

University of São Paulo
"Luiz de Queiroz" College of Agriculture

Legacy phosphorus in Brazilian agriculture and cover crops contribution to
enhance this nutrient bioavailability in Oxisols

Marcos Rodrigues

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

Piracicaba
2018

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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RESUMO

Legado do fósforo na agricultura brasileira e a contribuição de plantas de cobertura para aumentar a biodisponibilidade deste nutriente em Latossolos

Fósforo (P), um dos elementos essenciais às plantas e animais, é um recurso não-renovável e altamente demandado na agricultura atualmente. O crescimento da população mundial e as estimativas futuras da demanda de alimentos aumentam os questionamentos sobre a sustentabilidade dos sistemas agrícolas atuais, especialmente no que se refere a baixa eficiência no uso do P na agricultura. A agricultura brasileira requer altas quantidades de P e muitas pesquisas tem sido desenvolvidas para aumentar a produtividade das culturas e, conseqüentemente, a eficiência da produção de alimentos. Entretanto, este desenvolvimento tem sido alcançado a altos custos com uso de fertilizantes minerais (sobretudo P) e as perspectivas de longo prazo sobre os custos da intensificação da agricultura tropical estão atualmente em debate. Nesse contexto, no **capítulo dois** da presente tese avaliamos a evolução da área cultivada no Brasil e das demandas de P, quantificando o legado do P no Brasil e, baseado em cenários envolvendo a expansão da área cultivada e da produtividade, estimamos o futuro (até 2050) legado de P e as demandas por fertilizantes fosfatados. Ainda estimamos a potencial redução no consumo de fertilizantes fosfatados que pode ser alcançada pela utilização e melhorias na reciclagem do P oriundo de dejetos. Alternativamente, considerando a intensificação dos sistemas e seus efeitos na disponibilidade de P, propusemos cenários alternativos visando a utilização do “Legacy P” em sistemas agrícolas melhorados para garantir a expansão agrícola sem drásticos incrementos no consumo nacional de fertilizantes fosfatados. Um avanço internacionalmente reconhecido na agricultura Brasileira é o Cerrado, onde extensas áreas agrícolas são cultivadas duas vezes ao ano, incrementando assim a produção de alimentos e fibras sem qualquer custo aos ecossistemas naturais. Nesse contexto, a agricultura sob plantio direto (PD) tem sido amplamente difundida e, como consequência, a fertilidade dos solos tem-se elevado, o que pode também favorecer a disponibilidade de P, quando são introduzidas rotações com espécies de plantas que apresentem mecanismos de mobilização do P, como o uso de plantas de cobertura. Entretanto, os efeitos desses sistemas melhorados de rotação na biodisponibilidade de P em solos tropicais no longo prazo permanecem incertos na literatura e foram objeto de estudo no **capítulo três**, onde está descrito um estudo envolvendo a avaliação das mudanças de longo-prazo nas frações de P em Latossolos, oriundos de dois experimentos de campo de longa duração no Cerrado, envolvendo a avaliação de sistemas de preparo (PD e preparo convencional, PC) e plantas de cobertura (milheto, braquiária milho e um controle, apenas pousio). O cultivo por longo tempo resultou em grandes quantidades de “Legacy P” acumulado no solo e, com o uso de braquiária como cultura de cobertura, maior quantidade de P foi reciclado para as camadas superiores do solo e as frações de P lábil e todas as frações orgânicas aumentaram. Baseado nos efeitos das plantas de cobertura, sugerimos que a biodisponibilidade do “Legacy P” pode ser representada pelo P extraído nas frações lábeis + o P de frações moderadamente lábeis. No **capítulo quatro**, utilizamos outras técnicas atualmente difundidas para a especiação de P, aplicando-as em solos tropicais influenciados pela conversão do Cerrado nativo em agricultura sob PD e PC. Uma abordagem múltipla para identificação e quantificação de formas de P em solos tropicais foi proposta através da utilização do fracionamento de P proposto por Hedley em conjunto com a especiação de P por XANES da borda K do P para identificação de ligação do P a matrix inorgânica do solo, e espectroscopia de ressonância magnética nuclear (NMR) para complementação da identificação de espécies orgânicas de P em solos tropicais.

Palavras-chave: Fertilizantes fosfatados; Disponibilidade de P; Frações de P; Formas de P; Fracionamento de Hedley; Cerrado; Especiação de P; Ressonância magnética nuclear; ³¹P-NMR; Raios-X; NEXAFS; XANES; XAS

ABSTRACT

Legacy phosphorus in Brazilian agriculture and cover crops contribution to enhance this nutrient bioavailability in Oxisols

Phosphorus (P) one of the essential elements to plants and animals, is a non-renewable resource and actually high demanded in agriculture. The World's population growth and future estimatives on food demands raises the question about the sustainability of the current agriculture systems, specially concerns about the low P use efficiency in agroecosystems. Brazilian agriculture requires high P inputs and many researches has been developed to increase the crops yield and, consequently, food production efficiency. However, this development was achieved at the high costs of mineral fertilisers (specially P) and long-term perspectives about the costs of the intensification in the tropical agriculture is actually in debate. In this context, in the present thesis **chapter two** we evaluated the evolution of the Brazilian cropland basis and P demands, quantifying the Brazil P Legacy and, based on scenarios involving the cropland area and yield expansion, we estimated the future (up to 2050) Legacy P and P fertilisers demand. We also estimated the potential alleviation that could be achieved in the mineral fertilizers consumption by utilisation and improvements on manure P recycling. Alternatively, considering the systems intensification and its effects on P availability, we proposed alternative scenarios to promote the Legacy P utilisation in Brazil's improved agriculture systems to grant the agriculture expansion without huge increments on the national P fertilisers consumption. A worldwide recognized advance in Brazilian agriculture is the Cerrado, where large cropland areas actually are cultivated twice a year, improving the food and fiber production without any additional disturbance of natural ecosystems. In this context, no-tillage (NT) agriculture has been widely used and, as consequence, soil fertility has been improved, which could be also favorable to the P availability, when rotation with crop species with P mobilization mechanisms are introduced, such as cover crops utilisation. However, the effects of these improved rotated systems on P bioavailability in long-term in tropical soils remains uncertain in the literature and was our objective in the **chapter three**, where is described a study involving the evaluation of long-term changes on P fractions in tropical Oxisols, from two long-term field trials involving tillage systems (NT and conventional tillage, CT) and cover crops (millet, brachiaria, maize and control, fallow). The long-term cultivation generated large amounts of legacy P in the soil and with the brachiaria introduction as cover crop, more P was recycled in the topsoil and labile P and all the organic P fractions increased. Based on the cover crop effects, we proposed that the bioavailability of the legacy P could be represented by the P extracted by the labile fractions + moderately labile P fractions. In the **chapter four**, we used other techniques actually widely applied to P speciation, and we applied in tropical soils affected by the native Cerrado conversion into NT and CT agriculture. A multiple approach to identify and quantify the P forms in tropical soils were proposed by the utilisation of Hedley's fractionation together with P K-edge XANES for P-bindings into inorganic soil matrix and liquid ^{31}P -NMR spectroscopy to complement the organic P species in tropical soils.

Keywords: P fertilisers; P lability; P fractions; P forms; Hedley's fractionation; Cerrado; P speciation; Nuclear magnetic resonance; ^{31}P -NMR; X-Ray; NEXAFS; XANES; XAS

1. GENERAL INTRODUCTION

Phosphorus (P) is an essential element to higher plants (Marschner, 1995) and often required in high amounts in the tropical agriculture (Novais et al., 2007; Sousa and Lobato; 2004), which results frequently in a low P use efficiency in these regions, often not over than 50% (Roy et al., 2016). Phosphorus plays an essential role in soil fertility and world food security and, since phosphate rocks are non-renewable resources, recently, interest in phosphorus emerged from the discussion about its availability and costs (Cordell and White, 2014; Mew, 2016).

Despite the P fertilizer essential demand to maintain or increase the productivity of crops in tropical agriculture systems (Condrón and Newman, 2011; Johnston et al., 2014) the small P portion effectively used by plants, remaining the majority fixed into the soil, mainly sorbed on the surface of crystalline oxides (Novais et al. 2007) raises a question about the sustainability of the tropical agriculture to sustain the world food production (Rockstrom et al., 2017; Roy et al., 2016).

Brazil play a key role in the global food security and the country is internationally recognized as a global farm (Tollefson, 2010), one of the most important food and fiber producer in the world (Rockstrom et al., 2017). However, due to the Brazil's reliance on large P fertilizer inputs (Roy et al., 2016) to maximize agricultural production and meet future global food demands, the sustainability of the food production in the country in longer-term perspectives is currently on debate (Cordell et al., 2009; Withers et al., 2015). Expansion of Brazilian cropland to meet future food demands (Strassburg et al., 2014) will occurs either by converting degraded low-input pasture or native savanna (MAPA, 2015). These aspects denote the requirement of considerable amounts of P fertiliser (Lopes and Guilherme, 2016; Roy et al., 2016; Sattari et al., 2016). This high demand for supplying plant demand and overcome soil P-fixation is a major question to Brazilian agriculture, due to the reliance on imported P to supply about 60% of inorganic P fertiliser used in Brazilian agriculture (ANDA, 2017). Therefore, Brazilian agriculture is particularly vulnerable to future P scarcity and/or P price sudden abnormal raising.

Farming systems and soil and fertilizer management alter P dynamics and availability by changing P forms and their relative quantities in soil (Agbenin and Goladi, 1998; Matos et al., 2006). In agricultural systems, annual additions of inorganic fertilizer become the main P source, increasing total P content in soil over time.

Soil P can be found in inorganic and organic compounds that range from ions in solution to many stable solid compounds. Organic (Po) and inorganic (Pi) forms are found in soils and, according to their biogeochemistry, can exist as ionic forms in soil solution to highly stable compounds with mineral phase, such as primary mineral-P (e.g. apatite), P sorbed (adsorption or co-precipitation) with amorphous and crystalline Fe, Al and Ca, or organic P compounds, usually present as soil microbial biomass and soil organic matter decomposition compounds of varying recalcitrance, including phosphomonoesters, phosphodiester and organic polyphosphates (Nash et al., 2014).

The P compounds and forms in which P accumulates depends on soil type and soil management. In highly weathered acid soils rich in Al- and Fe-oxides (e.g., Oxisols predominant in tropical regions), Pi is strongly sorbed on the edges of silicate clay minerals and on pedogenic Al- and Fe-oxides. However, Po pools in the soil can represent 20-80% of the total soil P and its availability is variable and dependent on soil organic matter decomposition (Condrón and Tiessen, 2015). The Po forms in soils are far less understood than inorganic

P, not least due to analytical limitations (Turner et al., 2005). However, this fraction is highly relevant to the supply of P to crops in highly weathered soils because of the limited availability of Pi (Nziguheba and Bunemann, 2005), giving to Po a crucial importance for maintaining the P availability in tropical soils (Bünemann et al., 2011; Bünemann, 2015).

Alternative management strategies to reduce reliance on imported P and use it more efficiently will therefore become increasingly important if Brazilian farming systems keep increasing the nutrients availability and improve soil fertility. For example, no-tillage (NT) agriculture promotes both inorganic P (Pi) and organic P (Po) accumulation in surface horizons (Tiecher et al., 2012a, 2012b; Rodrigues et al., 2016). Pi accumulates at the soil surface under NT because higher P affinity adsorption sites become more saturated, lowering P binding energies and increasing P diffusion to the soil solution (Gatiboni et al., 2007). Po increase in the soil due to the concentration of crop residues at the soil surface leading to greater organic matter and its decomposition products (Rheinheimer and Anghinoni, 2003). However the relative importance of Pi and Po forms to sustain the P availability and crops nutrition in highly weathered soils of Brazil's intensively farmed is poorly understood (Zamuner et al., 2008; Roy et al., 2016).

The plants can mobilize different P forms from soil with specific mechanisms i.e. mycorrhizal associations, exudates such as low molecular-weight organic acids, phosphatases, phytases and protons (Hinsinger et al., 2011), also some changes in root morphology like density of roots, distribution, elongation and root hairs presence (Lambers et al., 2006), increasing the P lability P as well as decreased the non-labile P. With these schemes the plants make a strong drain to mobilize P from occluded-P and non-labile P to less stable P forms such labile-P, there is a continuous exchange of P between organic and inorganic pools, and between the soil solution and exchangeable and fixed pools (Suriyagoda et al., 2014). In this context, cover crops with some of the mentioned physiological characteristics not only help to have substantial effect on soil conservation by covering the soil surface from erosion (Calegari et al., 2013), but also could improve the soil P utilization (Boer et al., 2007; Tiecher et al., 2012b), contributing to enhance P bioavailability and the yield of subsequent cash crops (Calegari et al., 2013; Carvalho et al., 2011).

Different sequential-P-fractionation schemes have been developed to quantify P in the above described inorganic and organic forms, as recently summarized by Pierzynski et al., (2005). HEDLEY et al. (1982) developed a sequential extraction technique for separating soil P into various inorganic P (Pi) and organic P (Po) fractions. These schemes have been widely applied for P dynamic in tropical soils (Gatiboni, 2003; Cherubin et al., 2016; Costa et al., 2016; Rodrigues et al., 2016). However, long-term changes and specifically the P forms accumulation in intensively cultivated tropical agroecosystems are not fully comprehended.

More insights about P forms in the soils with the advances achieved with new analytical technologies, such as X-Ray absorption spectroscopy (XAS) (Condrón and Newman, 2011; Kruse et al., 2015). Since the early 1990s the use of synchrotron radiation (electromagnetic radiation produced in a synchrotron) has enabled the development and application of techniques for P speciation and molecular-scale processes in soil and related samples (Kruse et al., 2015). P speciation by X-Ray analysis using near Edge Structures (XANES) spectroscopy (P K-edge), using Synchrotron light as source of radiation, has been successfully used to characterize inorganic P forms in soils (Hesterberg, 1999; Hesterberg, 2010; Kruse et al., 2015) and has been used to identify Ca-bound P (e.g., apatites, brushite, di-calcium, tri-calcium and octocalcium phosphates), Fe-bound P (e.g., strengite), and Al-bound P (e.g., variscite) in soil samples (Kizewski et al., 2011). However, for organic P forms XANES has

been less successfully used, due to the lower crystallinity of organic P forms to XANES (low sensitivity with no specific spectra signature). For organic P, phytic acid (myo-inositol hexakisphosphate, IHP) is often used in P K-edge XANES data evaluation (Prietz et al., 2013; Liu et al., 2013). However, due to the organic P (Po) speciation difficulties inherent to IHP similarities in spectral variations to P sorbed to Fe, Al and Ca minerals (pre-edge peak, white line, post-edge shoulder and features) when metal phytates exist, it has been advisable to combine P K-edge XANES spectroscopy with complementary methods that are more sensitive to Po chemistry, such as ^{31}P -NMR (Kruse et al., 2015).

The ^{31}P nuclear magnetic resonance (NMR) has been suggested to provide a better understanding about organic P dynamics in environmental samples, such as soils (Negassa et al., 2010; Liu et al., 2013; 2014; Cade-Menum, 2015) and could be helpful to obtain detailed insights into soil organic P speciation. Phosphorus has only one isotope, ^{31}P , which is NMR-active with the highest sensitivity among the major elements cycling in soil (e.g., ^{13}C and ^{15}N) and, since the ^{31}P is the only natural occurring P isotope, all the P atoms in a sample can be accessed by ^{31}P -NMR (Cade-Menum, 2005; Kruse et al., 2015).

Therefore, we proposed in the present study to evaluate the current P fertilizer usage in Brazilian agriculture and, based on future cropland expansion and P demands by the crops scenarios, determine the fate of soil legacy P with trends for its utilization as a key aspect to improve P use efficiency in tropical agriculture. We also evaluated long-term changes on soil P fractions promoted by tillage systems and cover crops introduction in the soybean/cotton succession system in Brazilian Oxisols. Additionally, we performed a study on the P species present in Brazilian Oxisols under long-term conventional tillage and native soil by combination of the sequential chemical fractionation techniques (Hedley's procedure) with the most actual methods used to P speciation, P K-edge XANES and ^{31}P -NMR spectroscopies.

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2. TRANSITIONS TO SUSTAINABLE MANAGEMENT OF PHOSPHORUS IN BRAZILIAN AGRICULTURE

ABSTRACT

Brazil's large land base is important for global food security but its high dependency on inorganic phosphorus (P) fertilizer for crop production (2.2 Tg rising up to 4.6 Tg in 2050) is not a sustainable use of a critical and price-volatile resource. A new strategic analysis of current and future P demand/supply concluded that the nation's secondary P resources which are produced annually (e.g. livestock manures, sugarcane processing residues) could potentially provide up to 20% of crop P demand by 2050 with further investment in P recovery technologies. However, the much larger legacy stores of secondary P in the soil (30 Tg in 2016 worth over \$40 billion and rising to 105 Tg by 2050) could provide a more important buffer against future P scarcity or sudden P price fluctuations, and enable a transition to more sustainable P input strategies that could reduce current annual P surpluses by 65%. In the longer-term, farming systems in Brazil should be redesigned to operate profitably but more sustainably under lower soil P fertility thresholds.

Keywords: P fertilisers; Legacy P; P consumption; P availability; P fractions; manure P; P inputs; P outputs; P surplus

2.1. Introduction

Two main strategies exist to meet rising global food and biofuel demand: intensify the existing agricultural land area as much as possible, and/or expand into areas with native vegetation which would be detrimental to global biodiversity. A recent analysis¹ suggested that global crop yields are not increasing sufficiently rapidly to avoid the less sustainable second option (i.e. agricultural expansion), unless more effort is focused on the first option (agricultural intensification). A key challenge for society is to achieve agricultural intensification sustainably without further depletion of natural capital, degradation of the environment, or threats to human well-being²⁻⁴. Brazil is one example of a nation whose agricultural output has increased rapidly in recent decades due to advances in agronomic practices (e.g. improved varieties, double cropping and no-tillage cultivation systems), investment in agrochemicals (e.g. lime and fertilizers) and expansion of the cultivated land area⁵⁻⁷ (Figs. S1, S2 and supplementary discussion on the green revolution in Brazil). Important frontier areas of cropland expansion have been in Mato Grosso and Pará States, and in the Matopiba region (Maranhão, Tocantins, Piauí and Bahia states), and these areas are expected to continue expanding up to 2030 and beyond⁸.

Tollefson⁹ considered Brazil a global farm because of its large potential contribution to world food production. It is the second largest global supplier of food and agricultural products, and is forecast to be the foremost supplier to meet future global food demand⁷. The country's enormous land base, generally favourable climate and deep soils provide large potential to further expand agricultural output through both agricultural intensification and controlled cropland expansion. For example, an increase in both crop yield and cropping area is likely needed to meet future sugar and bioethanol demand¹⁰. The intensification of beef production (e.g. through higher stocking rates, improved grass varieties and better grassland management) could release existing low-input, degraded pastureland for conversion to intensive cropland without further disturbance to natural

ecosystems^{11,12}. According to Sparovek et al.¹², 469 Mha of native vegetation (57% of Brazil's total land area) is currently under government protection, and it remains an important national sustainability goal to preserve these areas¹³. However, this still leaves 114 Mha of native vegetation potentially exploitable for cropland expansion within the current government limits on deforestation¹². The considerable importance of agriculture to Brazil's economy, and the large contribution Brazil's agriculture makes to global trade as an exporter of soybean (*Glycine max*) and meat, reinforce the need to merge its future agricultural intensification with sustainable use of natural resources and limited environmental impact.

A major economic and environmental consideration in expanding Brazilian agriculture is the increased fertilizer requirement for higher agricultural output, and in particular phosphorus (P). Since most Brazilian soils are highly P fixing, large inputs of P fertilizers over and above crop P offtake are regularly applied to overcome the rapid immobilization of inorganic P that occurs in highly weathered soils rich in iron (Fe) and aluminium (Al)^{14,15}. Expansion of Brazilian cropland by either converting degraded low-input pasture or native savanna will therefore require considerable amounts of P fertilizer^{5,14,16}. Currently, Brazil's phosphate rock (PR) mines supply only limited amounts of P due to the igneous rock's low P solubility and high processing costs, and about 60% of inorganic P fertilizer used in Brazilian agriculture is imported¹⁷. More recent geological prospecting has identified several other PR reserves in Brazil, including those of sedimentary origin with higher P solubility¹⁸. However, logistical, environmental and tax issues are preventing these reserves from being explored in the short term. With increasing geopolitical concerns over future global PR accessibility and price volatility, a continued reliance on P imports may considerably increase the costs of food production in Brazil^{19,20}. The high demand for fertilizer P, and heavy reliance on imports, makes Brazilian agriculture particularly vulnerable to future P scarcity, or sudden fluctuations in the cost of P; for example, such as occurred in 2008 when the price of PR rose by 800% over a 12-18 month period¹⁹. It is also widely recognized that global PR reserves are a finite and critical natural resource^{21,22}, and that unused P is a costly pollutant of inland and coastal waters^{23,24}. Alternative management strategies to reduce reliance on imported P, and use P more efficiently, will therefore become increasingly important if Brazilian farming systems are to be sustainable well into the future.

Here we examine the current and future P demand of Brazilian crop production, and investigate transitional strategies for meeting this demand more sustainably by reducing reliance on costly and finite PR resources. We hypothesized that there is large scope to improve the efficiency and sustainability of P use in Brazil by accounting for the potential stocks of secondary P that could substitute for P imports and increase the resilience of Brazilian agriculture to future P scarcity, or sudden P price fluctuations. We further investigated how radical any change in fertilizer P inputs up to the year 2050 needs to be to reduce Brazil's P surplus in agriculture to near zero.

2.2. Results

2.2.1. Brazil's phosphorus demand: past, present and future

Total annual P fertilizer use in Brazil has increased from an average of 0.04 Tg in 1960 to ca. 2.2 Tg in 2016 (Fig S3A). This rapid rise in P fertilizer use has contributed substantially to the green revolution in Brazil, but fertilizer P inputs are twice plant demand, and have been since 1970 (Fig S3A). The vast majority of

this mineral P fertilizer is applied to cultivated crops (particularly maize (*Zea mays*), soybean and sugar cane (*Saccharum sp*)). Only about 1.5% of national P fertilizer consumption is attributed to pastureland, despite occupying substantial areas of marginal and degraded land (166 Mha)⁸. The average annual P fertilizer rate on all crops is currently ca. 25 kg P ha⁻¹ yr⁻¹ (Figure S3B), but there is large regional variation. For example, while the P rate applied to soybean is around 25 kg P ha⁻¹ in Paraná state (fertile soils), the average rate is 35 kg P ha⁻¹ in Goiás state, and 50 kg P ha⁻¹ in the Matopiba region, where a higher proportion of the soils are still responding to P fertilizer²⁵. Typical annual fertilizer P rates on maize range from 35-60 kg P ha⁻¹²⁵, whilst sugarcane typically receives 50-80 kg P ha⁻¹ for its establishment, and a further annual application of 10-15 kg P ha⁻¹ after third year of the usual up to 5-7-year continuous growing cycle²⁶.

The rate of increase in P fertilizer use over the last 20 years (5.5% yr⁻¹) is much greater than the average rate of cropland expansion over the same period (2.6% yr⁻¹, Figure 1A, B). This largely reflects the higher starter rates of P fertilizer used to overcome P fixation in new frontier areas converted to cropland, but also includes the additional P fertilizer inputs needed for the intensification of the existing cropland, especially where farmers have adopted double cropping (typically soybean and maize). For example, the areas with double cropping have increased from 3M ha to nearly 12 M ha in Brazil over the last 10 years²⁷, and this is evident as a sharp increase in total annual crop P offtake (Figure 1C).

The increased use of fertilizer P has led to considerable surplus P accumulation in Brazil's soils enabling increased soil P fertility. For example, the annual P surplus (calculated as total fertilizer and manure P inputs minus crop P offtake) increased nearly fivefold from ca. 0.34 Tg in 1974 to ca. 1.49 Tg in 2016 (Figure 1D). The total P surplus in 2016 represented nearly 70% of the P fertilizer used in that year and emphasizes how inefficient current P use is in Brazil. These trends could be a significant future drain on national and global PR resources if they were to continue.

