of variance of data from maize shoot dry matter production as function of doses of P applied in 10 soil samples (first cultivation)

Source of variation	D.F.	S.S.	M.S.	F
Block	3	9.84	3.28	0.432 ^{ns}
Soil	9	6,035.62	670.62	88.335***
Dose of P	5	3,858.50	771.70	101.648***
Dose of P*Solo	45	2,288.17	50.85	6.698***
Residue	177	1,343.76	7.60	
Total	239	13,535.89		

Coefficient of variation = 22.58 %. Total average: 12.20 g/plant. ^{ns} $p \geq 0.10$; *** $p < 0.001$. D.F. = Degrees of Freedom; S.S. = Sum of Squares; M.S. = Mean Square.

Table 3. Analysis of variance of data from maize shoot P content as function of doses of P applied in 10 soil samples (first cultivation)

Source of variation	D.F.	S.S.	M.S.	F
Block	3	0.187	0.063	2.757*
Soil	9	5.349	0.594	26.189***
Dose of P	5	4.948	0.989	43.603***
Dose of P*Solo	45	2.352	0.052	2.303***
Residue	177	4.017	0.023	
Total	239	16.854		

Coefficient of variation = 20.64 %. Total average: 0.74 g kg⁻¹. * $0.05 > p \geq 0.01$; *** $p < 0.001$. D.F. = Degrees of Freedom; S.S. = Sum of Squares; M.S. = Mean Square.

Table 4. Analysis of variance of data from maize shoot total amount of P as function of doses of P applied in 10 soil samples (first cultivation)

Source of variation	D.F.	S.S.	M.S.	F
Block	3	105.51	35.17	5.910***
Soil	9	6,039.08	671.01	112.762***
Dose of P	5	4,673.70	934.74	157.082***
Dose of P*Solo	45	2,234.61	49.66	8.345***
Residue	177	1,053.26	5.95	
Total	239	14,106.16		

Coefficient of variation = 24.20 %. Total average: 10.08 mg plant⁻¹. *** $p < 0.001$. D.F. = Degrees of Freedom; S.S. = Sum of Squares; M.S. = Mean Square.

Table 5. Soil available P content by Melich-1, Mehlich-3, and Ion Exchange Resin extractants in response to five levels of P applied in ten soil samples (first cultivation)

Soil	Level of applied P						Average
	0.0	0.5	1.0	2.0	3.0	5.0 ^{5/}	
Mehlich-1							
	----- mg dm ⁻³ -----						
GU-UC ^{1/}	3.0	6.2	14.5	28.6	38.6	84.7	29.2
GU-C ^{2/}	15.3	27.5	41.5	55.9	80.9	132.0	58.9
TA-UC	22.1	26.7	39.1	57.3	83.5	121.1	58.3
TA-C	66.1	68.9	94.1	122.0	137.7	257.5	124.4
RP-UC	3.0	4.6	6.6	15.7	47.3	70.8	24.7
RP-C	78.3	85.6	99.0	135.4	154.4	173.7	121.1
PD-UC	3.0	10.3	12.3	18.2	53.4	98.8	32.7
PD-C	47.0	66.4	57.2	121.4	114.3	120.8	87.9
PR-CS ^{3/}	3.1	5.0	13.5	24.0	51.6	83.0	30.1
PR-SS ^{4/}	8.2	17.2	24.9	34.9	45.7	128.0	43.1
Average	24.9	31.8	40.3	61.3	80.7	127.0	61.0
Mehlich-3							
	----- mg dm ⁻³ -----						
GU-UC	2.9	6.1	11.6	18.8	25.6	64.5	21.6
GU-C	13.9	22.4	26.3	40.7	45.2	60.9	34.9
TA-UC	44.2	45.5	74.8	71.9	84.8	121.1	73.7
TA-C	90.1	90.1	112.5	128.7	144.4	204.7	128.4
RP-UC	6.8	15.0	20.4	30.0	40.2	70.5	30.5
RP-C	113.9	103.7	116.6	139.6	153.8	158.5	131.0
PD-UC	8.0	11.9	15.1	18.6	45.9	83.5	30.5
PD-C	86.1	93.5	92.4	119.8	109.1	127.5	104.7
PR-CS	4.8	10.2	17.9	33.3	38.5	64.6	28.2
PR-SS	11.8	20.9	27.7	44.0	51.5	73.4	38.2
Average	38.3	41.9	51.5	64.5	73.9	102.9	62.2
Ion Exchange Resin							
	----- mg dm ⁻³ -----						
GU-UC	10.2	17.5	21.1	36.7	72.0	131.1	48.1
GU-C	44.9	68.7	75.7	130.6	151.0	219.7	115.1
TA-UC	35.5	37.7	54.5	81.4	140.9	119.0	78.2
TA-C	101.0	104.7	129.8	154.2	182.8	231.8	150.7
RP-UC	27.7	37.9	56.9	132.7	142.6	217.6	102.5
RP-C	292.1	305.0	329.6	329.8	405.1	386.4	341.3
PD-UC	10.8	16.6	25.9	30.6	58.3	94.4	39.4
PD-C	85.2	123.2	116.1	157.1	194.0	186.4	143.7
PR-CS	8.4	18.7	26.3	27.7	53.9	120.6	42.6
PR-SS	12.8	26.6	28.0	47.6	60.3	71.6	41.4
Average	62.8	75.6	86.4	112.8	146.1	177.9	110.3