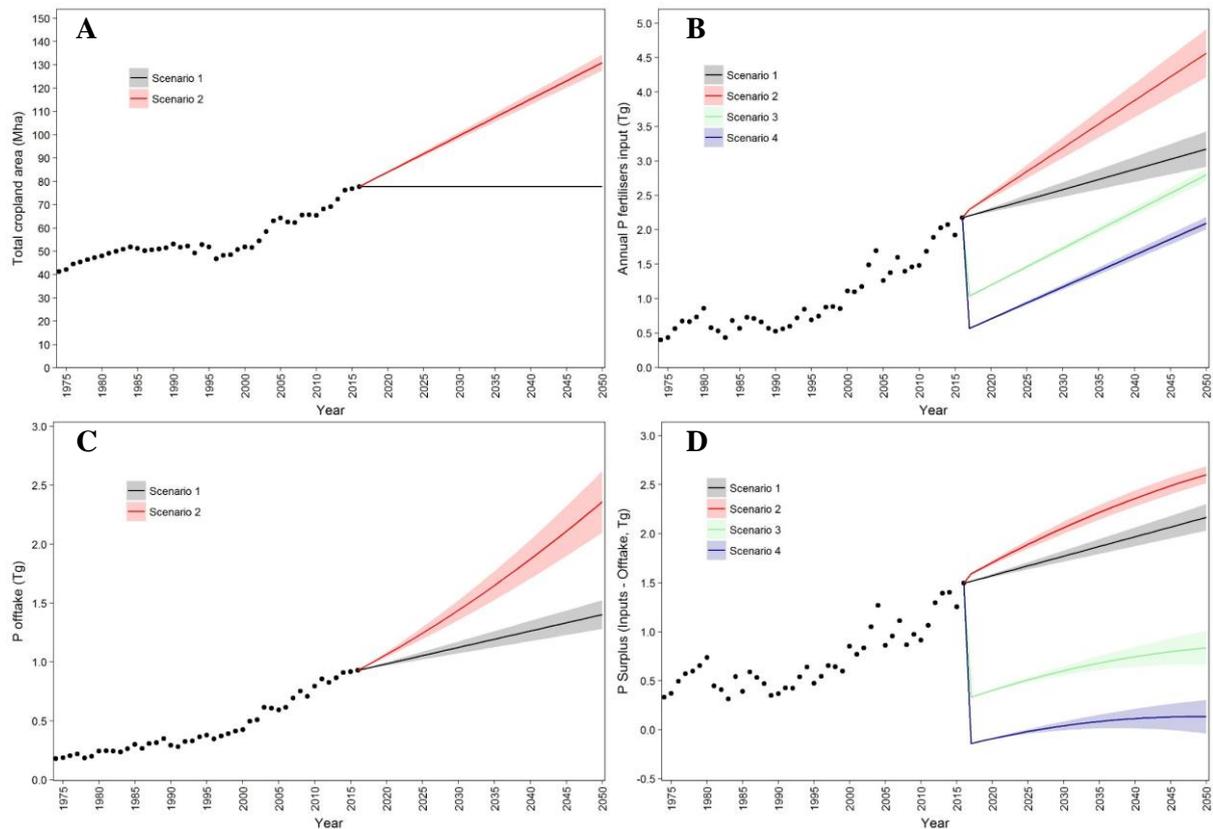


Figure 1. Brazil's cropland area from 1975 to 2050, and effects of future cropland intensification (scenarios 1 and 2) and P input reduction strategies (scenarios 3 and 4) on annual P fertiliser demand (B), annual crop P offtake (C) and annual P surplus (D). Scenario 1 - intensify existing cropland area; Scenario 2 - intensify existing cropland area + expand cropland into native Cerrado and degraded pasture areas; Scenario 3 – ratio of P inputs:outputs in cropland areas is 1:1; Scenario 4 – ratio of P inputs:outputs in cropland areas is 0.5:1. Coloured bands give the uncertainty surrounding each scenario prediction.

To assess the magnitude of future P fertilizer demand and potential P surpluses in Brazil up to the year 2050, we examined two cropping intensification/expansion scenarios:

Scenario 1: Crop production will intensify only on existing land areas

Further sustainable intensification of Brazilian agriculture without increasing the cropland area could be achieved through adoption of improved agronomic practices and technologies: for example, through the zoning of areas with different climates to optimise crop varietal choice^{7,9}. Annual yields of the three major cultivated crops (maize, soybean and sugarcane) have increased at a national average rate of 1.17, 2.84 and 0.60% per year, respectively over the last 20 years (Fig S2). If sustained, these rates of intensification would increase the mean yields of these crops to 9.6, 4.2 and 92.4 t ha⁻¹ by the year 2050, respectively. These forecast yield levels are well within the maximum yield potential of these crops considering both natural limitations (e.g. water availability)^{10,28}, and data from field trials²⁹. Combining the trends in yield for these three main crops gives an average rate of yield increase of 1.5% per year, which is identical to the annual increase in crop yields reported globally by Ray et al.¹. Although there is large regional variation in crop yields across Brazil, we predicted that if the average yield of all annual crops continued to increase at 1.5% yr⁻¹, and if P fertilizer use

increased commensurately with increased yield potential (as currently recommended to farmers), then fertilizer demand could reach 3.2 Tg ($41 \text{ kg P ha}^{-1} \text{ yr}^{-1}$) by 2050, leading to an annual surplus of 2.2 Tg (Fig. 1D). This demand represents an increase of 1 Tg (46%) compared to 2016 usage, and 15% of the anticipated global demand for inorganic P fertilizer on cropland (20.8 Tg) in 2050¹⁶.

Scenario 2: Crop production will expand into frontier areas and Intensify on existing areas

Rising global market demand for food has encouraged the recent rapid expansion of cultivated crops into native Cerrado and existing degraded pasture areas in central and North Brazil⁶. Regional variation in this rate of cropland expansion is wide. For example, maize production in Matopiba is expanding into degraded pastureland at over twice the national rate³⁰. This frontier area now produces over 10% of the country's soybean production, and is forecast to nearly double its production over the next decade^{30,31}. If the current average annual rate of all cropland expansion ($2.6\% \text{ yr}^{-1}$) across Brazil continues, we estimated the cropland area would increase to 131 Mha by 2050 (Fig 1A). This represents an increase of 53 Mha in cultivated annual crops compared to 2016, which is slightly greater than the increase of 38 Mha (mainly in soybean and sugar cane) projected by the Brazilian Ministry of Agriculture^{7,30}, but well within the 130 Mha (native vegetation plus degraded grassland) that is potentially suitable for conversion¹². Conversion to cropland would require additional P fertilizer to overcome soil P fixation, termed by Roy et al.¹⁴ as a P fixation tax directly linked to soil P retention capacity⁵. For example, initial fertilizer inputs to increase P availability to critical levels in Cerrado soils can range from 26 to 122 kg P ha^{-1} for sandy to clayey soils, respectively²⁵. In our analysis we assumed a conservative P fixation tax of 35 kg P ha^{-1} in the first year of cropping in addition to an average annual rate of P application of 25 kg P ha^{-1} for all new frontier cropping areas. Combining the forecast P inputs for the intensification of existing cropland (Scenario 1) together with the forecast P inputs to new frontier areas, the annual P fertilizer demand in Brazil in the year 2050 was estimated at 4.6 Tg ($35 \text{ kg P ha}^{-1} \text{ yr}^{-1}$), leading to an annual surplus of 2.6 Tg (Fig. 1D). This demand represents an increase of 110% in total P consumption compared to 2016, and 22% of the anticipated global fertilizer P demand in 2050. Future fertilizer demand in Brazil does therefore constitute a significant drain on finite global P resources, requiring transitional strategies to reduce this demand and improve efficiency.

2.2.2. Secondary sources of phosphorus in Brazil

A key P stewardship strategy to improve the efficiency and sustainability of P use in the food chain is to re-use (recover and/or recycle) secondary sources of P as a substitute for the imports of highly soluble inorganic fertilizers derived from PR^{32,33}. This requires an understanding of the potential stocks of secondary P present in Brazil.

Secondary bioresources in Brazilian agriculture

Agriculture produces a number of bioresources or processing residues that could be potentially recycled back to land as secondary sources of P. Bioresources have the added advantage over mineral fertilizers in that they contain useful organic matter and water holding properties for improving general soil quality and reducing P fixation in tropical environments³⁴⁻³⁶.

Animal numbers in Brazil have increased rapidly in recent decades and generate significant amounts of manure P (Figure 2A, B). The manure from confined cattle, pigs and poultry that is currently recycled to cropland is estimated to contain 267 Gg P (Table 1), with pigs and poultry providing the majority (93%) of this secondary P resource. Manure from unconfined cattle was not included in this analysis because it is largely dispersed within pasture areas since animals graze all year in Brazil at low (1.1 head ha^{-1}) stocking densities¹¹. Based on the average rate of increase in cattle, pig and poultry numbers over the last 10 years (Fig. 2B), the total manure recycled to land can be expected to provide 421 Gg P yr^{-1} by the year 2050, or 13 and 9% of Brazil's future P demand in 2050 for scenario 1 and 2, respectively (Table 1). Amounts of biosolid P currently produced from wastewater treatment was estimated at only about 1 Gg (Table 1). According to Andreoli et al³⁷, only one third of the human population have combined sewage collection and treatment, and only about 15% of the treated wastewater biosolids that are produced are recycled to land. Biosolid P production from Brazil's population is increased to only ca. 1.1 Gg in the year 2050 if current collection and treatment infrastructure remains the same, and suggests there is considerable underutilization of potential wastewater P resources in Brazil.

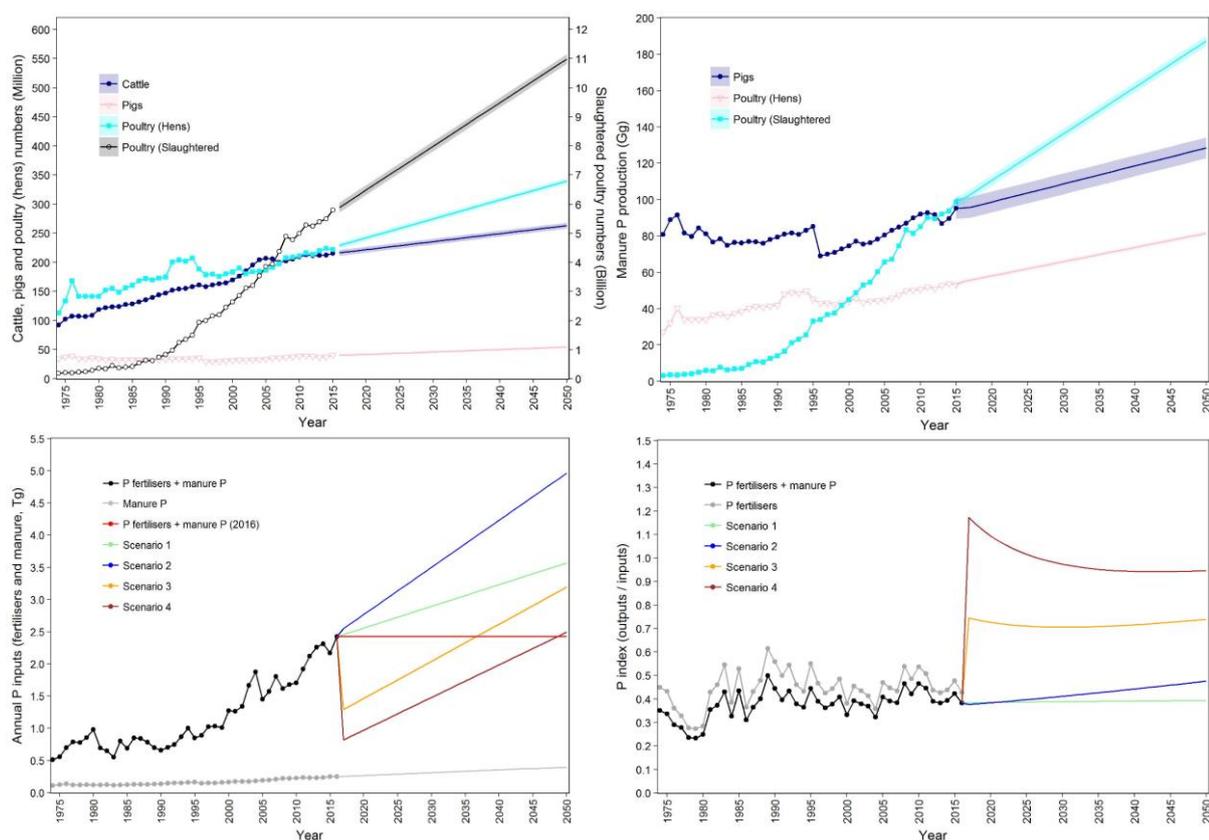


Figure 2. Brazil's animal numbers (A) and manure P production from pigs and poultry (B) from 1975 to 2050, and the contribution of manures to total annual P inputs (C) and P efficiency index (D) up to 2050 and for each scenario. Scenarios are as for Figure 1 and coloured bands give the uncertainty surrounding predictions.

The processing of sugarcane to produce sugar and ethanol produces a filter cake (30% dry matter) and a liquid effluent (vinasse) that can be beneficially recycled to land³⁸. Total production of filter cake is currently estimated to be ca. 3.2 Tg of dry solids (DS) containing 32 Gg of P (Table 1). Based on the forecasted increase in

sugarcane area and yields, the amount of potentially recyclable filter cake P was estimated at 87 Gg by the year 2050, or 2-3% of P demand. Liquid vinasse is more difficult to apply and contains much less P than filter cake³⁸; thus it was not considered to be a significant P bioresource. As pre-harvest burning is now being phased out in Brazil, the sugarcane crop (as do other crops) produces significant amounts of straw residue (ca. 90 Tg yr⁻¹) which can be generally beneficial for erosion control, nutrient cycling (e.g. contains 45 Gg of P) and improved soil C content³⁹. However, this straw is more likely to be partially, or fully, removed for use as a biofuel, or for cellulosic ethanol production. Other crop residues exported off the field (e.g. coffee (*Coffea*) and cotton (*Gossypium*)) have been combined with localized sources of manures to make composts, but we considered the amounts returned to land are relatively small nationally as these crops occupy relatively small areas²⁷.

Hence, we estimated that secondary bioresources currently produced on an annual basis in Brazil contain 0.3 Tg of P (Table 1), which represents 14% of current annual fertilizer P use. This secondary P resource will rise to 0.5 Tg of P in 2050, which would still only represent 16% and 11% of Brazil's anticipated fertilizer use for scenarios 1 and 2, respectively. Alternative secondary sources of P therefore need to be explored.

Table 1. Amounts of P contained in different secondary bioresources in Brazil annually. For 2050 predictions, confidence intervals are given in parenthesis.

Resource type	Numbers or dry solids (2015) (M or Tg DS)	Quantity of P in 2015 (Mg)	Numbers or dry solids (2050) (M or Tg DS)	Quantity of P in 2050 (Mg)	Reference
Animal manures					
Confined cattle	3.2	19,200	4.0	24,000	64
Pig	40.3	95,314	54.4 (± 2.4)	128,472 ($\pm 5,695$)	61
Poultry	-	-	-	-	-
Hens	222.1	53,309	339.7 (± 4.9)	81,532 ($\pm 1,180$)	62
Slaughtered poultry	5,796.2	98,840	10,985.9 (± 167)	187,335 ($\pm 2,850$)	These authors
Total (animal manures)	-	266,663	-	421,339	-
Sugar cane					
Filter cake	3.2	32,164	8.7	87,318	33
Wastewater biosolids	0.8	956	0.9	1,062	32
Total (biosolids)	-	33,120	-	-	-
Total (animal + biosolids)	-	299,783	-	509,719	-

Legacy soil P

Overall, Brazilian agricultural fields have received more fertilizer P than required for crop needs since 1970 (Fig. S3A) and has therefore accumulated significant legacy soil P reserves. We calculated that a cumulative total of over 45.7 Tg of inorganic fertilizer P has been applied in Brazil since early 1950s, when fertilizers were first regularly used, and that 22.8 Tg of this input remains in the soil. Moreover, an additional 7 Tg of manure P from pig and poultry farms has been recycled to cropland since 1974 based on numbers of animals and their average excreta P content, and assuming all manure is recycled in some form to land (data for confined cattle are available only from 2000²⁷). Hence, although a slight underestimate of total manure P loading, the manure input from pigs and poultry represents at least 16% of the total fertilizer P inputs since 1960 (Fig. 1C). Manure inputs therefore make a significant contribution to the surplus P accumulating in Brazilian farming systems and soils but their use lowers the national P efficiency index (defined here as ratio of P inputs in fertilizers and manures to P outputs in crop P offtake), (Fig. 2D). After accounting for cumulative crop P offtake, the total cumulative surplus P accumulating in Brazil's soils as legacy P since 1960 therefore amounts to at least 29.8 Tg (Fig. 3), with the vast majority of this residing in cropland, since very little P is applied to pasture in Brazil. We estimated the corresponding cumulative amounts of legacy P remaining in the soil by the year 2050 could reach 92 and 103 Tg, respectively for each cropland intensification scenario (Fig. 3). These reserves of P are substantial and in principle could potentially meet Brazil's crop P demand for many years depending on its bioavailability.

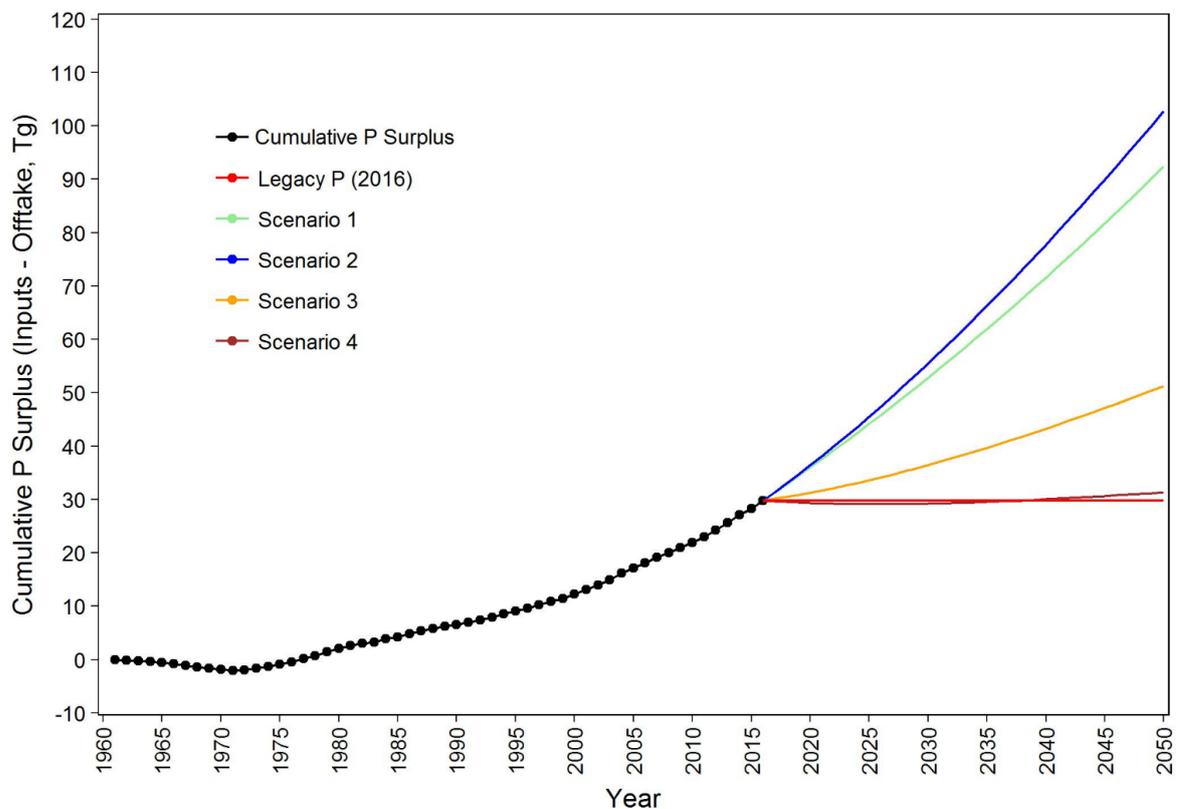


Figure 3. Total legacy P present in Brazilian soils from 1960 to 2050 for each scenario. A P input strategy of applying 50% of plant P offtake (scenario 4) is needed to limit further accumulation of P surpluses in the soil at current cropland expansion rates in Brazil. Scenarios 1-4 are as described in Fig.1.

To evaluate the potential crop availability of this legacy soil P, we examined soil P dynamics in six long-term trials (14 - 38 years) representing typical acidic, clayey, highly P fixing (high Fe levels) soils of the Brazilian Cerrado region, where most of the future agricultural expansion and intensification is anticipated to occur. All site details are given in Table S1. In summary, the sites had soybean, maize and cotton as the main crops during the summer, and cover crops or legumes as the winter crop. At sites 1-5, no tillage (NT) was compared with conventional tillage (CT) systems, but site 6 was cultivated only under a NT system. To compare the accumulation of legacy soil P forms, an adjacent area of native vegetation (NV) close to the experiments, with similar soil type, was also included as a natural reference system (except site 6). At site 6, a high P input system (HP) was compared with a low P input system (LP).

A detailed P balance for each site showed that the net P balances accumulating in the soils at each site were fully accounted for by the changes in soil total P between cultivated treatments and the native Cerrado control (Fig. 4A, B and Table S2).

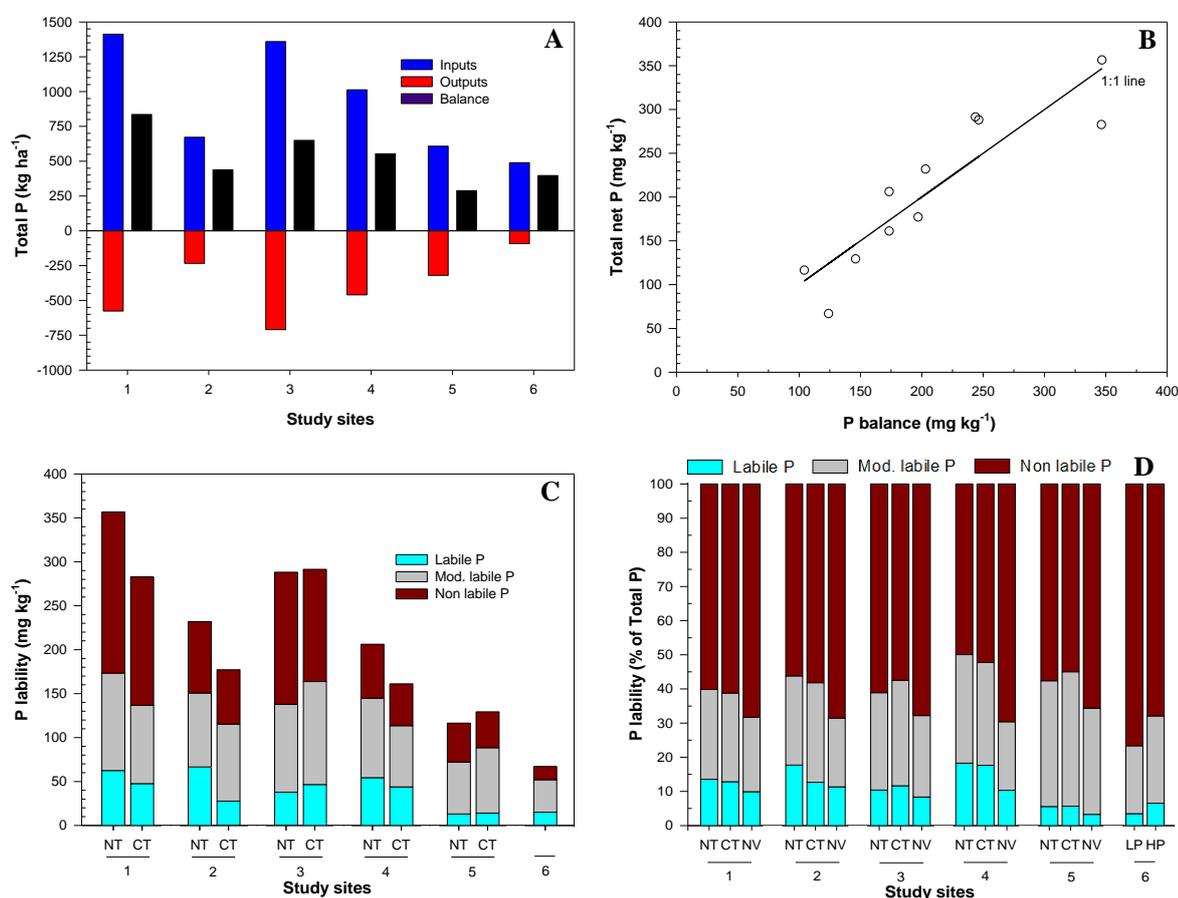


Figure 4. Legacy soil P in six different Brazilian long-term field sites; **A)** P inputs, outputs and balance since conversion from native Cerrado vegetation; **B)** Change in soil total P as a function of the total P surplus balance with the 1:1 line drawn; **C)** Increase in soil P for each tillage/P input treatment as a function of P lability according to the Hedley sequential fractionation method; **D)** P lability as a percentage of total soil P in native vegetation and cropped areas for each tillage/P input treatment. NT – no tillage; CT – conventional tillage; NV – native vegetation, LP – low P; HP – high P.

Soil total P typically increased up to two-fold as a result of the cumulative surplus P additions under cultivation (Table S3). Of this total P increase, 11-26% was in labile forms (crop available), 32-58% was in moderately labile forms (partly crop available) and 22-52% was in non-labile forms (crop unavailable) according to the Hedley fractionation procedure (Table S3)⁴⁰. More non-labile P tended to accumulate at sites 1 and 3 (Fig. 4C), which had greater clay and Fe oxide contents, and more P tended to accumulate in inorganic P forms than in organic P forms (Table S3). At all sites, and despite continuous fertilizer inputs for up to 38 years, the majority of total P in the soils (i.e. legacy P + native P) still resided in a non-labile form (Fig. 4D). However, the amounts of labile and moderately labile legacy P potentially available to crops still represent a considerable P reserve (up to 7.7 and 15.5 Tg, respectively).

2.2.3. Transitions to more sustainable phosphorus use

With limited potential to substitute secondary bioresources for annually imported P fertilizer, more radical strategies for improving the efficiency of P use must place a greater reliance on the value of legacy soil P in Brazil^{41,42}. Sorption theory suggests soil P immobilization should gradually decrease (i.e. P tax diminishes) as P fixation sites in the soil become blocked leading to an increase in both soil labile P and the P efficiency index⁴³. For example, in the long-term sites 1-4 (Table S1), the P efficiency index in maize-soybean rotations gradually increased from 0.47 to 0.80 over the experimental period, and with a notable increase in 2008 when fertilizer inputs were reduced due to excessive pricing of PR on the world market (Fig. S4C). Continued legacy soil P accumulation should therefore eventually increase soil labile P sufficiently to enable a transition to a near-maintenance fertilizer strategy that aligns P inputs more precisely to actual crop offtake⁴⁴. Field trials in Brazil suggested that the combined use of NT, cover crops, correction of acidity and adoption of the 4R principles of nutrient management (Right rate, Right time, Right place and Right form⁴⁵), could reduce inorganic fertilizer P inputs from their current high level to a near maintenance level to improve overall P use efficiency^{46,47}. The current farmer practice of generous overuse of P may therefore not be justified. We estimated that a transition to maintenance P applications (from all annually available P sources) on existing cropland from 2016 onwards (Scenario 3) would reduce the annual surplus in 2050 to 0.83 Tg (i.e. a reduction of 65%), and limit the cumulative P surplus in the soil to 51.3 Tg, or nearly half the corresponding estimates for scenarios 1 and 2 (Fig. 3). This suggests that technology transfer will need to play an important role in the future adoption of more sustainable P input strategies by farmers in Brazil.

Since a significant proportion of the increase in legacy soil P is in labile or moderately-labile forms, we argue here that legacy soil P could further be relied upon to buffer the economic impact of future PR price volatility by applying less P than crop P offtake. It may be possible to eliminate or reduce further P applications if there is sufficient available legacy P to maintain soil P supply and prevent any decline in crop yield. Recent evidence for a clay soil under NT management in Southern Brazil suggested that moderately labile P was at least partially utilized by crops (Table S4). Other field evidence suggested that the recovery of legacy P could be as high as 80% depending on cropping system and the amount of available legacy soil P present⁴⁶. However, it is difficult to predict the bioavailability of legacy soil P, and, at this point, total reliance on legacy soil P to supply sufficient crop P is risky. Although unfertilised areas with high levels of legacy P have generally shown yield losses of soybean and maize, as compared with fertilized areas, an intermediary P input strategy with plant

demand being in part supplied by the soil P reserve could be achieved without yield loss⁴⁶. In support of this strategy, a range of recent studies in China have shown how crop yield can be maintained, or even increased, while fertilizer application is reduced below offtake requirements by targeting the P supply to the crop root zone to increase P uptake efficiency⁴⁸. We estimated that a transition to 50% below-maintenance P applications on existing cropland from 2016 onwards (Scenario 4) would be needed to limit the cumulative P surplus in 2050 to close to its 2016 level of ca. 30 Tg (Fig. 3).

2.3. Discussion

The investment cost of increasing soil P fertility to optimize crop yields in Brazil is high because of the P fixation tax demanded by Cerrado soils following conversion to cropland^{5,14}. Fertilizer P inputs are still double crop P offtake nationally (Figure 2D), and Roy et al.^{14,49} argued that this high rate of P input will need to continue for many years, especially on clayey soils. This P input strategy may not be sustainable in the long-term if Brazilian cropland continues to expand at its current rate of 2.6% yr⁻¹, and continues to rely on imports of primary fertilizers derived from finite global P reserves. Brazil's own reserves of exploitable PR are of igneous origin and of relatively poor quality (i.e. low P content and low solubility in citric acid) compared to the higher quality sedimentary-derived PR currently imported from Morocco, Tunisia, Algeria, Egypt, USA and Peru⁵⁰. Total reserves of PR currently available for exploitation in Brazil are estimated at 320 Mt⁵¹, which at current extraction rates (ca. 6.7 Mt yr⁻¹) will be exhausted in about 50 years. However, investment in geological research over the last decade has identified new potential reserves in Brazil that are not yet accounted for, although their quality and the costs to process them remain uncertain. Investments in new PR processing technologies will be needed to capitalise on these PR resources of marginal quality, and the price of PR on the world market (driven by PR supplies from Morocco) will determine decisions on new mine investments¹⁹. Brazilian agriculture is therefore likely to remain heavily dependent on inorganic P imports to satisfy its growing fertilizer P demand and therefore vulnerable to future P scarcity. Continued overuse of P fertilizer relative to crop demand will also lead to further soil P accumulation and potential long-term eutrophication problems^{52,53}.

Secondary bioresources in Brazil could make an important contribution to annual P fertilizer demand, provided these materials are available to apply in both existing and expanding frontier areas, and have adequate P bioavailability. Brazilian farmers will require evidence that these materials are satisfactory alternatives to highly soluble mineral fertilizers that they have come to depend on. For example, research suggests that while filter cake is an effective nutrient source³⁸, the P availability of Fe-rich wastewater biosolids may be more limited⁵⁴. Alternative biotechnologies to treat human and industrial wastewater will be necessary to continually provide a renewable resource which is of better value to soil quality and nutrient provision in Brazilian soils. Our analysis also highlights a major underinvestment in recovering P from the food chain in Brazil. If 80% of Brazil's 210 million people were connected to a central sewerage collection system (over 80% of Brazil's population live in cities), and 80% of this collected waste was treated, and 80% of the biosolids produced was recycled to land, we estimate a potential secondary resource of 10.3 Gg of P annually. This is over ten times more than is currently recycled. Similarly, food waste in Brazil will be considerable, but this resource is currently not being conserved and recycled. Assuming food waste per person in Brazil is the same as in Europe (173 kg)⁵⁵, and that food waste contains approximately 1.5 g P kg⁻¹ of waste⁵⁶, this bioresource could provide 45

Gg of P annually. Developing an effective circular economy for P in Brazil will therefore require further investment in P recovery infrastructure, and the creation of suitable markets for these secondary P resources, including those linked to the production of bioenergy, although this will remove the value of the organic matter present in the original material⁵⁷⁻⁵⁹. For example, (partial) recovery of P from different bioresources would enable their more widespread distribution. With additional investment in P recovery technologies for wastewater and food waste (as discussed above), and using all potentially available secondary bioresources as detailed in Table 1, we estimate that annually produced bioresources could provide no more than ca. 0.68 Tg of P by the year 2050, or 15-20% of projected fertilizer demand. Although a potentially significant and nutritionally useful secondary P resource, fertilizer substitution value of annually produced bioresources is therefore limited in capacity.

The largest potential source of secondary P that might be used as a substitute for fertilizer P imports is clearly the legacy soil P that has already accumulated in Brazilian soils (28 Tg). This secondary P resource can be accessed *in situ* and incurs no external costs of recovery. The utilization of legacy soil P in existing cropland areas is attractive because it not only provides immediate financial savings on inputs of inorganic P fertilizers, but also reduces the longer-term risk of water eutrophication arising from P in land runoff⁴²: for example, on more steeply sloping land converted from pasture where erosion risk might be expected to be greater. Our estimate of legacy soil P is greater than the 20 Gg estimated by Roy et al.¹⁴ who accounted for mineral fertilizer P inputs only, and used FAOSTAT land areas⁶⁰ rather than Brazilian census data. At a current P fertilizer price of \$1.4 kg⁻¹ of P, this legacy P is valued at over \$40 billion if it could all be utilized by crops. Regional variation in soil legacy P will be large, especially in areas where livestock manures cannot be distributed evenly due to transport costs, and where they are applied to crops to meet the nitrogen (N) requirements of crops due to a low manure N:P ratio. The strong relationship between surplus P accumulation and soil total P across the field sites reported here (Fig. 3B) suggests that simple measurement of soil total P in cropped and naturally vegetated areas will provide a good guide to the total legacy P reserves present.

The major barrier to the use of legacy P in tropical soils for profitable crop production is whether it can be mobilized sufficiently to provide all or part of crop P demand, and over what time period this soil P store can be utilized. While labile P fractions can be assumed to be fully crop available, the fertilizer replacement value of moderately labile and non-labile P forms of legacy soil P for Brazilian farming systems requires further clarification. However, just considering the significant amounts of labile P remaining in the soil, there is clear potential value in utilizing legacy soil P as a buffer against future P scarcity, or fluctuating P prices, by allowing short-term cessation of P inputs without risk of yield penalty. In the longer-term, sustainable P use and increased resilience of food production systems in Brazil, as elsewhere, will require crop production systems to be redesigned to lower crop P demand, operate under lower soil P fertility and maximise soil P acquisition and P use efficiency through advanced crop, microbial and fertilizer engineering – termed agro-engineering by Rowe et al.⁴². This redesign could be achieved through the development of more integrated farming systems linked to land use capability⁶¹, more P-efficient crop cultivars with lower seed total P⁶², crop rotation design to exploit the plants innate ability to scavenge for soil P⁶³, the use of targeted bio-inoculants⁶⁴, and the development of novel fertilizers that by-pass the soil⁶⁵ (See further supplementary discussion on Agro-engineering).

In summary, there is large scope to expand cropland output in Brazil without impeding the nation's extensive grazed beef production systems or contravening deforestation rules. Brazil's high P fertilizer demand

leads to large inefficiency because it does not account for legacy soil P reserves. Crop production to meet demand will continue to represent a drain on global P resources unless more sustainable P input strategies are adopted. These strategies should include (a) national and regional investment in P mining and P recovery technologies to provide cost-effective domestic PR sources, or secondary P resources that can substitute for imported P, and (b) transition to maintenance, or below maintenance, P input strategies where legacy soil P stocks provide all, or a portion, of crop P requirements and reduce reliance on diminishing PR reserves. With a bank of soil P fertility for long-term P security, farming systems could be redesigned to perform profitably and efficiently under lower levels of soil P fertility.

2.4. Material and Methods

2.4.1. Intensification Scenarios

Two scenarios for agricultural intensification in Brazil to 2050 were constructed based on past trends and national census data of Brazil's total cropland area²⁷, crop production⁶⁶, animal numbers²⁷, and P fertilizer consumption⁶⁷.

Scenario 1: Brazil's agricultural intensification will occur only on the existing cropland area and crop yields will increase by an average 1.5% per year based on the combined average yield trends of the major crops maize, soybean and sugarcane over the last twenty years.

Scenario 2: Brazil's agricultural intensification will occur on the existing cropland area *and* new croplands (i.e. excluding commercial forests and pasturelands) will expand into both native Cerrado areas and degraded pasture areas at a combined rate equivalent to the increase in the annual cropland area over the last 20 years (2.6% yr⁻¹).

Cropland and crop production: Total cropland includes all annual temporary crops and permanent crops, but since the permanent cropland area has remained very stable (Fig. S1A), cropland expansion to 2050 was based on the rate of change in annual cropland area over the last twenty years (1996-2015). Trends in crop yields were based on the major crops maize, soybean and sugarcane, which represent 82% of all annual crops in Brazil (Fig. S1B), and where most of the future agricultural expansion will occur. The predicted expansion of these crops to 2050 was based on average yield trends (1996-2015 Fig. S2). This was a period of improved economic stability in Brazil and provides a robust base for future forecasts³⁰. Yield trends were estimated using the autoregressive (AR) integrating (I) moving-average (MA) model (commonly named ARIMA), accounting for autocorrelation in the time-series, selected based on Akaike information criteria (AIC)⁶⁸. The time-series analyses were performed in R 3.4, using the forecast package⁶⁹. The annual yield increment trends for each of the three crops were normalised for yield level and pooled by the parametric bootstrap method⁷⁰ to provide a statistically-based combined average yield trend, which was then applied to all cropland.

Animal numbers and manure P production: Trends in numbers of total cattle (dairy + beef), pigs and poultry (data available from 1974²⁷) were based on incremental rates over the last decade (2006-2015): cattle, 0.38%, pigs, 1.70%, layer hens, 1.76% and broiler chickens, 4.17% (Table S1). Amounts of P excreted by

grazing cattle were assumed to be totally recycled to pasture areas and were not included further. Data on confined cattle were not included in the trend analysis for manure P production (Fig. 2B) because future expansion of livestock sector is forecast to be largely in pig and poultry numbers³⁰. However, manure P from confined cattle is included in the bioresource estimates for 2016 and 2050 (see below). Average amounts of P excreted by pigs and poultry annually were based on the typical volumes of excreta produced by each type of livestock and average values of P in excreta modified to take account of larger liveweights in Brazil. For pigs, we assumed a daily excretion of 7 L day⁻¹ of slurry with a density of 1.014 kg dm⁻³ and P content of 0.91 g P kg⁻¹⁷¹. For poultry hens, we assumed a daily excretal P production of 0.24 kg P animal⁻¹ yr⁻¹⁷². For litter from broiler chickens, we assumed a standard chicken house with 20,000 birds and six production cycles of 60 days would generate 220 Mg of litter in each cycle with a P content of 9.3 g P kg⁻¹. All P excreted by pigs and poultry is assumed to be recycled to cropland areas.

Fertilizer P consumption: Trends in total fertilizer P use (data for deliveries on farm available from 1960⁶⁷) to 2050 for scenario 1 assumed that the P fertilizer requirement will increase proportionally to crop yield (i.e. 1.5% yr⁻¹). This maybe an overestimation in practice if farmers are economically constrained and limit P applications within the next 35 years. For scenario 2, fertilizer P inputs to the existing cropland area were assumed to the same as for scenario 1, but for new areas we assumed an initial single input of 35 kg P ha⁻¹ to overcome soil P fixation²⁵, followed by a constant input of 25.2 kg ha⁻¹ which is the average annual fertilizer P use on crop land over the last 10 years.

P surpluses and efficiency index: P surpluses for each scenario were calculated as the difference between the total P inputs (fertilizers + manure) and total P outputs (crop offtake) and did not include P losses in surface runoff which were anticipated to be minimal in a P-fixing soil environment⁴⁹ and flat cerrado landscapes. Total P inputs were calculated as above. Annual average crop P offtake was calculated for both annual and permanent crops based on data from Roy et al.¹⁴. Predictions of crop P offtake for the years 2016-2050 assumed that P offtake would increase at the same rate as yield (1.5% per year), and that crop yields in new areas (Cerrado and degraded pasture) would be the same as the national average when P fertilizer was applied. A P efficiency index was calculated as the ratio of P inputs (fertilizer + manure) and crop P offtake.

2.4.2. Secondary P Resources in Brazil

The amounts of P contained in potential secondary P resources that could be used as a substitute for mineral fertilizers derived from PR were calculated for the year 2016 and projections made to the year 2050 (Table 1).

Livestock manures: Estimates of P in pig and poultry manure were made as detailed above. National averages conceal large variability in the P content of livestock diets on individual farms and this can influence local excretal P concentrations. Numbers of confined cattle in 2015 and 2050 assumed that 15% of total cattle are slaughtered annually²⁷ and 10% of slaughtered cattle are confined feedlot cattle (according to ANUALPEC,

cited by Costa Junior et al.⁷³). Export of P in cattle excreta was taken as 6 kg P animal⁻¹ yr⁻¹ assuming a medium P diet⁷⁴.

Sugarcane: P contained in the filter cake produced as a by-product from the processing of sugar cane assumed 5 kg of cake is produced per tonne of sugar cane harvested with a P content of 10 g kg⁻¹^{38,75}. We assumed there would be no transport restriction of filter cake to a sufficient area of agricultural land for recycling up to 2050 and a long-term P bioavailability of 100%, as is usually assumed in fertilizer recommendation systems when assessing the value of bioresources to long-term soil P fertility⁷⁶.

Wastewater biosolids: P contained in biosolids assumed that 48% of the population were connected to a sewerage collection system, 66% of collected sewage was actually treated, 12 kg of biosolids (100% dry matter) were produced per capita of the treated population, and 15% of the biosolids produced after treatment were recycled to land with a P content of 8.5 g P kg⁻¹ biosolid DM³⁷. Population estimates for 2050 were taken from the United Nations database⁷⁷.

Legacy soil P: Legacy P remaining in the soil since 1960 was taken as the cumulative surplus calculated from the balance between P inputs (fertilizers and manures) minus crop P offtake as calculated above.

Six long term trials in Brazil's main cropland production areas were selected to investigate the amounts and forms of legacy soil P that have accumulated since conversion of the native Cerrado vegetation. A summary of the background site details is given in Table S1. At sites 1-5, soil P was measured under no-tillage (NT) and conventional tillage (CT) treatments with different cover crops and compared with soil P under natural cerrado vegetation (NV) as a reference area¹⁵. At site 6 under a NT cultivation system, soil P was measured for treatments comparing low P (LP) fertilizer (6.9 kg P ha⁻¹) and high P (HP) fertilizer (34.9 kg P ha⁻¹) inputs. The experimental treatments were arranged in a randomised block with three replicates. These sites are representative of a large area of cultivated cropland in the main Cerrado region of Brazil, with acid, clayey, high P-fixing soils and typical cropping systems that include soybean, maize and cotton. They provide a suitably large range in the legacy period over which a P input-output balance can be calculated (14-38 years) (Table S1).

For each site, a detailed P balance was estimated for each treatment considering the total fertiliser P inputs and the total P offtake in the harvested crop since the area was converted from the native forest vegetation to cropland (Table S2). Annual P inputs (fertilizer P) and P outputs (yield x grain P content) were based on measured values for the period of the replicated trial, and estimated for the period between deforestation and when the trial began (when measured data were not available) by considering the average values from the first three trial years (sites 1, 3 and 4). Soil samples to a depth of 20 cm were collected from all treatments and natural reference areas at each site by auger in 2011 (site 5), 2012 (sites 1 and 2), 2013 (3 and 4) and 2015 (site 6) for P soil P analysis. Previous work showed that P did not migrate below this depth¹⁵. Changes in soil P forms were determined by the Hedley sequential P fractionation method⁷⁸, with modifications by Condon et al.⁷⁹. This method uses chemical extractants to remove progressively from the same sample the most available to the most stable forms of inorganic (Pi) and organic P (Po). Sequentially-extracted P was grouped into three fractions of P lability: a) labile P, corresponding to the inorganic P extracted by anion exchange resin and the inorganic and organic P extracted by 0.5 mol L⁻¹ NaHCO₃; b) moderately-labile P, corresponding to the inorganic and organic

P extracted by 0.1 mol L⁻¹ NaOH, and inorganic P extracted by 1.0 mol L⁻¹ HCl; and c) non-labile P, corresponding to the inorganic and organic P extracted by 0.5 mol L⁻¹ NaOH, and the P in the remaining residue¹⁵. The soluble reactive P concentration in each extract was measured according to colourimetry using the Murphy and Riley⁸⁰ procedure for acid extracts, and Dick and Tabatabai⁸¹ procedure for alkaline extracts. Organic P in each extract was determined by the difference between total P and inorganic P. A summary of the amounts of P extracted in each sequential P fraction is given in Table S3.

2.4.3. Sustainable P management scenarios

Two scenarios of more sustainable P management in Brazil up to the year 2050 were devised (Scenarios 3 and 4) to examine their potential impact on fertilizer P demand and surplus soil P accumulation compared to the previously discussed scenario 2, which is the most likely to occur and includes both intensification of existing cropland and cropland expansion into native Cerrado and degraded grassland.

Scenario 3: Total annual P inputs (all sources) into existing cropland in Brazil will not exceed annual crop P offtake. This scenario assumes that adequate levels of soil labile P for optimum yields in Brazil have already been attained. Surpluses of P will still occur in this scenario because of P inputs to overcome P fixation in new frontier areas converted from native Cerrado, or degraded pastureland.

Scenario 4: Total annual P inputs (all sources) into existing cropland in Brazil will not exceed 50% of annual crop offtake. This scenario was developed to produce no further increase in the cumulative legacy P in the soil in 2050. It represents a more extreme P input strategy that relies on the bioavailability of legacy soil P reserves to sustain yields and still needs to be proven feasible.

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3. TILLAGE SYSTEMS AND COVER CROPS AFFECT SOIL PHOSPHORUS AVAILABILITY IN BRAZILIAN CERRADO OXISOLS

ABSTRACT

Crop rotations and soil tillage management cycle soil phosphorus (P) and keep it stored in topsoils in a bioavailable form for subsequent cash crops. Changes in soil P fractions promoted by no-tillage (NT) and conventional tillage (CT) in soybean/cotton crop systems with different cover crop residues were investigated in long-term Brazilian Oxisols (Ox-1 and Ox-2), and compared to native Cerrado vegetation. Hedley's P fractionation was performed in soil samples taken from 0-5, 5-10 and 10-20 cm depth layers and P fractions grouped by their predicted lability. After long-term cultivation, similar P availability was observed in the surface layers (0-5 and 5-10 cm). Long-term cultivation generated large amounts of legacy P in the soil but only a small portion remained in labile fractions, with a slight increase in non-labile P and Po concentrations under NT cultivation. The brachiaria cover crop recycled more P than other crops, increasing the labile P and all the organic P fractions. Based on the cover crop effects, we propose that the bioavailability of legacy P could be represented by the amount of inorganic P extracted by the labile fractions + moderately labile P fractions.

Keywords: P lability; P fractions; Hedley's fractionation; Cerrado

3.1. Introduction

Phosphorus (P) is an essential nutrient to maintain or increase the productivity of crops in tropical agriculture (Novais et al., 2007; Novais and Smith, 1999). However, P fertilizer is a costly and price volatile agrochemical input, and may become more scarce and/or expensive as finite mineable reserves of phosphate rock are gradually used up (Cordell et al., 2009; Elser et al., 2014). There are strong economic and environmental arguments to increase the efficiency of use of this non-renewable resource, especially in emerging economies such as Brazil, where future P demand is expected to further increase in line with agricultural intensification (Roy et al., 2016; the author, chapter 2)

The expansion of cash crop agriculture into Cerrado areas since the 1970's has contributed significantly to world food production (Lopes and Guilherme, 2016). However, this expansion has led to a high P fertilizer demand on the P-fixing soils. However, soil P saturation and fertility has increased only slowly as the vast majority of added reactive inorganic P is rapidly adsorbed onto the surface of Fe and Al (hydr)oxides (Novais et al., 2007; Rodrigues et al., 2016; Sousa and Lobato, 2004; Roy et al., 2017). This strategy is not

profitable to improve the P use efficiency in the future (Rowe et al., 2016; Withers et al., 2014), especially in the tropics (Roy et al., 2016).

The Cerrado agriculture has been practiced in low P use efficiency systems, often lower than 50% (Roy et al., 2016). Brazil's reliance on large P fertilizer inputs to maximize agricultural production for achieving the global food demand is both inefficient and resource demanding, and therefore unsustainable in the long-term perspectives (Cordell et al., 2009; Withers et al., 2015). Thus, the adoption of alternative practices to use fertilizer P more efficiently is urgently required, for example by crop engineering to select more P-efficient varieties and optimizing the integration of cropping system, P fertiliser use and soil management to increasing P bioavailability (Hinsinger et al., 2011; Lambers et al., 2006; Rowe et al., 2016; Withers et al., 2014).

In tropical agriculture, P fertiliser management strategies are commonly integrated with the crop intensification and the adoption of no-tillage cultivation to reduce or retard the fixation of P fertilizers (Gatiboni et al., 2007; Rheinheimer et al., 2008). Rotation systems with less soil disturbance have shown positive effects on soil P bioavailability (Rodrigues et al., 2016; Tiecher et al., 2012a, 2012b). Also, different plants have evolved biochemical, physiological, and structural mechanisms to exploit less labile soil P fractions including mycorrhizal associations, changes in root morphology and modifications in rhizosphere biochemistry through exudation of low molecular-weight organic acids, phosphatases, phytases and protons (Hinsinger et al., 2011; Lambers et al., 2015, 2006). Cover crops not only help to have substantial effect on soil conservation by covering the soil surface from erosion processes (Calegari et al., 2013), but also take up soil P and store it in their roots and shoots resulting in improved P recycling (Boer et al., 2007; Tiecher et al., 2012a). This enhanced P bioavailability and storage in degradable organic fractions (Soltangheisi et al., 2017; Teles et al., 2017; Tiecher et al., 2012a) improved the yield of subsequent cash crops (Calegari et al., 2013; Carvalho et al., 2011).

Near continuous cropping during the year increases root density in the soil, resulting in greater exudation of organic compounds, and organic P (Po) accumulation in the soil, which modulates P bioavailability in tropical soils such as Oxisols (Cross and Schlesinger, 1995). The Po fraction in tropical soils is important because it is less susceptible to strong adsorption on functional groups of Fe and Al oxides and hydroxides compared to inorganic forms, and the organic decomposition products can also compete for P adsorption sites (Dakora and Phillips, 2002; Guppy et al., 2005; Hinsinger et al., 2011; Pavinato and Rosolem, 2008)

Soil Pi and Po forms have been successfully grouped and quantified (although not identified) by sequential chemical extractions, with the widely used Hedley's P fractionation procedure, by its ability to concomitantly determine Pi and Po fractions (Hedley et al., 1982). Despite several analytical limitations of this P fractionation procedure (Condrón and Newman, 2011), the conceptual P lability distribution predicted by the sequential reagents in the Hedley's P fractionation is considered useful for interpreting the P form dynamics (Cross and Schlesinger, 1995), investigating the fate of native and applied P in agroecosystems (Negassa and Leinweber, 2009) and quantifying the P lability i.e., the P distribution in different fractions according to their potential to supply plants and the soil biota (Yang and Post, 2011).