^{1/}UC: soil samples collected in uncultivated areas. ^{2/}C: soil samples collected in cultivated areas. ^{3/}CS: clayey soil. ^{4/}SS: sandy soil ^{5/}The dose of P corresponding to level 5.0 was 650 mg.dm⁻³ to the soils GU-UC and GU-C; 350 mg.dm⁻³ to the soils TA-UC and TA-C; 560 mg.dm⁻³ to the soils RP-UC and RP-C; 410 mg.dm⁻³ to the soils PD-UC, PD-C and PR-CS, and 300 mg.dm⁻³ to the soil PR-SS.

Table 6. Analysis of variance of data from soil available P content by Mehlich-1 soil test as function of doses of P applied in 10 soil samples (first cultivation)

Source of variation	D.F.	S.S.	M.S.	F
Block	3	2,017.76	672.59	2.545°
Soil	9	306,398.23	34,044.25	128.833***
Dose of P	5	293,381.72	58,676.34	222.047***
Dose of P*Solo	45	46,619.32	1,035.98	3.920***
Residue	177	46,772.49	264.25	
Total	239	695,189.52		

Coefficient of variation = 26.64 %. Total average: 61.0 mg dm⁻³. ° 0.10 > p ≥ 0.05. *** p < 0.001. D.F. = Degrees of Freedom; S.S. = Sum of Squares; M.S. = Mean Square.

Table 7. Analysis of variance of data from soil available P content by Mehlich-3 soil test as function of doses of P applied in 10 soil samples (first cultivation)

Source of variation	D.F.	S.S.	M.S.	F
Block	3	9,176.81	3,058.94	11.909***
Soil	9	412,717.62	45,857.51	178.530***
Dose of P	5	115,927.56	23,185.51	90.265***
Dose of P*Solo	45	16,828.24	373.96	1.456*
Residue	177	45,464.55	256.86	
Total	239	600,114.79		

Coefficient of variation = 25.77 %. Total average: 62.2 mg dm⁻³. * 0.05 > p ≥ 0.01. *** p < 0.001. D.F. = Degrees of Freedom; S.S. = Sum of Squares; M.S. = Mean Square.

Table 8. Analysis of variance of data from soil available P content by Ion Exchange Resin soil test as function of doses of P applied in 10 soil samples (first cultivation)

Source of variation	D.F.	S.S.	M.S.	F
Block	3	3,621.4528	1,207.1509	2.575°
Soil	9	1,812,122.0662	201,346.8962	429.432***
Dose of P	5	395,120.2549	79,024.0510	168.542***
Dose of P*Solo	45	72,511.6430	1,611.3698	3.437***
Residue	177	82,989.6847	468.8683	
Total	239	2,366,365.1016		

Coefficient of variation = 19.64 %. Total average: 110.3 mg dm⁻³. ° 0.10 > p ≥ 0.05. *** p < 0.001. D.F. = Degrees of Freedom; S.S. = Sum of Squares; M.S. = Mean Square.