In a previous study by the present authors (Rodrigues et al., 2016) a significant accumulation of all P fractions in the soil after decades of Cerrado cultivation due to high P fertiliser inputs (usually applied as twice as the offtakes) was observed. Although the P use efficiency was over 55%, these inputs still generated a significant amount of residual, or legacy soil P (Haygarth et al., 2014). This legacy soil P a relevant source of secondary P that can be utilised in a potential future scenario of P scarcity (Sattari et al., 2012; Rowe et al.,

2016). In the soybean/cotton succession system typical of the Brazilian Cerrado, this legacy P could represent a potential P supply for decades of cultivation in long-term Cerrado no-tillage systems, and buffer the impact of a sharp increase in the price of P fertiliser (Rodrigues et al., 2016; see chapter 2). However, whether and how this sorbed P could be accessed by plants and how much of the residual P in tropical systems is potentially plant available is largely unknown. The introduction of no tillage and cover crops which concentrates P at the soil surface, and potentially reduces P fixation into non-labile fractions might be expected to enhance the P bioavailability of legacy P in Brazilian Oxisols. In this study, we investigated the impact of no tillage cultivation and cover crops in soybean/cotton rotations on the forms and lability of legacy soil P accumulation.

3.2. Material and Methods

3.2.1. Study sites description and experimental design

Two long-term field experiments established in the Brazilian Cerrado region were evaluated. Both sites have Oxisols (Ox) classified as Typic Hapludox (Soil Survey Staff, 2014), but differ in the conversion time from the native Cerrado to agricultural use (Table 1) and in their clay contents (Table 2).

Both soils Ox-1 and Ox-2 are located in the main soybean production area of central-west Brazil, currently the most important for agriculture in the country. The sites are located on Cerrado biome, where a spring/summer crop season of soybean is typically followed by a second crop (usually maize), or simply a fallow season, depending on the farmer's technology and prevailing weather conditions. The winter/fall period is notably very dry. Cotton is also included in the crop rotation to build up soil fertility. In this case, one season of soybean (spring/summer) and maize (summer/fall) is followed by a year of cotton cultivation.

At Ox-1 the Cerrado deforestation occurred in 1974, and soybean production was grown under a conventional tillage system until 1993. In September 1993, no-tillage (NT) and conventional tillage (CT) main plots were established. In 2004, different cover crop treatments were established after soybean harvesting. At Ox-2 the deforestation occurred in 1997, and soybean was cultivated under CT for three consecutive seasons. In 2000, the CT and NT main plots were established. After the soybean harvesting in 2005, different cover crop treatments were established. A summary of site history, location and average climate is presented in Table 1.

The experimental design adopted at both sites was randomized blocks within a split-plot design, with three replicates. The tillage systems (CT and NT) were the main plots and the subplots at both sites consisted of the following cover crops: fallow (no cover, with chemical weed control), millet (*Pennisetum glaucum* L.), ruzigrass (*Brachiaria ruziziensis*, syn. *Urochloa ruziziensis*) and maize (*Zea mays* L.). Each sub-plot had dimensions of 9.6 x 110 m and 8.3 x 107 m (width x length) for Ox-1 and Ox-2, respectively. The CT system used ploughs (twice annually) before sowing, whereas in the NT system the soil was not disturbed (except for sowing operations), and the crop residues were chemically desiccated and left on the soil surface. A native Cerrado area adjacent to the experimental area, and with the same topography, soil morphology and mineralogical characteristics, was used as reference plots for calculating a legacy soil P balance (see Chapter 2).

Table 1. Geographical information and general characteristics of the experimental areas.

Description	Study Sites	
	Ox-1 (Costa Rica, MS)	Ox-2 (Sapezal, MT)
Cerrado deforestation (years)	1974 (42)	1997 (18)
Time of trial establishment (years)	22 (1994 – 2016)	14 (2001 – 2015)
Location	Lat.: 08°15'10" S	Lat.: 13°56'33" S
	Long.: 03°12'41" W	Long.: 58°53'43" W
Altitude	790	640
Annual temperature (mean, °C)	24.8	23.5
Annual precipitation (mm)	1,950	2,150

Table 2. Selected soil physical and chemical characteristics in cultivated and native Cerrado areas at each site.

Study site	Soil layer cm	CEC ⁽¹⁾ mmol _c kg ⁻¹	pH CaCl ₂	SOC ⁽²⁾	DCB ⁽³⁾		Oxalate ⁽⁴⁾		Granulometry ^S			
					Fe	Al	Fe	Al	Sand	Silt	Clay	
					----- g kg ⁻¹ -----							
Ox-1	Trial	0-10	91.8	5.0	25.9							
		10-20	67.8	4.7	20.8	48.8	10.7	1.78	6.73	101	243	656
	Native	0-10	68.8	4.8	26.2							
		10-20	52.9	4.6	20.0							
Ox-2	Trial	0-10	93.7	4.9	23.9							
		10-20	77.9	4.8	19.5	48.1	13.1	1.76	7.29	185	363	452
	Native	0-10	78.7	3.8	26.3							
		10-20	97.2	4.0	18.4							

§ Granulometric analysis by pipette method (EMBRAPA, 1997) after dispersion in 1 mol L⁻¹ NaOH. Analysed and presented by Pavinato (2009).

⁽¹⁾ CEC: cation exchange capacity; ⁽²⁾ SOC: soil organic carbon; ⁽³⁾ DCB: dithionite, citrate and bicarbonate, extracted according to Mehra and Jackson (1960); ⁽⁴⁾ Oxalate: acidic ammonium oxalate extraction according Schwertmann (1964).

The mean annual P fertilizer input since deforestation was estimated at 37.2 kg P ha⁻¹ yr⁻¹ in Ox-1 and 44.8 kg P ha⁻¹ yr⁻¹ in Ox-2, whereas the mean annual offtake of P by the cash crops across tilled plots were 15.2 and 13.1 kg P ha⁻¹ yr⁻¹ in Ox-1 and Ox-2, respectively. This left a net annual P surplus of 22.0 and 31.7 kg P ha⁻¹ yr⁻¹ for Ox-1 and Ox-2, respectively.

3.2.2. Soil sampling and chemical analysis

A general sample (0-10 and 10-20 cm) from each site (field trial and native soil) was collected (composed of 4 sub-samples in each sub-plot) for soil physical and chemical characterization (Table 2). The amorphous/crystalline Fe ratio ($Fe_{Oxalate}/Fe_{DCB}$, Table 2) was lower than 0.05 (0.036 and 0.037 for Ox-1 and Ox-2, respectively) demonstrating that in both soils the crystalline oxides are predominant.

X-Ray diffraction (DRX) analysis of the clay fraction was performed according to the method described by Jackson (1969), but modified by saturating the clay fraction with 1 M KCl or 1 M $MgCl_2$ after Fe-oxide removal to identify the phyllosilicates. The following post-treatments were applied: K saturation at room temperature (25°C); K saturation and heating up to 300°C; K saturation and heating up to 500°C; Mg saturation at room temperature (25°C); Mg saturation with solvent ethylene glycol. Another sample without removing the Fe was prepared to identify the Fe oxides present in the soils, followed by the oriented glass slide preparation to analysis. The diffractograms were collected in a Miniflex II Desktop X-Ray Diffractometer (RIGAKU), CuK α radiation, analysis interval from 10 to 50°2 θ to the clay fraction without Fe samples and 5 to 30°2 θ to the samples of clay+Fe. The results showed the presence of Kaolinite, and the Fe oxides Hematite and Goethite, also Gibbsite as the Al hydroxide, in both study sites. These minerals will be subsequently referred to Al and Fe oxides in the following sections.

Soil samples were collected after desiccation of the cover crops (August 2015 in Ox-2 and August 2016 in Ox-1). The soil was sampled 0-5, 5-10 and 10-20 cm depth using a spade. Four trenches were opened in each plot and subsamples from each trench were mixed to form a composite sample. Additionally, for sampling in the adjacent Cerrado native areas, six different plots (around 30 m distance from each other and at least 30 m from the forest edge) were delimited, and subsamples were collected from each plot at each depth and mixed to obtain a composite sample.

Samples were air dried and sieved (2 mm) prior to sequential P fractionation according to the method proposed by Hedley et al. (1982) with modifications by Condon et al. (1985). The sequential extraction order and interpretation of the each extractant was based on the literature, as given below. It should be noted that there is some uncertainty and possible overlap in the precise forms which these extractants remove across variable agroecosystems (Condon and Newman, 2011; Negassa and Leinweber, 2009), but such fractionation nevertheless provides useful insights into the lability of soil P.

The P fractions of Hedley's fractionation procedure are: I) Anion exchange resin (P_{AER}), which extracts labile inorganic P readily diffusing into solution and trapped by a resin membrane (2.0 cm²); II) 0.5 mol L⁻¹ $NaHCO_3$ at pH 8.5 (P_{Bic}), removing labile inorganic P (P_{iBic}) weakly sorbed on the surface of crystalline compounds and labile organic P (P_{oBic}) from compounds that present low recalcitrance (like ribonucleic acid and glycerophosphate) in the soil (Tiessen and Moir, 1993); III) 0.1 mol NaOH L⁻¹ ($P_{Hid0.1}$), extracting moderately labile inorganic P ($P_{iHid0.1}$) related to P strongly adsorbed onto Fe and Al oxides and 1:1 clay minerals (Hedley et al., 1982) and also moderately labile organic P ($P_{oHid0.1}$) associated to fulvic and humic acids adsorbed onto mineral and SOM surfaces (Linguist et al., 1997); IV) 1.0 mol HCl L⁻¹ (P_{HCl}), the fraction representing inorganic P associated with apatite and other sparingly-soluble Ca-P compounds (Gatiboni et al., 2007), originally described as non-labile P (Cross and Schlesinger, 1995; Hedley et al., 1982), but for tropical acid soils can be considered as a moderately labile (mod-labile) P fraction; V) 0.5 mol NaOH L⁻¹ ($P_{Hid0.5}$), removing more recalcitrant inorganic P ($P_{iHid0.5}$) also associated with Fe and Al and 1:1 clay minerals and non-labile forms of

organic P ($P_{\text{Hid}0.5}$) associated with fulvic and humic acids and considered here as non-labile P fraction since it removes P inside soil aggregates (Condon et al., 1985); VI) Residual P (P_{Residual}), occluded P (Cross and Schlesinger, 1995) remaining in the soil after all other sequential extractants, determined after acid digestion with $\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2 + \text{saturated MgCl}_2$ (Olsen and Sommers, 1982).

At each step of Hedley's procedure, 10 mL of extractant was added to 0.5 g soil in a 15 mL centrifuge tubes (1:20 soil:solution ratio) and shaken end-over-end (vertical agitator, 60 rpm) for 16 h at room temperature ($\pm 25^\circ\text{C}$). After each extraction, the soil suspensions were centrifuged at 3278 g for 30-40 min to collect clear supernatants. Total P (Pt) in the alkali extracts (P_{Bic} , $P_{\text{Hid}0.1}$ and $P_{\text{Hid}0.5}$) was obtained by digestion of an extract aliquot with sulphuric acid 50% and ammonium persulfate 7.5% in an autoclave at 121°C for 2 h (USEPA, 1971). In order to avoid most of the organic P hydrolysis onto inorganic P, Pi in alkali extracts was measured colorimetrically by the method proposed by Dick and Tabatabai (1977). The molybdate reactive Pi in acid extracts (P_{AER} and P_{HCl} , and digestion of P_{Bic} , $P_{\text{Hid}0.1}$ and $P_{\text{Hid}0.5}$) was measured by the colorimetric method of Murphy and Riley (1962). The organic P in alkali extracts was obtained by difference between the measured Pt and Pi.

Soil P fractions were grouped according to their lability predicted by the extractants. Labile P included P_{AER} , P_{Bic} and P_{OBic} ; moderately labile P included $P_{\text{Hid}0.1}$ (Pi and Po) and P_{HCl} , and non-labile P included $P_{\text{Hid}0.5}$ (Pi and Po) and P_{Residual} . In addition, the P fractions were grouped into biological (P_{Biol}) and geochemical (P_{Geoc}) P (Cross and Schlesinger, 1995), wherein the biological fraction includes all the organic fractions ($P_{\text{OBic}} + P_{\text{Hid}0.1} + P_{\text{Hid}0.5}$) and the geochemical fraction includes all the inorganic fractions plus residual P ($P_{\text{AER}} + P_{\text{Bic}} + P_{\text{HCl}} + P_{\text{Hid}0.1} + P_{\text{Hid}0.5} + P_{\text{Residual}}$).

In order to evaluate the cumulative legacy soil P present in the soil since deforestation, P fractionation was also performed on native Cerrado soil samples and the values subtracted from the cultivated plots. Thus, the net P values were evaluated in relation to their distribution in labile, moderately labile and non-labile P, and also relative to the biological and geochemical P pools.

3.2.3. Statistical analysis

All the data on P fractions, P lability and legacy P pools were previously analysed to verify their normality (Shapiro-Wilk test) and homoscedasticity (Barlett test). After checking these statistical presumptions, a two-way analysis of variance (ANOVA) was performed considering the randomized blocks within the split-plot design, where the soil cultivation systems were considered as main plots and the cover crops as subplots. When the treatment effect was significant by F-test ($p < 0.05$), the means were compared by the Tukey's test ($p < 0.05$). All the statistical analyses were performed in the R Environment v.3.4 (R Core Team, 2017) and the split-plot design ANOVA and Tukey's test were performed using the ExpDes R package (Ferreira et al., 2013).

3.3. Results

3.3.1. Inorganic and organic P fractions

The statistical treatment differences observed in the P fractions within the labile, moderately labile and non-labile pools at 0-5 and 5-10 cm depths were similar in both study sites (Tables S1 and S2). In order to simplify and clarify the effects of tillage systems and cover crop treatments, these two layers were averaged to 0-10 cm and then analysed.

P_{AER} levels in the topsoil (0-10 cm) were affected by the interaction between tillage system and cover crop at both sites (Table 3). At Ox-1, fallow under NT resulted in 44% and 50% higher P_{AER} compared to the CT and the average of other cover crops, respectively, whereas at Ox-2 it was the brachiaria cover crop which increased P_{AER} levels under NT. Under CT, maize maintained the highest P_{AER} level in the topsoil, which was 55%, 54% and 33% higher than under fallow, millet, and brachiaria, respectively.

Tillage system and cover crop did not affect P_{iBic} and P_{oBic} fractions in Ox-1 in either the 0-10 or the 10-20 cm layer (Table 3). At site Ox-2, significant effects on P_{iBic} were observed only under brachiaria in the NT system, which increased P_{iBic} concentrations by 94% and 81% in 0-10 and 10-20 cm, respectively compared to other cover crops. When the soil was managed under CT, the positive effect of brachiaria was observed only in 10-20 cm layer, being significantly higher than fallow and millet. The P_{oBic} concentrations were not affected by cover crops, but there was 47% more P_{oBic} in the CT system than in the NT system in 10-20 cm layer at Ox-2.

At Ox-1, mod-labile $P_{iHid0.1}$ was reduced by 11%, 13% and 21% under millet, maize and brachiaria, respectively, when compared to fallow in the top 10 cm (Table 4). Interaction between tillage and cover crop in $P_{iHid0.1}$ at Ox-2 (Table 4). Under NT, brachiaria increased this pool by 35% compared to the other cover crops, while under CT, maize increased $P_{iHid0.1}$ by 28% relative to the other crops. At both sites, $P_{iHid0.1}$ was not affected by either tillage systems or cover crops below 10 cm.

Cover crops significantly affected $P_{oHid0.1}$ at both study sites and soil depths (Table 4). At Ox-1, brachiaria cultivation enhanced $P_{oHid0.1}$ by 28% and 22% in 0-10 and 10-20 cm, respectively, compared to the other cover crops. However, at Ox-2, brachiaria only changed this P fraction in the 10-20 cm layer under the NT system. Tillage system had a major affect on $P_{oHid0.1}$ concentrations at Ox-2 with considerably higher values (+109%) under NT than under CT.

Since the P_{HCl} fraction contributed the lowest (<1%) portion of total P at both sites (Table 4), and did not show any difference between either the cover crops or tillage systems, the results for this fraction were not statistically evaluated neither discussed.

Cover crops \times tillage system interactions did not affect $P_{iHid0.5}$, $P_{oHid0.5}$ or residual P fractions at both sites and at all depths (Table 5). At Ox-1, cover crops did not have any significant effect and the tillage system only slightly changed $P_{oHid0.5}$ in the top 10 cm, where NT increased $P_{oHid0.5}$ by 29% compared to CT. Cover crop only affected $P_{iHid0.5}$ in Ox-2, with increments in this fraction (in relation to fallow) of around 20% and 23%, respectively for maize and brachiaria.

The $P_{Residual}$ was not affected by the tillage systems and cover crops in both study sites (Table 5). In both trials, residual P ranged from 36 to 46% of the total P and neither tillage systems nor cover crops influenced this fraction (Table 5). However, brachiaria cultivation reduced slightly the proportion of residual P (2% in site Ox-1 and 4% in site Ox-2) in 10-20 cm depth layer. This effect was not related to $P_{Residual}$ depletion but mostly due to the increment in total P levels as consequence of brachiaria cultivation (see sec. 3.2, total P).

Table 3. Labile P fractions (mg kg⁻¹) in two Brazilian Cerrado Oxisols (Ox-1 and Ox-2) after decades of no-tillage (NT) and conventional tillage (CT) cultivation and cover crops rotation in soybean/cotton cash crop production. Individual data are presented only when there was a statistically significant (P <0.05) interaction effect between tillage and cover crop treatments.

Cover Crops	Ox-1						Ox-2					
	0 – 10 cm			10 – 20 cm			0 – 10 cm			10 – 20 cm		
	NT	CT	Mean	NT	CT	Mean	NT	CT	Mean	NT	CT	Mean
Inorganic P extracted by anion exchange resin (P_{AER}, mg kg⁻¹)												
Fallow	35.1 Aa	24.4 Ba	-	-	-	19.4 ns	37.1 Aa	27.7 Bb	-	-	-	26.7 ns
Millet	20.6 Ab	24.4 Aa	-	-	-	20.8	26.8 Ab	27.8 Ab	-	-	-	22.7
Brachiaria	24.7 Ab	24.0 Aa	-	-	-	24.9	43.4 Aa	32.1 Bb	-	-	-	28.3
Maize	25.0 Ab	27.8 Aa	-	-	-	18.3	37.7 Aa	42.8 Aa	-	-	-	24.4
Mean	-	-	-	21.4 ns	20.3	-	-	-	-	27.5 ns	23.5	-
Inorganic P extracted by 0.5 M NaHCO₃ (P_{iBic}, mg kg⁻¹)												
Fallow	-	-	24.4 ns	-	-	15.5 ns	28.9 Ab	25.5 Aa	-	38.5 Ab	19.5 Bb	-
Millet	-	-	17.8	-	-	16.7	25.6 Ab	22.2 Aa	-	34.1 Ab	16.3 Bb	-
Brachiaria	-	-	17.3	-	-	16.5	41.0 Aa	24.7 Ba	-	62.7 Aa	33.0 Ba	-
Maize	-	-	21.1	-	-	15.5	23.9 Bb	31.0 Aa	-	30.9 Ab	31.6 Aa	-
Mean	20.2 ns	20.1	-	15.0 ns	17.1	-	-	-	-	-	-	-
Organic P extracted by 0.5 M NaHCO₃ (P_{oBic}, mg kg⁻¹)												
Fallow	-	-	22.7 ns	-	-	17.7 ns	-	-	42.1 ns	-	-	42.8
Millet	-	-	21.9	-	-	19.0	-	-	45.3	-	-	46.3
Brachiaria	-	-	23.8	-	-	20.4	-	-	47.5	-	-	37.6
Maize	-	-	24.8	-	-	18.2	-	-	45.7	-	-	46.9
Mean	21.6 ns	25.0	-	18.3 ns	19.3	-	44.8 ns	45.5	-	35.1 B	51.6 A	-

For each soil and within each depth and P fraction, means followed by the same capital letter in rows and small letter in columns were not significantly different by Tukey's test (p<0.05).

ns: not significant; NT, no-tillage system; CT, conventional tillage system.

Table 4. Moderately labile P fractions (mg kg^{-1}) in two Brazilian Cerrado Oxisols (Ox-1 and Ox-2) after decades of no-tillage (NT) and conventional tillage (CT) soil managements and cover crops rotation in the soybean/cotton cash crop production.

Cover Crops	Ox-1						Ox-2					
	0 – 10 cm			10 – 20 cm			0 – 10 cm			10 – 20 cm		
	NT	CT	Mean	NT	CT	Mean	NT	CT	Mean	NT	CT	Mean
Inorganic P extracted by 0.1 M NaOH ($\text{P}_{\text{Hid}0.1}$, mg kg^{-1})												
Fallow	-	-	218.9 a	-	-	140.1 ns	83.7 Ab	88.0 Aab	-	-	-	71.0 ns
Millet	-	-	195.3 ab	-	-	120.6	76.6 Ab	70.8 Ab	-	-	-	72.4
Brachiaria	-	-	172.9 b	-	-	135.2	104.5 Aa	69.7 Bb	-	-	-	85.3
Maize	-	-	190.8 ab	-	-	132.8	71.8 Bb	97.2 Aa	-	-	-	80.5
Mean	196.8 ns	192.2		136.7 ns	127.7		-	-		78.9 ns	75.7	
Organic P extracted by 0.1 M NaOH ($\text{P}_{\text{Hid}0.1}$, mg kg^{-1})												
Fallow	-	-	121.6 b	-	-	100.2 b	-	-	54.1 a	29.6 Ab	24.4 Aa	-
Millet	-	-	132.4 b	-	-	113.9 b	-	-	39.7 b	23.4 Ab	17.4 Aa	-
Brachiaria	-	-	164.5 a	-	-	129.2 a	-	-	43.2 ab	65.6 Aa	31.4 Ba	-
Maize	-	-	130.9 b	-	-	104.5 b	-	-	48.9 ab	35.9 Ab	21.2 Ba	-
Mean	143.2 ns	131.5		102.8 ns	121.1		48.9 ns	44.0		-	-	
Inorganic P extracted by 1 M HCl (P_{HCl}, mg kg^{-1})												
Fallow	-	-	1.8 #	-	-	1.1 #	-	-	1.0 #	-	-	0.8 #
Millet	-	-	1.7	-	-	1.0	-	-	1.2	-	-	0.9
Brachiaria	-	-	1.6	-	-	1.2	-	-	1.6	-	-	1.2
Maize	-	-	1.8	-	-	1.1	-	-	1.3	-	-	0.9
Mean	1.8 #	1.6		1.1 #	1.1		1.4 #	1.1		1.1 #	0.9	

For each soil and within each depth and P fraction, means followed by the same capital letter in rows and small letter in columns were not significantly different by Tukey's test ($p < 0.05$).

#, not statically evaluated; ns: not significative; NT: no-tillage system; CT: conventional tillage system.

Table 5. Non-labile P fractions (mg kg^{-1}) in two Brazilian Cerrado Oxisols (Ox-1 and Ox-2) after decades of no-tillage (NT) and conventional tillage (CT) soil managements and cover crops rotation in the soybean/cotton cash crop production.

Cover Crops	Ox-1						Ox-2					
	0 – 10 cm			10 – 20 cm			0 – 10 cm			10 – 20 cm		
	NT	CT	Mean	NT	CT	Mean	NT	CT	Mean	NT	CT	Mean
Inorganic P extracted by 0.5 M NaOH ($\text{Pi}_{\text{Hid}0.5}$, mg kg^{-1})												
Fallow	-	-	48.0 ns	-	-	43.9 ns	-	-	66.6 b	-	-	61.2 ns
Millet	-	-	47.3	-	-	45.3	-	-	71.3 b	-	-	66.5
Brachiaria	-	-	47.7	-	-	47.3	-	-	81.9 a	-	-	67.2
Maize	-	-	49.2	-	-	44.0	-	-	79.8 a	-	-	66.5
Mean	47.5 ns	48.6		45.8 ns	44.4		74.6 ns	75.1		78.9 ns	75.7	
Organic P extracted by 0.5 M NaOH ($\text{Po}_{\text{Hid}0.5}$, mg kg^{-1})												
Fallow	-	-	76.6 ns	-	-	39.3 ns	-	-	27.9 ns	-	-	31.8 ns
Millet	-	-	69.0	-	-	36.1	-	-	43.9	-	-	32.2
Brachiaria	-	-	68.4	-	-	39.9	-	-	34.5	-	-	43.2
Maize	-	-	71.9	-	-	43.6	-	-	32.5	-	-	34.2
Mean	80.6 A	62.3 B		40.0 ns	39.5		34.6 ns	34.7		40.7 ns	30.0	
Residual P (mg kg^{-1})												
Fallow	-	-	307.3 ns	-	-	282.1 ns	-	-	245 ns	-	-	239.5 ns
Millet	-	-	312.5	-	-	289.9	-	-	260	-	-	243.2
Brachiaria	-	-	307.4	-	-	284.2	-	-	262	-	-	248.7
Maize	-	-	316.4	-	-	298.9	-	-	259	-	-	249.3
Mean	317.6 ns	304.2		295.0 ns	282.5		252.3 ns	261.6		235.7 ns	254.7	

For each soil and within each depth and P fraction, means followed by the same capital letter in rows and small letter in columns were not significantly different by Tukey's test ($p < 0.05$).

ns: not significant; NT: no-tillage system; CT: conventional tillage system.

3.3.2. Phosphorus lability

Labile P ($P_{AER} + P_{iBic}$ and P_{OBic}) in top 0-10 cm was not significantly affected by tillage system or cover crop at site Ox-1 (Figure 1A). However, in the 10-20 cm layer, brachiaria enhanced labile P by 17% compared to fallow. At site Ox-2, interactions between tillage system and cover crop influenced labile P at both depths (Figure 2A). In the top 0-10 cm, labile P increased by 22-35% when brachiaria was cultivated under NT compared to other crops. In the CT system, only maize cultivation improved labile P. In the 10-20 cm layer, labile P with brachiaria and fallow were similar under NT, but maize, brachiaria and millet increased labile P by 59%, 30%, and 18%, respectively compared to fallow under CT.

A tillage system x cover crop interaction significantly influenced moderately labile P at both sites in 0-10 cm (Figures 1B, 2B). Under CT, fallow and maize increased mod-labile P compared to brachiaria and millet at site Ox-2 (Figure 2B), while no cover crop effect was observed under CT at site Ox-1 (Figure 1B). Under the NT system, millet, brachiaria and fallow showed more mod-labile P at Ox-1 (Figure 1B), whereas at Ox-2 fallow and brachiaria increased mod-labile P more than other cover crops (Figure 2B). Significant effects of brachiaria under NT at Ox-2 in the 10-20 cm depth layer were also observed, with 39-59% more mod-labile P level than other cover crops, and there was 41% higher mod-labile P under NT than under CT (Figure 2B).

Non-labile P was not influenced by the tillage system nor by the type of cover crop at Ox-1 (Figure 1C). However, at Ox-2, some cover crops slightly increased non-labile P by 9-12% and 6-8% compared to fallow in the 0-10 and 10-20 cm layers, respectively (Figure 2D).

A significant treatment difference in total P content at Ox-1 was only observed in the topsoil (0-10 cm) (Figure 1D). Cover crops did not change total P concentrations, but NT under fallow and millet resulted in higher total P compared to the CT system. At Ox-2, a small interaction between tillage system and cover crop was observed at both depths (Figure 2D). Overall, brachiaria under NT increased total P concentrations by around 12% and 19% in 0-10 and 10-20 cm depth, respectively, whereas under CT, cover crop did not enhance total P in either soil layer.

The P_{Geoc} (geochemical P, total inorganic P) was also affected by cover crops in both study sites. At Ox-1, cover crops cultivation reduced P_{Geoc} compared to fallow in the top 10 cm, but no differences were observed in 10-20 cm (Figure 1E). Brachiaria was the most effective in reducing this P fraction (-10%), whereas millet and maize reduced P_{Geoc} by 5% and 4% (related to fallow), respectively. At Ox-2 (Figure 2E), significant small interactions between the tillage systems and the cover crops were observed in both soil layers, but the only notable difference in P_{Geoc} was observed under NT cultivation when brachiaria was grown, and under CT cultivation when maize was grown as the second crop.

The P_{Biol} pool (total organic P) behaved differently at the two sites. At Ox-1, it was affected by the interaction between tillage system and cover crop at both depths (Figure 1F). In the 0-10 cm layer, the highest P_{Biol} was recorded with brachiaria under NT, and significantly different to its cultivation under CT. Under CT, maize was as effective as brachiaria at enhancing P_{Biol} in the topsoil. In the 10-20 cm layer, brachiaria under CT gave the highest P_{Biol} , being significantly greater than for the other cover crops and also its cultivation under NT. In Ox-2, P_{Biol} was only influenced by cover crops in the 10-20 cm depth, whereas brachiaria resulted in 18-30% higher P_{Biol} compared to the other cover crop treatments (Figure 2F).

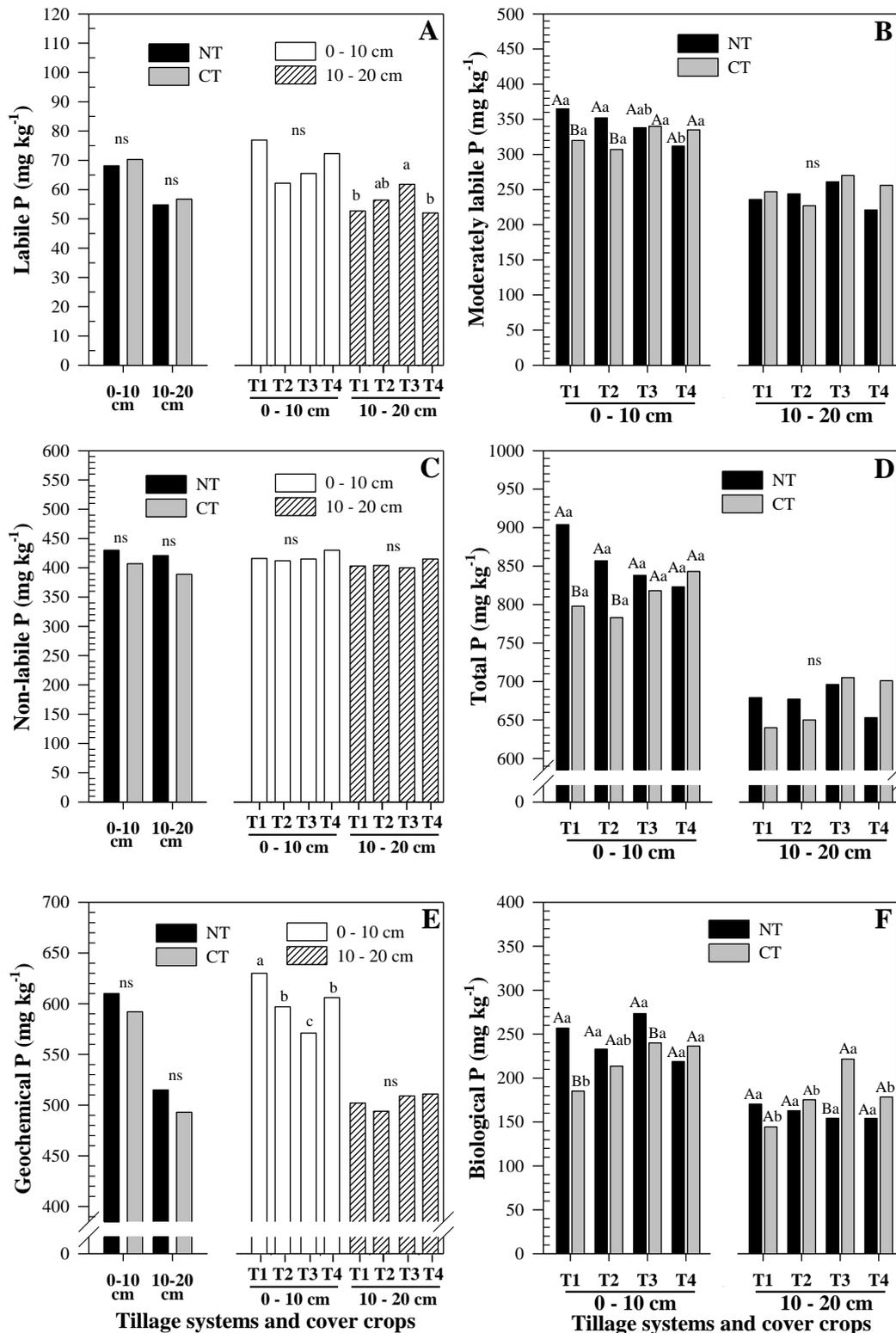


Figure 1. Soil phosphorus lability after long-term effects of tillage systems and cover crops in a Brazilian Cerrado Oxisol 1 (Ox-1). A) Labile P; B) Moderately labile P; C) Non-labile P; D) Total P; E) Geochemical pool; F) Biological pool. Within each depth and P fraction, means followed by the same capital letter (tillage systems) and tiny letter (cover crops) were not significantly different at $p < 0.05$ by Tukey test. ns: not significant differences observed ($n=3$). NT: no-tillage; CT: conventional tillage; T1: fallow; T2: millet; T3: brachiaria; T4: maize.

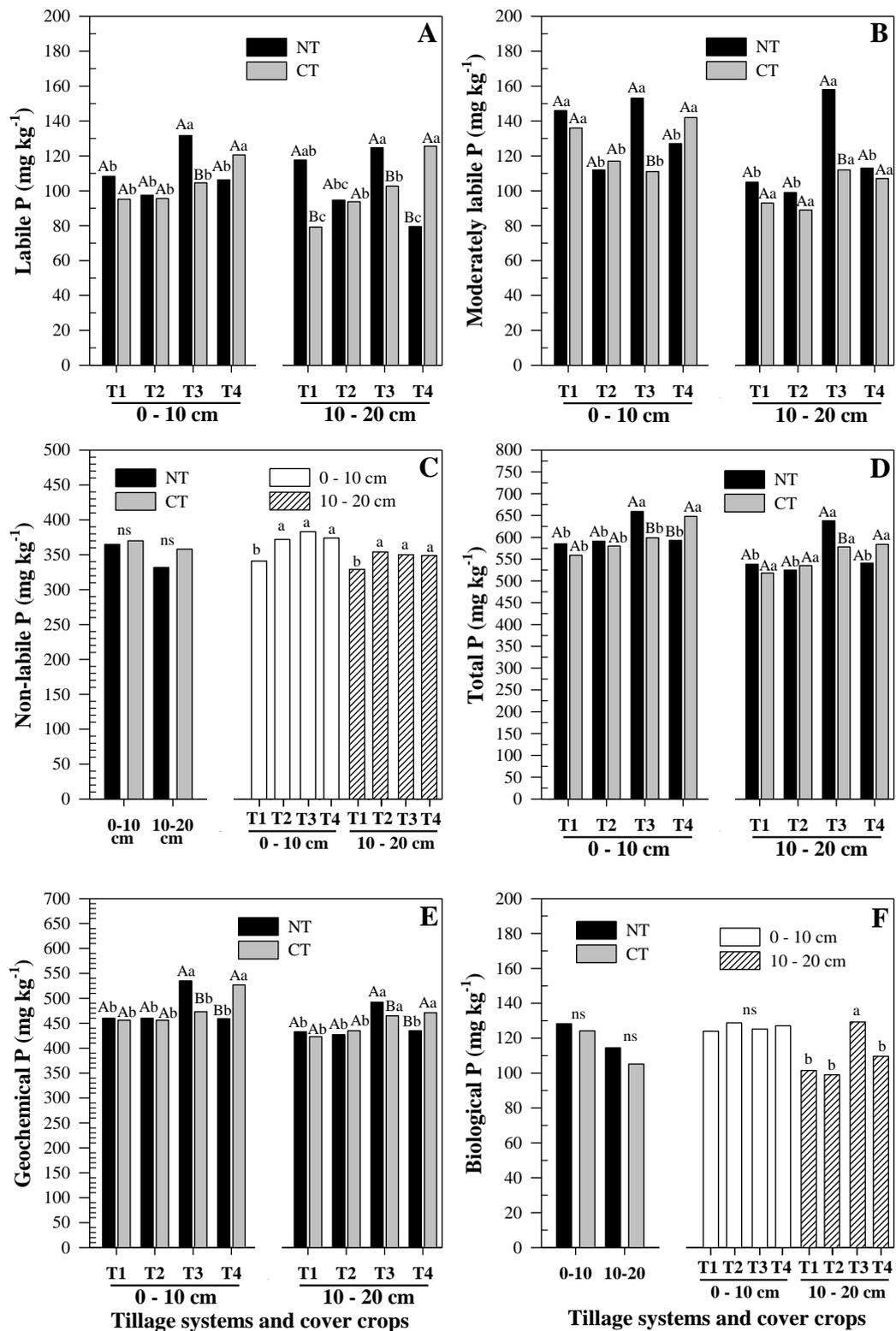


Figure 2. Soil phosphorus lability after long-term effects of tillage systems and cover crops in a Brazilian Cerrado Oxisol 2 (Ox-2). A) Labile P; B) Moderately labile P; C) Non-labile P; D) Total P; E) Geochemical pool; F) Biological pool. Within each depth and P fraction, means followed by the same capital letter (tillage systems) and tiny letter (cover crops) were not significantly different at $p < 0.05$ by Tukey test. ns: not significant differences observed ($n=3$). NT: no-tillage; CT: conventional tillage; T1: fallow; T2: millet; T3: brachiaria; T4: maize.

3.3.3. Legacy phosphorus

The legacy soil P that had accumulated at both sites since deforestation was obtained by calculating the difference between total P in the agricultural treatments (tillage systems and cover crops experiments) and the native Cerrado areas (Table S3). Labile, moderately labile, non-labile, geochemical and biological P pools were also calculated across the sampled 0-20 cm depth in cultivated areas and native vegetation.

Considering the P fertilizer inputs and the annual offtakes at the sites (see sec 2.1) since deforestation, the expected total soil legacy P is around 924 and 534 kg ha⁻¹ for Ox-1 and Ox-2, respectively (Table 6). The average legacy P determined by the P fractionation (0-20 cm soil depth) was 341.5 (±20) in Ox-1 (Figure 3A) and 184.5 (±15) mg kg⁻¹ in Ox-2 (Figure 3B). In order to verify the ability of the cover crops to prevent or enhance the availability of the legacy P, each separated pool was also analysed.

Cover crops did not significantly alter the lability of legacy P at Ox-1, as measured by the distribution of labile, moderately labile (Figure 3C) and non-labile (Figure 3E) pools. However, NT increased the non-labile portion of legacy P by 16% (Figure 3E). At Ox-2, brachiaria cultivation enhanced the legacy P from 44-51% under NT, whereas under CT both brachiaria and maize resulted in higher legacy P in the soil, 25% and 43% higher than fallow, respectively (Figure 3B). Overall, both the labile and moderately labile net P balances were increased by brachiaria cultivation under NT in Ox-2 (Figure 3D). However, the non-labile P was enhanced by 36-43% by cover crops in comparison to the fallow, with no differences between maize, millet and brachiaria (Figure 3F).

Table 6. Estimated soil Legacy P considering the P balance (P inputs – P outputs) in two long-term field trials in the Brazilian Cerrado (Ox-1 and Ox-2).

Study Site	Total P inputs	Total P outputs	Net P (Legacy)	Years since deforestation (trial begin)
	----- kg ha ⁻¹ -----			
Ox-1	1560.6	636.7	923.9	42 (22) [#]
Ox-2	806.3	272.6	533.7	18 (14) [#]

[#]: relative to the period of soil sampling (2016 for Ox-1 and 2015 for Ox-2) and the deforestation time.

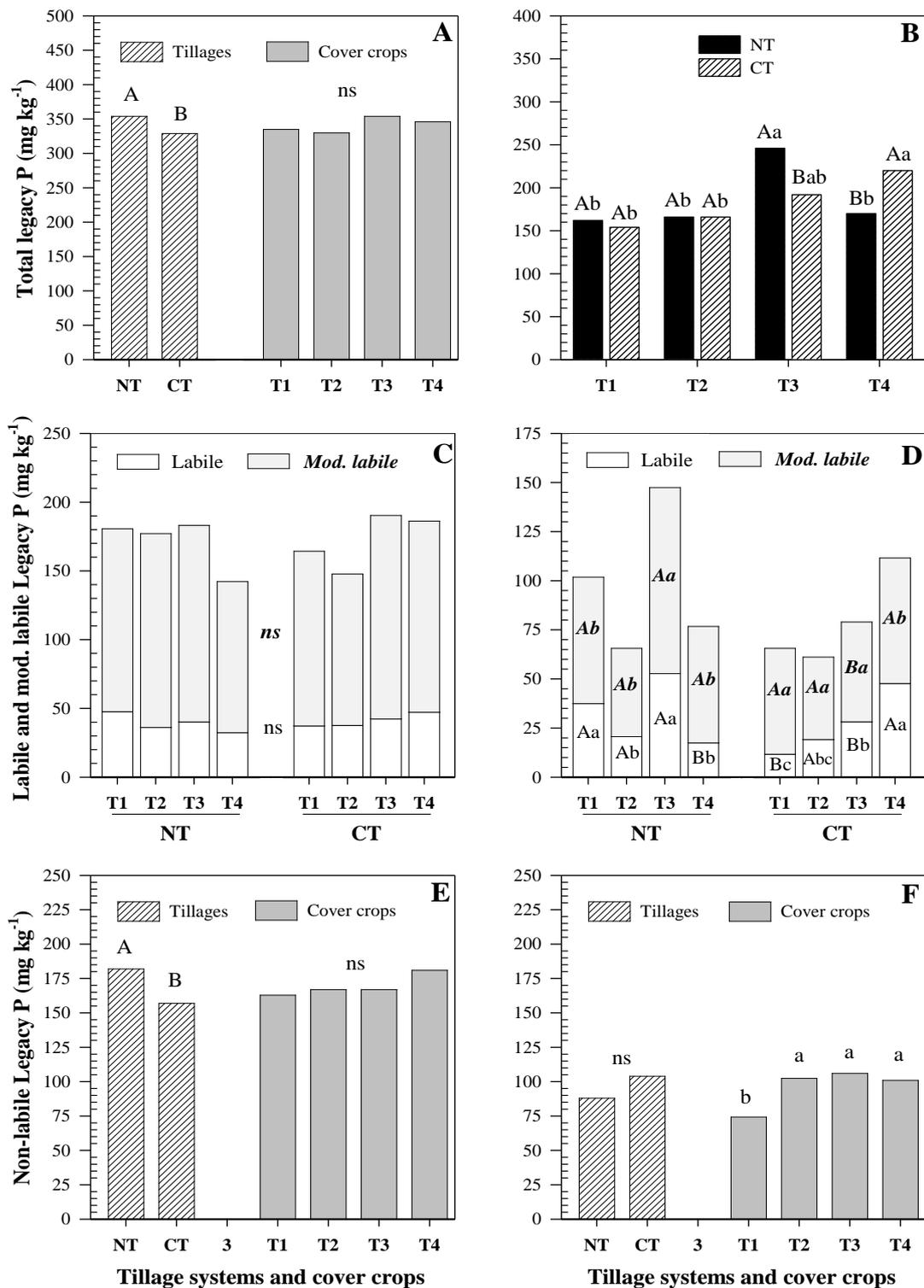


Figure 3. Soil P Legacy and its distribution in the labile, moderately labile and non-labile pools in soil top 20 cm of two Brazilian Oxisols after decades of tillage systems and cover crops cultivation. A) Total legacy P in the Ox-1; B) Total legacy P in the Ox-2; C) Labile and moderately labile Legacy P pools in the Ox-1; D) Labile and moderately labile Legacy P pools in the Ox-2; E) Non-labile P pool in the Ox-1; F) Non-labile P pool in the Ox-2. Within each depth and P fraction, means followed by the same capital letter (tillage systems) and tiny letter (cover crops) were not significantly different at $p < 0.05$ by Tukey test. ns: not significant differences observed ($n=3$). NT: no-tillage; CT: conventional tillage; T1: fallow; T2: millet; T3: brachiaria; T4: maize.

3.4. Discussion

3.4.1. Tillage and cover crops effects on P fractions and their lability

The 0-5 and 5-10 cm results were averaged over 10 cm as they showed similar behaviour, similar to previous studies evaluating the effects of tillage systems in tropical soils (Rodrigues et al., 2016; Vieira et al., 2016). However, other studies evaluating the effects of cover crops on P availability have reported higher P accumulation in the 0-5 cm depth, with small or no influence in deeper layers (Soltangheisi et al., 2017; Tiecher et al., 2012a, 2012b). There was a significant accumulation of soil organic matter (SOM) at the surface in those studies in response to the long-term deposition of organic residues, and P fertilizer which was broadcast on the soil surface. In our study, SOM levels were not very different between the 0-5 and 5-10 cm layers (Table 2) and P fertiliser was placed not broadcast, suggesting that it was appropriate to amalgamate the data to 0-10 cm depth.

At both study sites, higher P_{AER} levels were observed in fallow under NT compared to CT, and higher than for all other cover crops at Ox-1 (Table 3). Since the extractant (resin) removes just the P readily-available to plants (Gatiboni et al., 2007; Hedley et al., 1982), and the soils were sampled immediately after cover crops desiccation, this reflects the greater uptake of P_{AER} by the cover crops. Thus, since the cover crops accumulated P into their tissues (roots and shoots) and the soils were sampled immediately after cover crops desiccation, the P recycling capacity of cover crops was underestimated here and could contribute to reduce P fixation, i.e. the strong P adsorption and storage as non-labile P (Gatiboni et al., 2007; Rheinheimer et al., 2008) by P stocking in the soil as labile and moderately labile fractions (Boer et al., 2007; Calegari et al., 2013; Carvalho et al., 2014).

The use of brachiaria notably increased P_{AER} and P_{OBic} at Ox-2 (Table 3). This crop has previously been shown recently evaluated in intensification systems in Brazil and its use has been related to improve soil P availability over the time (Almeida and Rosolem, 2016), mainly because of this plant capacity in exudates significant amounts of organic acids or stimulating microbial enzyme activity around its roots capable to produce those compounds during the organic residues decomposition (Jones, 1998; McLaughlin et al., 2011). The P mineralization as result of the higher microbial and phosphatase enzyme activity around brachiaria roots, reported by Louw-Gaume et al. (2010) also contribute to increase the labile P fraction (Pavinato et al., 2008), observed in this study by decreasing P_{OBic} fraction and, consequently, increasing labile Pi fractions (P_{AER} and P_{iBic}) in site Ox-2, reinforcing here the These data suggest that brachiaria is a particularly useful cover crop for improving plant-available P in the long-term.

As expected from many years of intensive cultivation with high fertilizer P inputs (average annual surplus of 21.0 and 29.7 kg ha⁻¹ yr⁻¹ for Ox-1 and Ox-2, respectively), P_{AER} levels were above the agronomically-optimum critical level of 15 mg P kg⁻¹ soil adopted in Brazil's central region for the resin method (Sousa e Lobato, 2004). The increase in labile P (0-2) relative to the native area was equivalent to 80.4 and 59.0 kg ha⁻¹ (considering labile P at 0-20 cm, related to the labile P in the native area), and which means an annual addition of 0.91 and 1.64 mg P kg⁻¹ soil. Considering only P_{AER} concentrations increased by an average of 0.48 and 1.03 mg P kg⁻¹ soil for Ox-1 and Ox-2, respectively. Hence the critical soil P level was achieved after approximately 24 years in the Ox-1, but after only 3.5 years at Ox-2, because of the higher rate of legacy labile P accumulation and the higher P_{AER} in the native vegetation related to a lower clay content Table 2)

The $P_{OHid0.1}$ fraction can prove the ability of the brachiaria utilization as cover crop, due to store more organic P. However, this effect was more pronounced in site Ox-1, the longest time study site, which explain the

Po accumulation in this fraction, related to the organic P stored onto fulvic and humic acids adsorbed onto mineral and SOM surfaces (Linguist et al., 1997). In the shortest time study site (Ox-2), Po accumulation also occurred, but only with brachiaria cultivation and when NT was adopted, evidencing the benefits promoted by the brachiaria introduction in NT rotation systems. Brachiaria is capable of recycling and storage P into its root system, relating this crop as a high potential to explore more soil P (Boddey et al., 1996), leading to a higher plant P absorption and contributing to recycle this nutrient. Almeida and Rosolem (2016) reported a significant contribution of brachiaria in the labile P ($P_{AER} + \text{total } P_{BIC}$), indicating a relevant labile Po transformations into Pi, contributing to increase the plant P availability.

P_{OBic} and $P_{OHid0.1}$ (labile and moderately labile organic P) showed similar trends to the ones observed by Tiecher et al. (2012a), where the positive effect of cover crops was related to the authors to the P storing as organic labile fractions. Moreover, under CT system, this effect was also observed, but in deeper layers (10-20 cm), rather than restricted surface (topsoil, 0-10 cm) effects observed in NT.

The $P_{Hid0.5}$ fraction is related to the adsorbed P into mineral phases (Gatiboni et al., 2007; Hedley et al., 1982) and the organic phosphorus related to fulvic and humic substances (Condon et al., 1985), same as the first extraction with NaOH 0.1 mol L⁻¹, but this second extraction is performed to remove P trapped inside the soil microaggregates (Hedley et al., 1982), representing an important P fraction in Oxisols.

Tillage effects were most pronounced for the $P_{OHid0.5}$ fraction, with NT showing generally more organic P accumulation than CT at Ox-1. At Ox-2 (with shorter cultivation time), no effect of tillage system was observed in either the Pi or the Po fractions. Changes observed in this P fraction are attributed to an increment in the soil aggregation proportioned by the SOM levels increment and stabilization (Six et al., 2001, 2000), regulating the storage and turnover of organic carbon and nitrogen and, consequently, affecting the P availability and forms (McDowell et al., 2007; Wang et al., 2001). According to Wang et al. (2001) the P bounding into large aggregates was more readily desorbed and even with no differences in the total P content over different tillage systems are observed, the distribution of the organic P usually change over agricultural practices and distinct managements (Gatiboni et al., 2007, Rodrigues et al., 2016), due to differences in soil structure and carbon distribution in aggregates, what can promote greater P accumulation in the microaggregates (Fonte et al., 2014).

The residual P was not affected by either the tillage systems or by the cover crops in both study sites, similar to other reports (Rodrigues et al., 2016; Tiecher et al., 2012a, 2012b). The residual P is considered an occluded P form with high P binding strength and low reversibility. In tropical soils, the residual P fraction represents the majority of P present (Cherubin et al., 2016; Conte et al., 2003; Rodrigues et al., 2016; Tiecher et al., 2012b), and it is an important P fertilizer immobiliser (Soltangheisi et al., 2017; Teles et al., 2017) leading to low P use efficiency by crops. Although desirable, cover crops and tillage systems were not able to reduce the amount of P stored into highly recalcitrant fraction (residual P).

When evaluated the geochemical (P_{Geoc} , total inorganic P) and biological (P_{Biol} , total organic P) pools, in general, the effects of tillage systems and cover crops were observed only in the topsoil (0-10 cm) in Ox-1, whereas in 10-20 cm depth only P_{Biol} was significantly affected by tillage system and cover crops isolated. In NT, cover crops did not change P_{Biol} in 10-20 cm, whereas fallow under CT showed 18-35% lower P_{Biol} compared to the other crops. This result is probably related to the redistribution of organic P due to soil

disturbance in CT in deeper layers (Tiecher et al., 2012a), and consequently, generating more organic P accumulation, observed in the present study just in CT when cover crops were cultivated.

The P_{Geoc} and P_{Bio} were also significantly affected by cover crops and the behaviour showed the time of cultivation influence on the proportion of these P pools. In the site Ox-1 (longest time) the fallow presented the highest P_{Geoc} level (Figure 1E), whereas the cover crops, specially the brachiaria cultivation reduced the amount of P stored in inorganic pools, reinforcing that in long time cover crops introduction contribute to storing more P in organic fractions (Figure 1F). In the shortest study site (Ox-2), only the brachiaria was capable to store more P_{Bio} (Figure 2F) and its positive effects was not clear in the topsoil, but expressive in the 10-20 cm, the deeper depth evaluated. Also the brachiaria cultivation under NT and maize under CT resulted in more P_{Geoc} , but these results accomplish the total P (Figure 2D), which was directly affected by the increments promoted by the treatments on the labile (Figure 2A) and mod-labile (Figure 2B) P pools.

3.4.2. Tillage and cover crops effects on legacy phosphorus bioavailability

Soil P in the native Cerrado (Table 3S) in 0-5 cm was much higher than in the other layers, indicating the importance of plant P recycling in forests, especially in the tropics (Vincent et al., 2010). However, to estimate the legacy P and in order to compare the differences in the agricultural plots, this aspect was not taken in consideration in the present study and to avoid the tillage systems differences on P levels among the soil depths, only the average weighted 0-20 cm levels were taken in account.

Considering the average soil bulk density of 1.21 and 1.33 kg dm^{-3} for Ox-1 and Ox-2, respectively (Table 2), the total legacy P stock (in the 2000 dm^3 soil volume) calculated by the difference between the total P in the cultivated and native areas was 826.4 (± 48.4) and 490.8 (± 39.9) kg ha^{-1} in these respective sites, very close to the legacy P estimated by crop inputs and outputs (Table 6). This reinforces the potential use of the total P determination for legacy P quantification in agricultural fields. However, a very close native area is still needed because even the soil type is still the same, any small changes on clay contents can change the mineralogical characteristics of the soil or at least affect the P sorption (Fink et al., 2016) and, consequently, the total P levels.

The accumulated legacy P remaining in the soil amounted to 462 and 267 mg kg^{-1} for Ox-1 and Ox-2, respectively, which was 162 and 16 mg kg^{-1} higher than the legacy P predicted by the P fractionation for Ox-1 and Ox-2, respectively. This difference is probably related to the uncertainties involved in the first 20 and 4 years of cultivation for the Ox-1 and Ox-2, respectively, since that the database were not available and estimations were according to the common rates adopted in the beginning of the soybean cultivation in the Cerrado region (Lopes and Guimarães Guilherme, 2016; Sousa and Lobato, 2004), approach also investigated and validated by Roy et al. (2016). Overall, the legacy P obtained by the difference between the P data from the agricultural land and the native area provided a more useful approach, compared to the legacy P calculated by the P balance (P inputs – P outputs) in tropical soils (Withers et al., submitted).

The Brazil's farmland in the central region has been managed at the expense of high agricultural inputs since the conversion from native Cerrado (Lopes and Guimarães Guilherme, 2016), especially high P inputs according by the soil demand into increase its availability, denominated as the soil 'P fixation tax' (Roy et al., 2016). This aspect can be seen in the high P accumulation over the trials cultivation time (Table 6 and Figure 3A, 3B). Overall, a proportion of 48-51% and 47-57% of the legacy P was stored into non-labile P (Figure 3E,

3F) in the Ox-1 and Ox-2, respectively, reinforcing the high P input dependency (Roy et al., 2016), and consequently, the low P efficiency (Novais et al., 2007; Rodrigues et al., 2016) in tropical agricultural systems.

Despite the accumulation of the legacy P into the non-labile P fractions, the increase in labile and moderately labile legacy P promoted by the cover crops in Ox-2 (Figure 3D) indicates the potential of these plants, especially brachiaria, in preventing or retarding P fixation in tropical soils, generating a supplementary source of P potentially available for successive cash crops, consequently diminishing the P input rates (Rowe et al., 2016; Withers et al., 2014; Withers et al., *summitted*). This additional “source” of potentially available P can be estimated up to 50% (172 mg kg⁻¹, considering labile and moderately labile P) of the total legacy P in Ox-1. In site Ox-2, this value ranged from 37% (61 mg kg⁻¹) under millet in CT, rising up to 60% (147 mg kg⁻¹) when brachiaria was cultivated under NT, indicating the relevance of the NT system to enhance the P availability (Gatiboni et al., 2007; Rodrigues et al., 2016) and also the cover crops potential, especially brachiaria, into recycling P, increasing its lability and, consequently, prevent P fixation (Almeida and Rosolem, 2016), or even improve exploitation of less available P fractions (Merlin et al., 2016).

Therefore, a large proportion of the legacy P can be considered as potentially bioavailable P and therefore a supplementary P source for plants during periods of potential P scarcity or when fertilisers may become prohibitively expensive. Based on the P pools affected by the cover crops (Figure 3), we are suggesting the “bioavailable legacy P” as the nutrient amount stored into labile ($P_{AER} + P_{iBIC} + P_{oBIC}$) and moderately labile P pools ($P_{iHid0.1} + P_{oHid0.1} + P_{HCl}$). Although positive effects of cover crops on non-labile P_o were observed in the Ox-2, since this effect is due to an increase in a P fraction related to the soil aggregation development, it was not considered as a bioavailable in sustainable agricultural conditions. However, whether, and how much of, the moderately labile P can be used as a supplementary source of P by crops without yield penalties remains to be clarified and further investigations will be required to support the use of the bioavailable legacy P in the tropics.

3.5. Conclusions

A long cultivation period of up to 42 years generated a large amount of legacy P in the soil, but only a small portion of this legacy P remained in a labile form, and with a slight increase in non-labile P under NT cultivation compared to CT.

The use of brachiaria as a cover crop have the capability to recycle more P to the soil surface than millet, maize or fallow, promoting more total P accumulation and increasing labile P and all the organic P fractions in this topsoil layer (0-10 cm).

Based on cover crop accessibility to the soil P fractions observed in our study, “bioavailable legacy P” should be considered as the amount of P in labile ($P_{AER} + P_{iBIC} + P_{oBIC}$) and moderately labile P ($P_{iHid0.1} + P_{oHid0.1} + P_{HCl}$) pools.

Further investigations of the HCl extractor as bioavailable legacy P are necessary, regarding the absence of any Ca-P mineral phase (soil or low soluble P fertilizers) in the present study, resulting in no effects on P-HCl fraction. Also, the percentage of the moderately labile P to be considered as bioavailable legacy P without yield penalties requires further investigations to support the use of the soil legacy P in tropical soils.

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4. LONG-TERM LAND-USE AND TILLAGE CHANGING PHOSPHORUS SPECIES IN BRAZILIAN OXISOLS: A MULTI-TECHNIQUE APPROACH RELYING ON CHEMICAL FRACTIONATION OF SOIL P, ³¹P-NMR AND P K-EDGE XANES SPECTROSCOPIES

ABSTRACT

Phosphorus (P) interacts with soil constituents in many ways, forming different compounds, being found in a number of chemical forms. The identification of P species in tropical agro-ecosystems is relevant to understand P dynamics and storage in these common P-fixing soils, providing helpful information to improve P use. In this study, we investigated P partitioning and speciation in agricultural soils under different management systems on Brazilian Cerrado Oxisols, using sequential chemical P fractionation (SCF) associated with P speciation via P K-edge XANES and ³¹P-NMR spectroscopies. Topsoil (0-10 cm) samples were obtained from soils under three different management systems, comprehending the native vegetation (Cerrado), no-tillage (NT) and conventional tillage (CT) by long-term. Four representative agricultural sites in the Brazilian Cerrado were selected: Ox-1 (Costa Rica, Mato Grosso do Sul), Ox-2 (Sapezal, Mato Grosso), Ox-3 (Cristalina, Goiás) and Ox-4 (Tasso Fragoso, Maranhão). In all sites, the soils are classified as Typic Hapludox, though varying in cultivation time, soil clay and oxides types and contents. Long-term cultivation in NT and CT led P to accumulate in the topsoil. SCF data indicated that labile P was the smallest P pool observed across the studied soils and land uses. P-XANES identified the major solid-state P forms and showed that P is mostly sorbed to Fe and Al hydr(oxides) Goethite, Hematite and Gibbsite. Phytate was the predominant organic P species, with depletion following land use change to NT and CT management systems. Although the P extraction efficiency was very low (below 32%), ³¹P-NMR data was key to provide detailed information on organic P speciation, showing that monoester-P was present across all soils and management systems, whereas diester-P was found only in the Cerrado samples. The combined use of SCF, XANES and ³¹P NMR data was useful to obtain a deeper understanding of the inorganic and organic P chemistry and reactivity in Cerrado fields as it converts into agricultural lands.

Keywords: Hedley's fractionation; Phosphorus speciation; Phosphorus fractions; Nuclear magnetic resonance; X-Ray; NEXAFS

4.1. Introduction

The environmental and agronomic importance of phosphorus (P) is of concern throughout the world (MacDonald et al., 2011), as P is an essential nutrient often required in high amounts to sustain crop production (Novais et al., 2007; Novais and Smith, 1999). However, required additions of P fertilizers to keep up with food production for a population increasing demand will remain a big push for exploitation of phosphate, a non-renewable resource that has become scarce over time (Cordell et al., 2009; Elser et al., 2014). These aspects reveals the importance of the economic and environmental arguments to increase P use efficiency in tropical regions, like Brazil, where future P demands are estimated to increase following agriculture intensification (Roy et al., 2016; the author, chapter 2).

In tropical soils, such as the Brazilian Savanna (Cerrado), the high sorbing capacity and strong retention of P contributes greatly to the low bioavailability of this nutrient to plants. Brazil is one of the key countries where agriculture expansion is possible and represents a strategic location for future world food demand supply (Ray et al., 2013). However, in order to maintain or increase crop productivity in those areas, P fertilizer applications are necessary on a regular basis, which influences the chemical forms P presents in soils (Rodrigues et al., 2016) and its (re)cycling.

Successive and potentially excessive P inputs over time have resulted in a buildup of residual P in many soils globally (Condon et al., 2013), being called 'legacy P'. Exploring this legacy P by implementing best management practices to increase its bioavailability in soils has been discussed recently (Sattari et al., 2012; Condon et al., 2013). However, the soil management to prevent crop P deficiencies and guarantee higher P use efficiency requires a detailed understanding of P speciation, which allows a more accurate prediction of P bioavailability and mobility in soils (Negassa and Leinweber, 2009).

The Hedley's P fractionation scheme allows the quantification of P in different operationally defined pools, measuring concomitantly the inorganic and organic P in distinct fractions, increasing the recalcitrance of the pools according to the extractant strength (Hedley et al., 1982). With this technique is possible to evaluate the P dynamics among different agroecosystems (Condon and Newman, 2011) and, following a predicted lability (Cross and Schlesinger, 1995) it is possible to infer about the P fractions utilized by plants. Sequential chemical P fractionation data provides useful information on the relative solubility of different P pools in soils and can infer about P bioavailability. However, it is difficult to assign the environmental significance to the soil P fractions solely, because it is operationally defined by the extractants order, not allowing to determine specific P species (Negassa and Leinweber, 2009).

In chemical fractionation schemes the P measured colorimetrically without digestion is referred as "inorganic P" and the difference between the digested extract (total P) and this inorganic P is related as "organic P". This is not completely accurate since supposedly colorimetric analysis does measure inorganic P (Murphy and Riley, 1962), but it does not measure the total inorganic P in the extracts, being determining only the orthophosphate phase that can react with the colour reagent (molybdate). Thus, complexes inorganic P forms such as the pyrophosphates and polyphosphates are not determined in the process and will be included as organic P, although they are not linked to any carbon chain (Cade-Menun, 2005; Condon and Newman, 2011). Orthophosphate bounded (like colloidal P) in solution also cannot react with the colour reagent and will be included as well in the organic P pool, overestimating this. Eventually some degradation of organic P and polyphosphates can also happen during the colorimetric assay, because of the low pH required for colorimetric

analysis (Monaghan and Ruttenberg, 1999). These uncertainties about the organic and inorganic P determination could contribute to wrong interpretations about P dynamics in environmental samples like soils and the more accurate terms molybdate-reactive P (MRP) and molybdate-unreactive P (MUP) have been used to describe the P pools in sequential chemical fractionation schemes (Haygarth and Sharpley, 2000; Cade-Menun, 2015).

Phosphorus speciation via X-ray Absorption Near Edge Structure (XANES) spectroscopy (P K-edge) has been largely used to characterize inorganic P forms in soils (Hesterberg et al., 1999; Hesterberg, 2010; Kruse et al., 2015). It has been particularly employed to identify Ca phosphates (e.g., apatites, brushite, di-calcium, tri-calcium and octocalcium phosphates), Fe-P compounds (e.g., strengite), and Al-P compounds (e.g., variscite) as well as P bound to Al- and Fe- (hydr)oxide minerals in soils (e.g., Kizewski et al., 2011). However, P K-edge XANES has limited application to distinguish P species among phosphates associated to organic molecules, referred as organic P, due to the lack of more distinctive features in the XANES spectrum of organic P species, if compared to their counterpart inorganic ones.

The features seen in a P K-edge XANES spectrum have been used to distinguish among Ca-, Fe(III)- and Al-bound PO_4 species (Kruse and Leinweber, 2008; Hesterberg et al., 2010), being these spectral features also used for determination of P minerals and hosting phases in soils. When it comes to speciation of organic P, phytic acid (myo-inositol hexakisphosphate, IHP) is often used in P K-edge XANES data analysis (Prietz et al., 2013; Liu et al., 2014). However, due to the difficulties in organic P (Po) speciation inherent to IHP similarities in spectra features to those of P sorbed to Fe and Al minerals (pre-edge peak, white line, post-edge shoulder and features) when metal phytates exist, it is advisable to combine P K-edge XANES spectroscopy with complementary techniques that are more sensitive to Po chemistry (Kruse et al., 2015), such as ^{31}P nuclear magnetic resonance (NMR).

NMR has been suggested to provide a more accurate determination of organic P species in environmental samples, such as soils (Negassa et al., 2010; Liu et al., 2013; 2014; Cade-Menun, 2015). The use of ^{31}P NMR offers the possibility to concentrate the sample before analysis, increasing the detection limits of Po species characterization (Cade-Menun, 2005). However, a downside of the technique is that it provides limited information on inorganic P (Pi) speciation and requires an extraction step before analysis (Negassa et al., 2010; Doolette and Smernik, 2011). Solution-state ^{31}P NMR has been widely used to characterize Po forms, (Cade-Menun et al., 2015). To determine the P source, Cade-Menun et al. (2010) identified specific inorganic and organic P forms and characterized their distribution patterns with soil depth in a long-term tillage study. Few studies have investigated the effect of tillage systems (Redel et al., 2011) and P fertilizer use (Ahlgren et al., 2013) on the stratification of P forms in soil profiles employing ^{31}P NMR, which was the aim of our study.

Solution ^{31}P -NMR spectroscopy is by far the most widely used spectroscopic technique for the speciation of soil organic P because it provides the most detailed and accurate information in most circumstances (Doolette and Smernik, 2011). Phosphorus compounds (both organic and inorganic) of interest in studies of soil generally fall within a chemical shift range of 25 and -25 ppm. These include: phosphonates at 7 to 20 ppm; orthophosphate at 6 ppm; orthophosphate monoesters at 3 to 6 and 6 to 7 ppm; orthophosphate diesters at 2.5 to -3 ppm; pyrophosphate at -5 ppm; and polyphosphate from -5 to -20 ppm (Cade-Menun, 2015).

In more complex soil samples, where P may exist in a wider range of P forms under low concentrations, chemical fractionation has been combined with XANES (Kruse and Leinweber, 2008; Cade-

Menuum et al., 2015) and with ^{31}P -NMR (McDowell et al., 2007; Cade-Menum et al., 2015) for a more accurate interpretation of the results. However, to date, there have been only a handful of published studies combining all three techniques to characterize P in soil samples (Kruse et al., 2015). In this way, the aim of this study was to evaluate the changes in soil P species promoted by land use change from native Cerrado (Brazilian Savanna) into conventional and no-till cropping systems, by the combined use of sequential chemical P fraction (Hedley) along with P-K-edge XANES and ^{31}P -NMR spectroscopies.

The hypotheses of this research were:

1. the no-tillage adoption minimizes organic P depletion and can sustain more P in less strongly sorbed forms in tropical Oxisols;
2. the combined use of P chemical fractionation, P speciation by K-edge XANES aimed at characterizing inorganic P and ^{31}P -NMR aimed at characterizing organic P offers advantages over their isolated use to understand soil P dynamics in soils.

4.2. Material and Methods

4.2.1. Study sites characterization

In this study, four long-term field trials established in the Brazilian Cerrado region (Figure 1) were evaluated. All the soils used in this study are classified as Typic Hapludox (Soil Survey Staff, 2014), but vary in cultivation time, soil clay, and Fe and Al contents. Also, the conversion time from the native Cerrado into agricultural land diverged among the different sites. They were previously studied (Rodrigues et al., 2016) in relation to P accumulation (legacy P) and it was observed that P accumulation over the decades after native vegetation conversion into agricultural fields was significant, with a large portion of applied P via fertilizer remaining under P fractions of lower availability.

The climate across the four sites is relatively similar and classified as Aw – tropical, according to Köppen's classification system. The average annual precipitation (last 30 years) was 1,930; 2,150; 1,600 and 1,500, respectively for Ox-1 (Costa Rica, Mato Grosso do Sul, 08°15'10" S / 53°12'41" W, Lat/Long), Ox-2 (Sapezal, Mato Grosso, 13°56'33" S / 58°53'43" W, Lat/Long), Ox-3 (Cristalina, Goiás, 16°15'02" S / 47°37'02" W, Lat/Long) and Ox-4 (Tasso Fragosso, Maranhão, 8°30'57" S / 46°03'47" W, Lat/Long). The rainfall distribution is characterized by a well-defined rainy season from October to April and five month of dry season (with occasional rain totaling less than 80mm) from May to September.

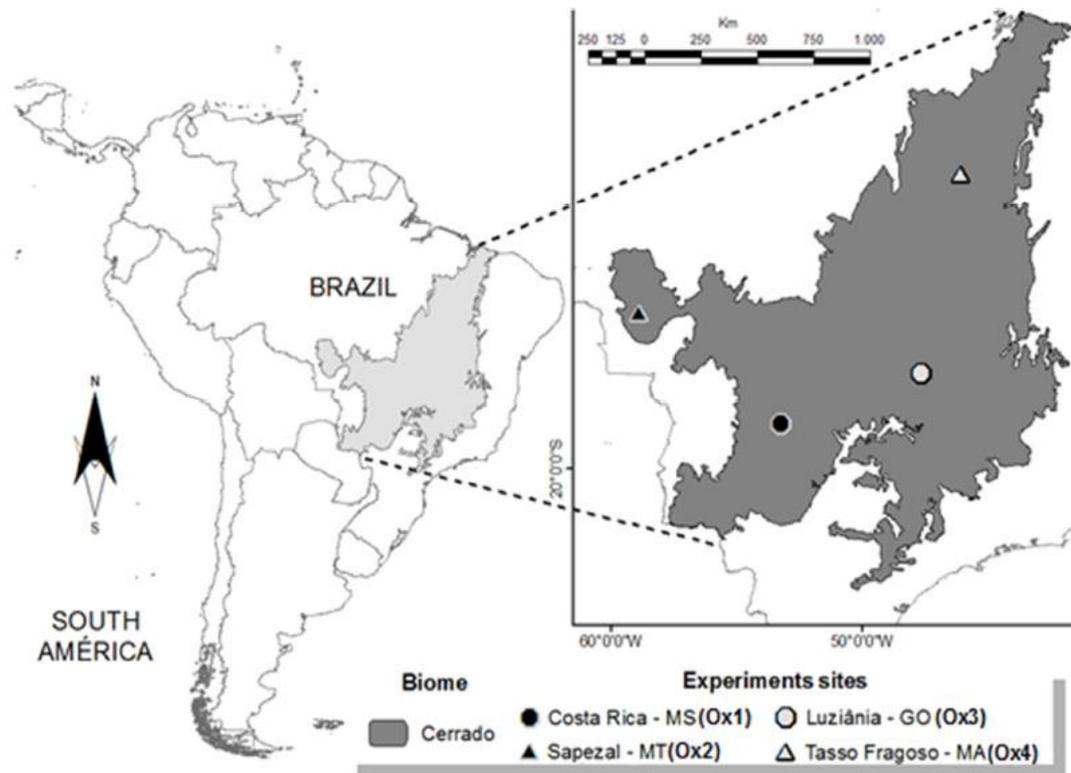


Fig. 1. Brazilian Cerrado biome and the specific geographic localization (inset) of the study sites Ox-1, Ox-2, Ox-3, and Ox-4. Source: Rodrigues et al. (2016).

The study sites were established at different times, providing an opportunity to study the long-term effect of land use and cultivation time on soil P accumulation, speciation and partitioning. Also, in all experimental sites, the no-tillage (NT) and conventional tillage (CT) systems were used, with a native vegetation (Cerrado) in adjacent areas, which allows for comparisons in terms of land-use change from Cerrado (NV) into agriculture, and the effect of tillage systems.

In the Ox-1 and Ox-3 sites, the Cerrado was deforested back in 1974 and 1977, respectively, whereas Ox-2 and Ox-4 experienced shorter cultivation times, with deforestation in 1997 and 1990, respectively. Soybean (*Glycine max* L.) and maize (*Zea mays* L.) were grown at Ox-1 and Ox-3 under a continuous CT system for 20 and 15 years, respectively, before the beginning of the experiment. At Ox-2, soybean and cotton (*Gossypium hirsutum* L.) were grown in CT, and occasionally under NT, until the experiment being establishment in 2001. At Ox-4 soybean was grown under CT from 1990 to 1995. Thereafter, crop rotations of soybean and maize alternated until the experiment was established in 2000. Ever since the tillage systems trial was established, a summer rotation between cotton and soybean (this one followed by millet, *Pennisetum glaucum* L., as cover crop in the winter/dry season) was run in all study sites, except in site Ox-3, where cotton/soybean/maize rotation was adopted.

4.2.2. Experimental design and soil sampling

The soils in all study sites have been previously analyzed for granulometric distribution and some chemical characteristics (Table 1). Ox-1 and Ox-3 both have high clay contents (65%), while Ox-2 has slightly less clay in its composition (45%) and Ox-4 has a high proportion of sand (70%) with only 25% of clay. In Ox-1 and Ox-2, a high content of crystalline Fe and Al were observed (via DCB analysis), while in Ox-3 and Ox-4 crystalline Fe content is much lower than in Ox-1 and Ox-2. Also, Ox-4 has much lower crystalline Al content compared to the other three soils.

Table 1 – Study sites soil chemical and granulometric characterization.

Study Sites	Tillage System	pH* CaCl ₂	Clay ----- g kg ⁻¹ -----	DCB ¹	DCB	Org. C ²	Mehlich-1	Total
				Fe	Al		P	P ³
			----- g kg ⁻¹ -----			----- mg kg ⁻¹ -----		
Ox-1	NT	6.0				23.2	10.8	739
	CT	5.5	656	48.9	11.2	19.2	12.9	714
	NV	4.6				20.7	2.7	477
Ox-2	NT	5.0				20.2	11.0	632
	CT	4.8	452	48.1	13.1	17.5	6.0	602
	NV	3.8				19.0	1.8	440
Ox-3	NT	5.6				18.4	6.8	734
	CT	5.2	653	26.6	12.1	15.7	7.8	747
	NV	4.0				18.9	0.9	419
Ox-4	NT	4.9				12.7	25.8	438
	CT	4.7	255	19.8	5.8	10.2	14.5	348
	NV	3.8				15.7	1.1	225

* Determined after equilibrium with 0.01 mol L⁻¹ CaCl₂ solution (1:2.5 soil:solution ratio).

¹ DBC: dithionite, citrate and bicarbonate extraction, according with method described by Mehra and Jackson (1960).

² According to the Walkley-Black method.

³ Extracted by the sulfuric digestion method (Olsen and Sommers, 1982) with colorimetric determination using the molybdate-blue method (Murphy and Riley, 1962).

Topsoil samples (0-10 cm) were collected from all four sites, under different land-uses (NT, CT and NV). Four subsamples were individually sampled by shovel in each plot to generate a representative composite sample. For NV three plots inside the native vegetation were established, with at least 30 m of distance from the border and at least 50 m of distance between the marked plots. Similarly to the agricultural plots, four subsamples were collected in each plot and then, homogenized into composite samples. After sampling, the soils were air-dried for 72 hours, sieved (2 mm sieve) and stored prior to the analyses.

4.2.3. Sequential chemical fractionation of soil phosphorus

The air-dried samples were subjected to a sequential chemical fractionation of P scheme according to the Hedley's procedure (Hedley et al., 1982), with modifications (Condon et al., 1985). The main advantage of this method is its capacity to determine both inorganic (Pi) and organic (Po) fractions concomitantly (Condon and Newman, 2011). Although the sequential P fractionation extractants give some uncertainty and may overlap in terms of the P forms extracted across variable agroecosystems (Condon and Newman, 2011), the method nevertheless provides useful insights into the lability of soil P.

The sequential P fractionation is: i) Anion exchange resin (P_{Resin}), that extracts soluble inorganic P readily available to plants (Van Rai et al., 1986); ii) $NaHCO_3$ (P_{Bic}) at pH 8.5 (0.5 mol L^{-1}), related also to labile inorganic P (P_{iBic}) weakly adsorbed on the surface of crystalline phase and labile organic P (P_{oBic}) with low recalcitrance, related to ribonucleic acid and glycerophosphate (Tiessen and Moir, 1993); iii) $0.1 \text{ mol NaOH L}^{-1}$ (P_{HID-1}), moderately labile inorganic (P_{iHID-1}) and organic (P_{oHID-1}) P fractions, related to strongly adsorbed Pi onto Fe and Al and clay minerals (Hedley et al., 1982) and Po mainly associated with fulvic and humic acids adsorbed onto mineral and SOM surfaces (Linguist et al., 1997); iv) $1.0 \text{ mol HCl L}^{-1}$ (P_{HCl}) extract mainly apatite P or other sparingly-soluble Ca-P compounds (Gatiboni et al., 2007); v) $0.5 \text{ mol NaOH L}^{-1}$ (P_{HID-2}), that removes more recalcitrant (non-labile) forms of inorganic (P_{iHID-2}) and organic P ($P_{oHID-0.5}$), associated with Fe and Al and clay minerals and Po from fulvic and humic acids inside aggregates (Condon et al., 1985); vi) Residual P ($P_{Residual}$), occluded (non-labile) obtained from remaining soil digestion with $H_2SO_4 + H_2O_2 +$ saturated $MgCl_2$ (Olsen and Sommers, 1982).

At each fractionation step, 10 mL of extractant was added to 0.5 g soil in a 15 mL centrifuge tubes and end-over-end (vertical agitator, 60 rpm) shaken for 16 h at room temperature ($\sim 25^\circ\text{C}$). After each extraction, the soil suspensions were centrifuged at 4000 rpm (3278 g) for 30 min to collect clear supernatants. Total P (Pt) in the alkali extracts (P_{oBic} , P_{oHID-1} and P_{oHID-2}) was determined by digestion with sulphuric acid (H_2SO_4) and ammonium persulfate in an autoclave at 121°C (USEPA, 1971). Pi in alkali extracts was measured by the colorimetric method of Dick and Tabatabai (1977). Pi in acid extracts (P_{AER} and P_{HCl} , and from digestion of P_{iBic} , $P_{iHID-0.1}$ and $P_{iHID-0.5}$) was measured by the colorimetric method of Murphy and Riley (1962) and Po in alkali extracts was obtained by difference between measured Pt and Pi.

Changes in P fractions have been previously reported by Rodrigues et al. (2016). The results presented herein are, however, limited to the topsoil layer (0-10 cm) only, regarded as the soil layer that is more affected by the tillage system in place. Since the aim of the current research was to evaluate the P accumulation in the soil and determine the remaining predominant solid-state species, quantitative aspects of our P fractionation results were not discussed. Rather, the relative proportions representing each discrete P fractions of the fractionation scheme in relation to the total P are presented and discussed. This approach provides enough information to compare the different soils and managements systems, minimizing the effects resulted from differences in terms of soil chemical and physical characteristics.

4.2.4. Phosphorus K-edge XANES spectroscopy analysis

Topsoil (0-10 cm) samples were finely ground in an automated ball grinder, followed by milling in an Agatha mill until the material could pass completely through a 100 mesh screen sieve (0.149 mm). This is important to avoid background noise in P K-edge XANES analysis.

The reference standards for P-XANES analysis were either obtained from commercial suppliers (amorphous Ca-phosphate, Na-phytate, tricalcium phosphate, octacalcium phosphate, deoxyribonucleic acid, lecithin), third persons (Gibbsite, kaolinite and hematite were obtained from Dr. Audrey Gamble from the Sparks' lab at the University of Delaware) or prepared in the lab by sorbing phosphate as a K_2HPO_4 salt to mineral analogues of commonly found clay minerals and oxides in soils. Goethite was synthesized according to Abdala et al., (2015). The sorption experiments were carried out by equilibrating each of the solids separately with Milli-Q DI water on a 1:20 w:v ratio at pH 5.5 ± 0.2 . The suspensions were end-over-end shaken at 60 rpm for 7 days, having the pH adjusted with 1M, 0.1M and 0.01M solutions of KOH or HCl every other day (3 times a day). After pH stabilization, the standards were centrifuged and reacted with a 0.8 mol L^{-1} P solution (as KH_2PO_4 , Sigma Aldrich), having the pH adjusted to 5.5 ± 0.2 (1:20 w:v ratio) over a 7 days reaction time. At the end of this period, the samples were centrifuged and the solutions stored for colorimetric P analysis (Murphy and Riley, 1962). In order to eliminate any residual of soluble P, the samples were washed three times with 0.01 M KCl (1:20 w:v ratio) for 15 min at 150 rpm, centrifuged and dried (45°C) for 72 hours.

Samples previously milled were analyzed by P K-edge X-rays Absorption Near Structure (XANES) spectroscopy. A composite sample representing soils from each land-use from each study site was chosen to perform the XANES analysis. The replicate equivalent to the median result of the total P determined by the sequential chemical fractionation was selected. Phosphorus K-edge XANES analyses were conducted at the Soft X-Ray beamline of the Brazilian Synchrotron Light Laboratory (LNLS), in Campinas. The operating conditions of the storage ring at the LNLS during data collection was 1.37 GeV with a current range of 110-250 mA.

Finely ground soil samples were uniformly spread on double-sided carbon tape to form a thin layer, mounted on a stainless steel sample holder and inserted into an experimental chamber that operates at vacuum conditions ($\sim 10^{-7}$ mbar). Phosphorus K-edge XANES data were collected in fluorescence mode using a Si-drift detector. Detector dead time was monitored throughout data collection to not exceed 8%, which was achieved by moving in and out the detector in the experimental chamber. A reference energy (E^0) value of 2150.7 eV (corresponding to the second derivative of a calcium phosphate standard used for energy calibration at beamline SXS) was used for energy calibration and scans were collected in energy ranging from 2120 to 2300 eV.

The P-XANES data were collected with varying step sizes of 1.0 eV from 2120 to 2145 eV, 0.2 eV from 2145.2 to 2180 eV, 1.0 eV from 2181 to 2220 eV and 3.0 eV from 2223 to 2300 eV and an accumulation time of 1.0 sec per data point. Each spectrum was obtained by averaging of 8 to 15 individual scans for the soil samples and at least three scans for the reference standards.

Background data was subtracted by fitting a first-order polynomial to the pre-edge region from $E^0 - 30$ to $E^0 - 10$ eV and the spectrum was normalized over the reference energy of 2151 eV by fitting a second-order polynomial over the post-edge region from $E^0 + 25$ to $E^0 + 140$ eV. The phosphate species included in the fit were the ones that best represented the phosphate species found in the studied soils and were chosen based on the pre-edge, white line maximum, first post-edge crest and second post-edge crest (eV) similarities between the standards and the soil samples.

Linear combination fitting (LCF) analysis of P-XANES data was performed using the ATHENA program in the DEMETER software package (Ravel and Newville, 2005) over the E0 -10 to E0 +30 eV energy range. Fitting was done iteratively, using up to five different reference spectra at a run. Reference spectra that yielded fit as negative or represented values lower than the XANES sensitivity (5%, Beauchemin et al., 2003; Werner and Prietzel, 2015) in the fit were eliminated. LCF was performed using all possible binary, ternary and quaternary combinations of the last five selected reference spectra (after fitting with all the reference compounds the higher percentage ones were selected for a final LCF with the most probable five species).

The E0 was allowed to vary and the weights of all P standards used were forced to sum to 1. The goodness-of-fit was judged by the chi-squared and R values, and P standards yielding the best fit (at the maximum quantity relative to the quaternary combinations) were determined as the most likely P species present in the investigated soil samples.

4.2.5. Solution ³¹P Nuclear Magnetic Resonance spectroscopy

Solution ³¹P NMR spectroscopy was performed using a modified version of the Cade-Menun and Preston (1996) procedure. For each soil type and land use, a duplicate air-dried soil sample (3g) was subjected to P extraction with 25 mL of combined 0.25 M NaOH and 0.05 M Na₂EDTA, shaking at 250 rpm for 6 hours at room temperature (~20°C). The extracts were then centrifuged (45 min, 5000 rpm) and filtered using Whatman No. 42 filter paper. A 1 mL aliquot was diluted for total P measurement by ICP-OES and the remaining filtrate was freeze-dried (36-48h). The lyophilized extracts were re-dissolved in 0.6 mL deuterium oxide (D₂O), 0.6 mL of 10 M NaOH and 0.4 mL of the NaOH-EDTA extracting solution, vortexed, stand for 15 min and then vortexed again (Cade-Menun and Liu, 2014). The extracts were centrifuged (30 min, 5000 rpm) to avoid remaining particles that might contribute to line broadening and transferred to 5 mm NMR tubes.

The ³¹P-NMR spectra were acquired on a Bruker Advance 400 MHz NMR spectrometer (7.5 T, 161.9MHz), equipped with a 5-mm broadband probe. The NMR instrumental parameters were a 90° pulse, 0.68 s acquisition time, 4.321 s pulse delay, controlled temperature at 20°C, without proton decoupling. For each sample, an amount of 3000-3500 scans (~ 4-5 h running time) were collected to achieve a reasonable spectral resolution (higher signal:noise ratio).

Three to five seconds of delay time has been reported to be enough to obtain quantitative spectra of NaOH-EDTA in similar soil extracts (McDowell et al., 2006; Stutter et al., 2015). The chemical shift (ppm) was referenced to an external 85% H₃PO₄ standard via the lock signal. Three parameters were observed to define the peaks: peak height and line width to determine the relative contribution of the species, and the chemical shift to identify them, by comparisons to literature data (Cade-Menun, 2015; Cade-Menun et al., 2010; Dooletteet al., 2009; Turner et al., 2003).

Integration of peak areas on spectra was processed with a line broadening of 3 Hz using a Bruker Topspin 2.0 software and MestReNova v.6.0. Quantification of P species was performed by spectra deconvolution analysis, providing the relative P concentration in the NaOH-EDTA extracts, based on the total NMR signal area for each species. If specific identification could not be made, they were grouped into compounds or compound classes (Cade-Menun et al., 2010; Dooletteet al., 2009).

4.3. Results and Discussion

4.3.1. Soil phosphorus fractions

Overall, higher inorganic P values were observed under NT in all studied sites, whereas the amounts of organic P were very similar in all studied sites (Figure 2A). The land use change from NV to long-term agriculture (both NT and CT) resulted on a significant increment in the organic and inorganic P pools, in all studied sites. Total P amounts did not vary much under NT and CT over time, neither in the inorganic or organic P pools, in all four different locations. Despite the differences in total P among the cultivated and native areas and across all studied sites, around 67-92% of it was present as inorganic P (Figure 2B). This corroborates the study by Abdala et al. (2015) showing that highly weathered agricultural soils of southern Brazil receiving application of manures over times spanning from 20 to over 40 years led P to accumulate preferentially under inorganic forms, ~80% of the total, regardless of management system, NT or CT. These observations suggest nothing but that accumulation of P in tropical and subtropical agricultural soils occurs mainly under inorganic forms, which predominate over its organic counterparts, regardless of soil management system, application time and P source, that is, organic (manures) or mineral fertilizer. Interestingly, in spite of the long-term of soil cultivation under NT being expected to lead P to accumulate preferentially under organic forms, as observed throughout the literature (Rheinheimer et al., 2002; Tiecher et al., 2012), CT was shown to lead to a greater accumulation of P in inorganic forms, from 67 to 83% under CT and 69 to 85% under NT of the total P.

A wide range, from 8 to 33% of the total P, was found to be present as organic P (Figure 2B), with the smallest values in Ox-4. The low clay content in Ox-4 (Table 1) perhaps explains the lowest content of P in that soil, suggesting that the weak stability of the P organo-complex formed limits P retention. Despite the dominance of the inorganic P forms, these relative organic P has been related as a potential sink of labile P in tropical soils, originally high P-fixing soils (Novais and Smith, 1999; Novais et al., 2007). The increment in the relative contribution of organic P after Cerrado conversion into agriculture, observed here for both NT and CT for all studied soils, could be related as a positive aspect in P dynamics in tropical soils (Dodd and Sharpley, 2016; Rodrigues et al., 2016). However, it is noteworthy to mention that in all sites the summer crops are followed by a cover crop cultivation (sec. 2.1), which resulted in increments in soil organic carbon levels (Table 1) and, consequently, increases in organic P.

Total P ranged from 225 mg kg⁻¹ (Ox-4) up to 478 mg kg⁻¹ under NV (Cerrado, Figure 1A). In cultivated areas, total P was much higher than NV in all sites, ranging from 349 up to 748 mg kg⁻¹. These differences between native and cultivated areas are a consequence of higher annual P inputs (chapter 2, sec. 2.2.1), a common practice in Cerrado agriculture in the last decades (Rodrigues et al., 2016; Lopes and Guilherme, 2016). In general, total P levels were very similar between NT and CT systems in all sites, indicating that over time the P balance was quite even between tillage systems, i.e., no relevant differences were observed in the annual P balance ascribed to P off-takes between NT and CT.

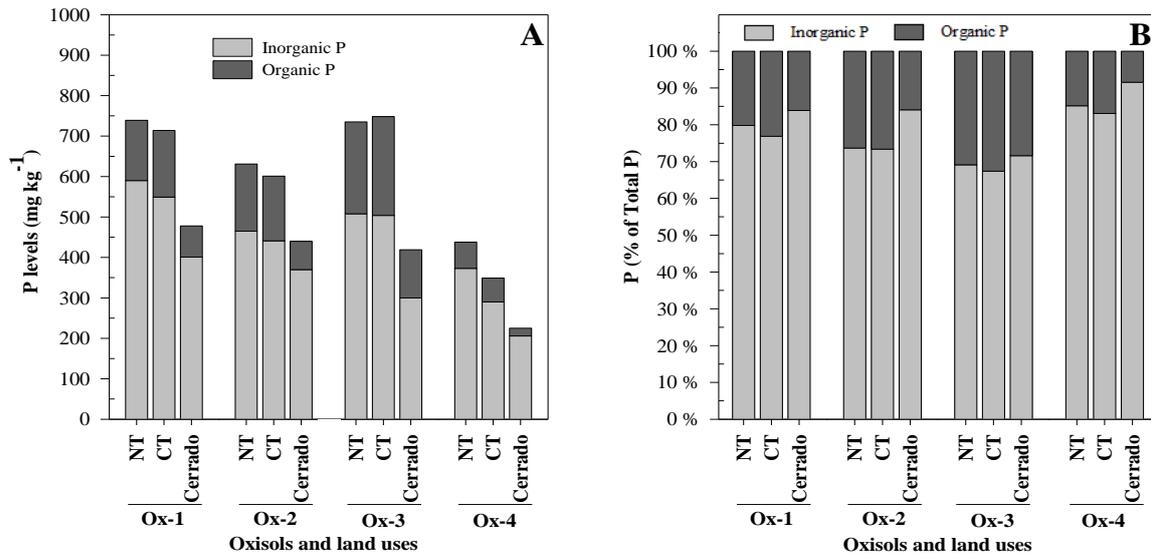


Figure 2. Inorganic and organic phosphorus levels (A) and their relative contribution in the total P (B) among different Cerrado Oxisols and land uses. NT: no tillage; CT: conventional tillage; NV: native vegetation (Cerrado).

The HCl-P fraction, representing the Ca phosphates (Hedley et al., 1982) was not observed in significant amounts (0.3-1.7%) in the studied sites (Table 2, Figure 3), and the explanation for this being the sole use of soluble P fertilizers over the years of cultivation and the absence of any calcium phosphates in the soils' parental material. Additionally, in all sites soil pH was lower than 6.5 (Table 1), known as the pH level below which solubilization of Ca-phosphates is favored (Andersson et al., 2016). Small though the observed Ca-P values are, they can be attributed to the formation of Ca-P during the bicarbonate (pH=8.5) and 0.1M NaOH (pH >12) extractions in the fractionation procedure used.

Inorganic and organic Hid-P fractions (0.1 and 0.5), representing P sorbed to Fe and Al forms (Pi and Po, Condron et al., 1985), corresponded to 60 to 82% of the total P (Figure 3), with similar proportions of Fe-P + Al-P forms in CT and NT systems in all sites. P forms associated with Fe-P + Al-P had a significant reduction in their relative contribution as consequence of NV to agriculture land-use (NT and CT), observed in all sites except in the Ox-3, where a reduced value of P bound to Fe + Al (hydr)oxides was observed due to the cultivation system (soybean and maize succession), which resulted in lower annual residual P (Rodrigues et al., 2016), resulting to the highest P bound in the soil matrix as organic P.

Table 2. Relative distribution of P according to Hedley's phosphorus fractions in topsoil (0-10 cm) samples of long-term field trials in Brazilian Cerrado Oxisols.

Land Use	P fractions (% , relative to total P)											Total-P
	Resin-P	Bic-Pi	0.1Hid-Pi	HCl-P	0.5Hid-Pi	Residual-P	Inorganic P	Bic-Po	0.1Hid-Po	0.5Hid-Po	Organic P	
Ox-1												
NT	3.5 (± 0.3)	4.3 (± 0.1)	14.5 (± 1.4)	0.7 (± 0.1)	18.2 (± 1)	38.4 (± 0.7)	79.7 (± 3.5)	4.8 (± 1.4)	9.9 (± 3.8)	5.5 (± 0.6)	20.2 (± 5.9)	100.0 (± 1.6)
CT	3.8 (± 0.3)	4.5 (± 0.2)	17.3 (± 2.1)	0.6 (± 0.1)	16.2 (± 1.2)	34.6 (± 2.1)	76.9 (± 6.1)	4.9 (± 0.7)	10.2 (± 0.7)	8.0 (± 2.7)	23.1 (± 4)	100.0 (± 3.5)
NV	1.1 (± 0.3)	2.8 (± 0.6)	11.1 (± 0.8)	0.4 (± 0.2)	20.6 (± 5)	47.8 (± 4.2)	83.9 (± 11.1)	4.8 (± 1.6)	8.6 (± 1.9)	2.7 (± 0.2)	16.1 (± 3.7)	100.0 (± 6.7)
Ox-2												
NT	4.2 (± 0.9)	7.6 (± 0.6)	11.6 (± 1.3)	0.6 (± 0.2)	15.6 (± 3.6)	34.0 (± 0.9)	73.6 (± 7.6)	5.0 (± 1)	12.8 (± 0.9)	8.5 (± 0.6)	26.3 (± 2.5)	100.0 (± 3.2)
CT	2.7 (± 0.4)	4.5 (± 0.7)	15.3 (± 1.6)	0.5 (± 0.1)	13.7 (± 1.6)	36.7 (± 6.5)	73.4 (± 10.9)	5.7 (± 1.2)	12.4 (± 3.6)	8.4 (± 0.5)	26.5 (± 5.3)	100.0 (± 5.1)
NV	2.0 (± 0.2)	4.3 (± 0.3)	13.5 (± 2.1)	0.4 (± 0.1)	14.9 (± 2)	48.9 (± 5.2)	84.0 (± 9.9)	6.6 (± 1.5)	6.3 (± 2.8)	3.1 (± 0.4)	16.0 (± 4.7)	100.0 (± 1.1)
Ox-3												
NT	2.4 (± 0.3)	5.4 (± 0.3)	11.0 (± 3.3)	0.5 (± 0)	13.8 (± 2)	36.1 (± 5.7)	69.1 (± 11.7)	2.0 (± 1)	18.7 (± 2.8)	10.2 (± 2.3)	30.9 (± 6.1)	100.0 (± 2.2)
CT	2.7 (± 0.4)	4.6 (± 0.4)	12.3 (± 3.3)	0.4 (± 0)	15.5 (± 4.1)	31.9 (± 4.4)	67.4 (± 12.6)	4.4 (± 1.8)	21.9 (± 2.7)	6.3 (± 0.5)	32.6 (± 4.9)	100.0 (± 0.3)
NV	0.9 (± 0.1)	3.9 (± 0.5)	7.1 (± 0.5)	0.3 (± 0.1)	14.6 (± 2.2)	44.9 (± 1.2)	71.6 (± 4.6)	3.9 (± 0.7)	16.6 (± 0.9)	7.8 (± 0.3)	28.3 (± 2)	100.0 (± 3.1)
Ox-4												
NT	4.7 (± 0.2)	9.2 (± 2.4)	23.1 (± 1.5)	1.4 (± 0.3)	20.0 (± 4.4)	26.9 (± 0.7)	85.3 (± 9.5)	5.5 (± 1.1)	5.5 (± 0.3)	3.9 (± 0.5)	14.9 (± 1.9)	100.0 (± 4.6)
CT	3.9 (± 0.7)	7.8 (± 0.5)	19.3 (± 1.1)	1.3 (± 0.6)	18.4 (± 2.4)	32.5 (± 2)	83.1 (± 7.2)	3.2 (± 0.7)	5.8 (± 1.1)	7.8 (± 2.7)	16.8 (± 4.5)	100.0 (± 3.7)
NV	2.6 (± 0.2)	4.4 (± 1.1)	15.1 (± 1.3)	1.7 (± 0.4)	23.9 (± 2.9)	44.0 (± 4)	91.6 (± 9.9)	3.6 (± 1.2)	2.1 (± 0.6)	2.5 (± 0.6)	8.2 (± 2.4)	100.0 (± 4)

NT: no tillage; CT: conventional tillage; NV: native vegetation (Cerrado). Values within brackets indicate the standard deviation (n=3).

The most recalcitrant P fraction (occluded P) did not differ between tillage systems, with similar values to the NV. It demonstrates that applied P via fertilizer in tropical soils becomes, in large part, associated to the Fe- and Al-P forms extracted by the NaOH, generally ascribed as the major sink for P in Oxisols (Novais and Smith, 1999; Novais et al., 2007).

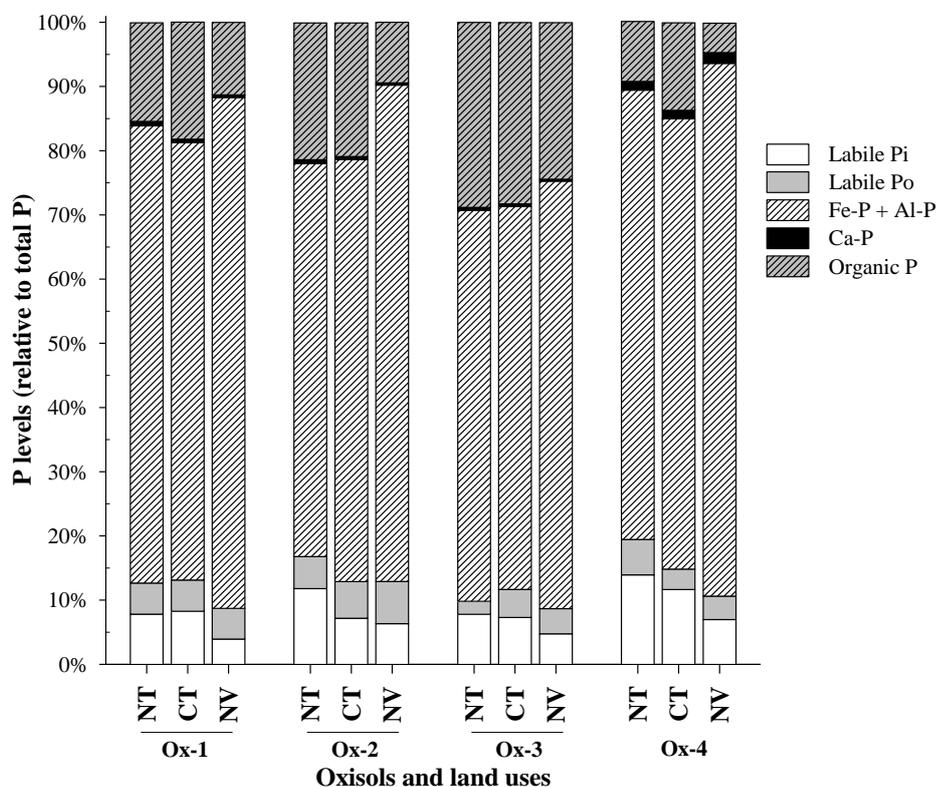


Figure 3. Distribution of P Hedley fractions associated to Fe+Al, Ca and organic compounds among different Cerrado Oxisols and land uses. NT: no tillage; CT: conventional tillage; NV: native vegetation (Cerrado).

4.3.2. P K-edge XANES spectroscopy

Prior to linear combination fitting (LCF) of our XANES data, reference spectra for P sorbed to mineral analogues, as described in *session 2.4.1*, were selected based on spectral characteristics upon visual inspection of P-XANES spectra. The observed characteristics are related to the presence of a pre-edge, edge or white line position and 1st and 2nd post-edge crests (Calvin, 2013; Werner and Prietzel, 2015).

A table containing the corresponding energies for the pre-edge, white line, and 1st and 2nd post-edge crests of the reference spectra for the mineral analogues used in LC analysis is presented below (Table 3).

The analysis of P K-edge XANES data of the soils was performed in three steps. To provide the first hint of chemical P species in the soils, the most improbable Ca-P species (at the number of 5, composed by the Ca-P brushite, dicalcium phosphate, tricalcium phosphate, octacalcium phosphate and amorphous Ca-P) were used in the LCF with the P sorbed in Goethite (common Fe oxide in tropical soils). The resulted best fit (lower R-factor and χ^2 values) granted the selection of the best Ca-P standard to follow the LCF. In the second step, the “best” selected Ca-P were analyzed with the organic P species (IHP, Lecithin and DNA) and P sorbed in

amorphous Fe and Al minerals. The “best” fitting (maximum of ternary combinations) was selected and added for the 3rd step, composed by the utilization of one Ca-P standard, the selected organic P specie(s) and the P sorbed in Fe and Al minerals (P-sorbed in amorphous Fe and Al, P sorbed in Goethite, P sorbed in Hematite, P sorbed in Gibbsite, P sorbed in Kaolinite).

Linear combination analysis was performed according to Abdala et al. (2018). Overall, P-XANES spectra showed white line maxima sitting around 2151.8 ± 0.2 eV (Table 4), that was indicative of P being mostly associated to the crystalline Fe-minerals (Table 3), for all soils, regardless of management system or region.

Table 3 – Phosphorus K-edge XANES spectral features and corresponding energies of reference spectra of P sorbed to mineral analogues used in the Linear Combination analysis.

Reference Standards	Pre-edge Energy (eV)	White line maximum	Post-edge shoulder	1 st post-edge crest	2 nd post-edge crest
Myo-inositol hexakiphosphate (phytic acid)	-	2,151.5	-	2,158.6	2,166.8
DNA	-	2,151.3	-	-	2,165.7
Lecithin	-	2,151.4	-	-	2,166.9
Am-Ca phosphate	-	2,151.4	2,153.2 – 2,157.3	2,161.4	2,168.0
Brushite	-	2,151.6	2,153.5 – 2,158.0	2,161.5	2,168.8
Tri-calcium phosphate	-	2,151.3	2,153.5 – 2,158.0	2,161.0	2,168.5
Octa-calcium phosphate	-	2,151.3	2,153.3 – 2,157.5	2,161.5	2,168.0
P sorbed to Ferrihydrite*	2,145.0 – 2,148.8	2,152.0	-	-	2,168.0
P sorbed to Am-Al hydroxide*	-	2,152.0	-	2,160.6	2,169.2
P sorbed to Goethite*	2,145.4 – 2,148.7	2,152.0	-	2,158.8	2,168.5
P sorbed to Hematite*	2,144.0 – 2,148.3	2,152.0	-	2,159.6	2,167.8
P sorbed to Gibbsite*	-	2,152.0	-	2,160.4	2,168.8
P sorbed to Kaolinite*	-	2,152.0	-	2,157.5	2,168.8

* Standards of P sorbed in different minerals analogues (sorption according sec. 2.1).

Table 4 – Phosphorus K-edge XANES spectra of the different soils and land-uses indicating the energies corresponding to the main diagnostic spectral features.

Soil	Land-Use	Pre-edge	White line maximum	Post-edge shoulder	1 st post- edge crest	2 nd post- edge crest
Ox-1	NT	-	2,151.8	-	~2,157.6	~2,168.5
	CT	-	2,151.9	-	~2,157.8	~2,168.5
	NV	-	2,151.9	-	~2,157.8	~2,168.1
Ox-2	NT	-	2,151.8	-	~2,157.6	~2,168.5
	CT	-	2,151.8	-	~2,157.6	~2,168.5
	NV	-	2,151.8	-	~2,157.8	~2,168.1
Ox-3	NT	-	2,151.8	-	~2,157.6	~2,168.5
	CT	-	2,151.9	-	~2,157.8	~2,168.5
	NV	-	2,151.9	-	~2,157.8	~2,167.8
Ox-4	NT	-	2,151.8	-	~2,157.2	~2,168.0
	CT	-	2,151.9	-	~2,157.8	~2,168.2
	NV	-	2,151.9	-	~2,158.2	~2,167.8

The LCF fitting of the samples was satisfactory, denoted by small values of R^2 . This value in the fits of P K-edge XANES analysis corresponds to a statistical parameter measuring the correlation between the measured and the predicted spectral feature. In the samples, the values ranged from 0.001-0.01 (Table 5). Also, the reduced- χ^2 values were low, ranging from 0.001 - 0.01. The anticipated major contribution of Fe minerals in soil via SCF was confirmed by LC analysis performed to the XANES data, with a predominance of P sorbed to Goethite (P-Goet) and Hematite (P-hema) in all soils, accounting for as much as 83 % of the total (Table 5). A smaller proportional of P sorbed to Gibbsite (P-Gib) was found, accounting for as much as 14% which did not vary much between cultivated soils (NT and CT) and native vegetation, irrespectively of the site where the sample came from.

The data clearly indicates the relevance of Fe- and Al- (hydr)oxide minerals in P retention in tropical soils (Table 5). Organic phosphates appeared only as phytate and ranged from 8 to 25% in the studied sites. Also, LC analysis indicated a depletion in organic P in the cultivated areas compared to the native vegetation, from 8% under NT to 13% under CT. The similarity of the organic P amounts under NT and NV indicate that these species tend to become more stable when suitable soil management practices are adopted for crop production.

Table 5. Relative proportions of the P chemical species determined by linear combination fitting (LCF) analysis of P K-edge XANES data from tropical soils with different land uses.

Land Use	Relative % of P species (from the standards used to combinations)										R factor	Reduced Square chi
	----- P sorbed in Fe oxides -----			P sorbed in Al (hydr)oxides		Kaolinite-P	----- Ca-P -----		----- Organic P -----			
	Goethite	Hematite	Am-Fe ¹	Gibbsite-P	Am- γ Al ²		Apatite	Other Ca-P ³	DNA	Phytate		
Ox-1												
NT	21 (\pm 8)	49 (\pm 7)	-	9 (\pm 3)	-	-	-	-	-	21 (\pm 2)	0.003	0.005
CT	30 (\pm 5)	49 (\pm 4)	-	7 (\pm 2)	-	-	-	-	-	14 (\pm 1)	0.002	0.002
NV	33 (\pm 4)	36 (\pm 4)	-	7 (\pm 3)	-	-	-	-	-	24 (\pm 7)	0.004	0.009
Ox-2												
NT	22 (\pm 8)	47 (\pm 6)	-	12 (\pm 5)	-	-	-	-	-	19 (\pm 2)	0.003	0.006
CT	25 (\pm 5)	50 (\pm 4)	-	11 (\pm 6)	-	-	-	-	-	14 (\pm 2)	0.004	0.009
NV	20 (\pm 8)	51 (\pm 9)	-	9 (\pm 5)	-	-	-	-	-	20 (\pm 3)	0.006	0.010
Ox-3												
NT	39 (\pm 6)	26 (\pm 5)	-	10 (\pm 1)	-	-	-	-	-	25 (\pm 2)	0.004	0.005
CT	41 (\pm 4)	25 (\pm 3)	-	14 (\pm 2)	-	-	-	-	-	20 (\pm 1)	0.001	0.001
NV	33 (\pm 4)	34 (\pm 4)	-	9 (\pm 1)	-	-	-	-	-	24 (\pm 1)	0.010	0.010
Ox-4												
NT	51 (\pm 6)	28 (\pm 5)	-	8 (\pm 2)	-	-	-	-	-	13 (\pm 1)	0.002	0.005
CT	50 (\pm 2)	33 (\pm 1)	-	9 (\pm 2)	-	-	-	-	-	8 (\pm 2)	0.010	0.010
NV	37 (\pm 1)	34 (\pm 1)	-	8 (\pm 1)	-	-	-	-	-	21 (\pm 2)	0.007	0.010

NT: no tillage; CT: conventional tillage; NV: native vegetation (Cerrado). Values within brackets indicate the predicted uncertainties according to LC analysis.

¹ Refers to P sorbed to the mineral analogue of Ferrihydrite.

² Refers to P sorbed to the mineral analogue of non crystalline Al hydroxide (non α -Al, as γ -alumina).

³ Other calcium phosphates (Brushite, dicalcium phosphate, tricalcium phosphate and beta octacalcium phosphate).

4.3.3. Solution ^{31}P -NMR spectroscopy

The amounts of phosphorus extracted in the NaOH+EDTA extraction (standard procedure) and measured by ^{31}P -NMR spectroscopy are shown in the Table 6. The extraction efficiency, i.e., the relative proportion between the P extracted in the NMR analysis and total P, in general, was not so expressive, ranging from 12 to 32% in the samples analyzed. This fact can be related to the high P fixation capacity observed in these soils (Oxisols), also identified in the SCF analysis, where around 27-49% (Table 2) of total P was observed to be present as residual P, after NaOH extractions, and in the P-XANES results.

Table 6 – Phosphorus levels extracted by NaOH+EDTA and extraction efficiency in the NMR procedure.

Study Sites	Tillage System	NaOH+EDTA P mg kg ⁻¹	NaOH+EDTA Extraction efficiency (% of Total P)
Ox-1	NT	220.5 (± 17.2)	29.8
	CT	203.0 (± 16.0)	28.4
	NV	68.8 (± 2.9)	14.4
Ox-2	NT	125.4 (± 12.3)	19.8
	CT	134.8 (± 25.6)	22.4
	NV	54.2 (± 5.9)	12.3
Ox-3	NT	191.8 (± 35.6)	26.1
	CT	220.8 (± 29.5)	29.6
	NV	68.8 (± 2.9)	16.4
Ox-4	NT	139.9 (± 21.1)	32.0
	CT	86.9 (± 16.8)	25.0
	NV	35.4 (± 2.9)	15.7

NT: no tillage; CT: conventional tillage; NV: native vegetation (Cerrado).

Values within brackets indicate the standard deviation (n=3).

In relation to the P species distribution observed in the ^{31}P -NMR analysis, the organic species observed in the samples were mainly identified as monoesters (mono-P) and diesters (diester-P). It was not possible to identify all the peaks in mono and diesters regions, due to the minor amounts of these compounds and limitations of the technique. Thus, the identified peaks were presented in the mono and diesters groups. The inorganic species were related to orthophosphate (P-ortho) and pyrophosphates. The observed species in our soil samples were composed, in large part, by a great proportion of inorganic-orthophosphate (observed in the 6ppm spectral region), and small amounts of mono-P (spectral region from 5.2 to 3 ppm), diester-P (spectral region between 2.5 and 2 ppm) and the inorganic pyrophosphates (around 4 ppm) (Table 7).

The relative distribution of the P-species is presented in Figure 5. In general, the inorganic-P was the dominant species among the sites, ranging from 75-92% of the total. In the NV (Cerrado), a higher contribution (~15-27%) of the organic species was observed, compared to the cultivated areas in Ox-2 and Ox-3 (3.5 to 4.4%)

and CT in Ox-1 (6.3%). Differences between land uses (NT and CT) were detected, in all studied soils, except Ox-3. the organic P in NT was 33% higher (Ox-4) up to 65% (Ox-1) and 214% (Ox-2) when compared to CT.

Table 7 and Figure 6 show the amounts of P (mg kg⁻¹) in each identified species, but in Table 7 the P levels are the ones in the P-NMR extracts and in Figure 6 the P levels were converted taking in account the total P and the solution P-NMR extraction efficiency (Table 6). It is possible to identify that the participation of the inorganic P is dominant and the organic P species are modified with the soil disturbance i.e., changing the natural agro-ecosystem into agricultural fields.

In general, the organic P values determined by ³¹P-NMR were smaller than the ones observed in chemical fractionation and XANES, being detected less than 10% of total P by NMR in all cultivated areas, this result was not expected since this technique should be very precise in estimating the organic P phases in soils (Cade-Menun, 2005). Otherwise XANES detected 14 to 25% of organic P (phytate), except soil Ox-4 with low clay and organic matter contents, and SCF detected 14.9 to 32.6% of organic P, also with smaller values in Ox-4, as expected.

Table 7 – Phosphorus levels (mg kg⁻¹) of ³¹P-NMR species in Brazilian Oxisols with long-term land use and distinct tillage systems.

Study Sites	Land use	P species by ³¹ P-NMR (mg kg ⁻¹)					
		Organic P		Total Po	Inorganic P		
		Monoesters (5.2 to 3.0)	Diesters (2.0 to -2.5)			Ortho-P 5.9 – 6.1	Pyro-P ~ -4
Ox1	NT	10.4 (±0.8)	-	10.4	202.4 (±15.8)	7.7 (±0.6)	210.1
	CT	6.3 (±0.5)	-	6.3	182.7 (±14.4)	14.0 (±1.1)	196.7
	NV	6.0 (±0.3)	3.6 (±0.2)	9.6	56.5 (±2.4)	2.7 (±0.1)	59.2
Ox2	NT	11 (±1.1)	-	11.0	103.6 (±10.2)	10.8 (±1.1)	114.4
	CT	3.5 (±0.7)	-	3.5	123.9 (±23.5)	7.4 (±1.4)	131.3
	NV	11.7 (±1.3)	2.5 (±0.3)	14.1	37.2 (±4.1)	2.8 (±0.3)	40.1
Ox3	NT	2.9 (±0.5)	-	2.9	176.1 (±32.7)	12.9 (±2.4)	188.9
	CT	4.4 (±0.6)	-	4.4	200.0 (±26.7)	16.3 (±2.2)	216.4
	NV	6.1 (±0.3)	8.7 (±0.4)	14.8	49.7 (±2.1)	4.3 (±0.2)	54.0
Ox4	NT	9.1 (±1.4)	-	9.1	126.2 (±19)	4.6 (±0.7)	130.8
	CT	6.8 (±1.3)	-	6.8	76.7 (±14.8)	3.4 (±0.7)	80.1
	NV	5.0 (±0.4)	-	5.0	28.5 (±2.3)	1.9 (±0.2)	30.4

NT: no tillage; CT: conventional tillage; NV: native vegetation (Cerrado).

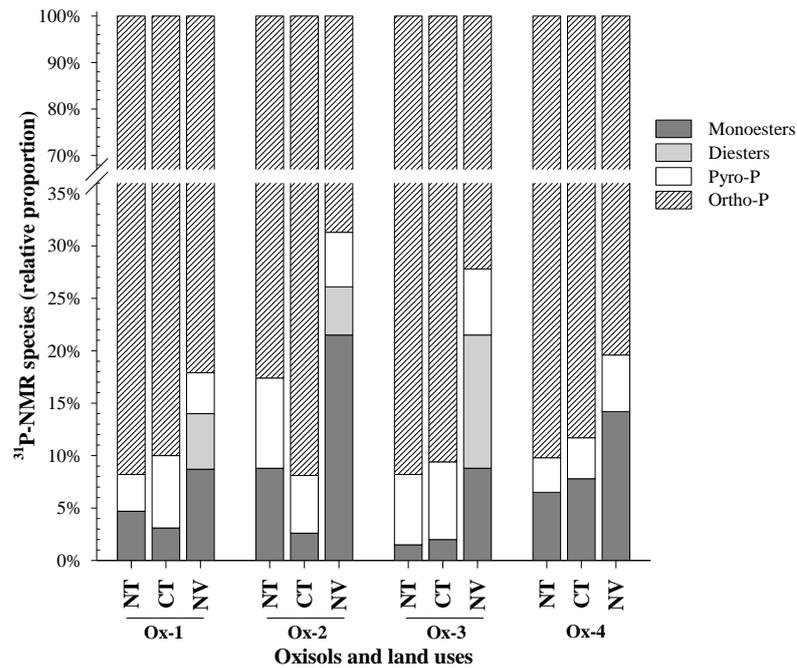


Figure 5. Relative distribution (%) of phosphorus species (^{31}P -NMR) in Brazilian Oxisols with long-term land use and distinct tillage systems. NT: no tillage; CT: conventional tillage; NV: native vegetation (Cerrado).

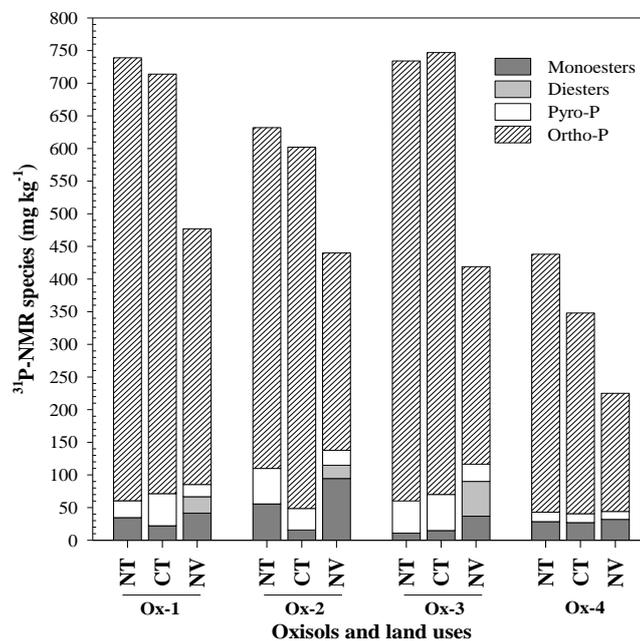


Figure 6. Phosphorus species (^{31}P -NMR) levels estimated to the total P (mg kg^{-1}) in Brazilian Oxisols with long-term land use and distinct tillage systems. NT: no tillage; CT: conventional tillage; NV: native vegetation (Cerrado).

4.4. Conclusions

Long-term cultivation under NT and CT generated a great P accumulation in the topsoil (legacy P), determined here by the sequential chemical P fractionation (SCF), which was useful to identify P labile fractions, which was the smallest portion of P observed in our soils and land-uses.

The P speciation by XANES was useful to identify preferential P-fixing forms, showing that the majority of the total P in Oxisols are sorbed in the Fe and Al hydr(oxides) Goethite, Hematite and Gibbsite. Only organic P as phytate was identified, with more relative contribution in the native Cerrado samples, with expressive depletion under long-term cultivation.

Although the P extraction efficiency was very low (below 32%) by NMR analysis and may not represent all the tropical soils P composition, ³¹P-NMR data was a key to provide detailed information on organic P speciation, showing that monoester-P was present across all soils and management systems, whereas diester-P was found only in the Cerrado samples. Inorganic P orthophosphate was the majority of the inorganic P species, but a significant amount of pyrophosphate was detected in all the samples (usually taken as organic P in SCF schemes).

The combined use of SCF, XANES and NMR was useful to a more specific understand of the inorganic and organic P forms in tropical soils and we recommend the following analysis sequence: SCF followed by NMR when the sample matrix is predominantly organic P, or SCF followed by XANES when the majority comprehend inorganic P in soils.

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APPENDIX

APPENDIX A. Supplementary material relative to chapter 2

Supplementary Material – TRANSITIONS TO SUSTAINABLE MANAGEMENT OF PHOSPHORUS IN BRAZILIAN AGRICULTURE

1. Phosphate fertilisers use

Phosphorus fertilisers became regularly applied in Brazilian agriculture after 1960 but their use increased more rapidly after 1990 (Fig 1A). Initially inputs did not match crop offtake, but since the mid 1970's, fertiliser P inputs have exceeded P offtake in harvested product by a factor of 2 (Fig. 1A). As the cropland area has also expanded rapidly into the native Cerrado and degraded pastureland in recent years, average fertiliser use over all cropland has stabilized and is currently 25-28 kg P ha⁻¹ yr⁻¹ (Fig. S1B).

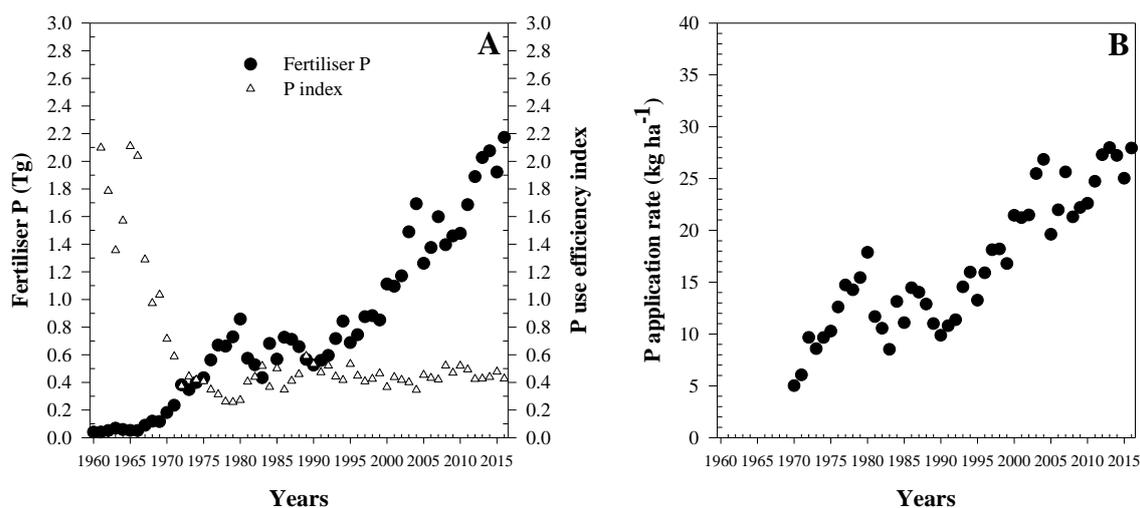


Figure S1. Amounts of phosphate fertilizers used in Brazil and P efficiency index (A) and average application rates to all cropland (B) from 1960-2016. The P efficiency index is the ratio of total cropland P offtake to total P fertiliser use.

2. Brazil's Green Revolution

Brazil has a total land area of approximately 243 M ha devoted to food production, of which ca. 166 Mha (68%) are currently in managed pasture, and ca. 77 M ha are managed cropland^{1,2}. Annually cultivated crops have increased rapidly since the mid 1990's when Brazil became economically more stable, while permanent crops (e.g. commercial forest and orchards) have remained constant (Fig. S2A). The three main cultivated crops are soybean (~31 Mha), maize (~15 Mha) and sugarcane (~9 Mha), which together account for 82% of Brazil's annual crops (Figure S2B). The areas of these three main crops have steadily expanded over the last 40 years, but with soybean showing a much steeper rise since 2000 (7.5% yr⁻¹) in response to a greater demand for export to Asia. Average crop yields and total production have also increased dramatically over this

period: from 1.6 to 5.7 t ha⁻¹ for maize, 1.3 to 3.1 t ha⁻¹ for soybean and 37 to 73 t ha⁻¹ for sugarcane (Fig. S3). Soil quality improvements resulting from the gradual conversion to no tillage cultivation systems, which now account for 54% of the total cultivated area in Brazil, have also contributed to increased crop production^{3,4}. In contrast to the cultivated cropland area, the area of pasture for cattle production in Brazil has remained relatively stable; for example from 154 Mha in 1974 to 166 Mha in 2006¹. Stocking rates and soil fertility in Brazil's extensive pasturelands still remain relatively low and well below their productivity potential⁵.

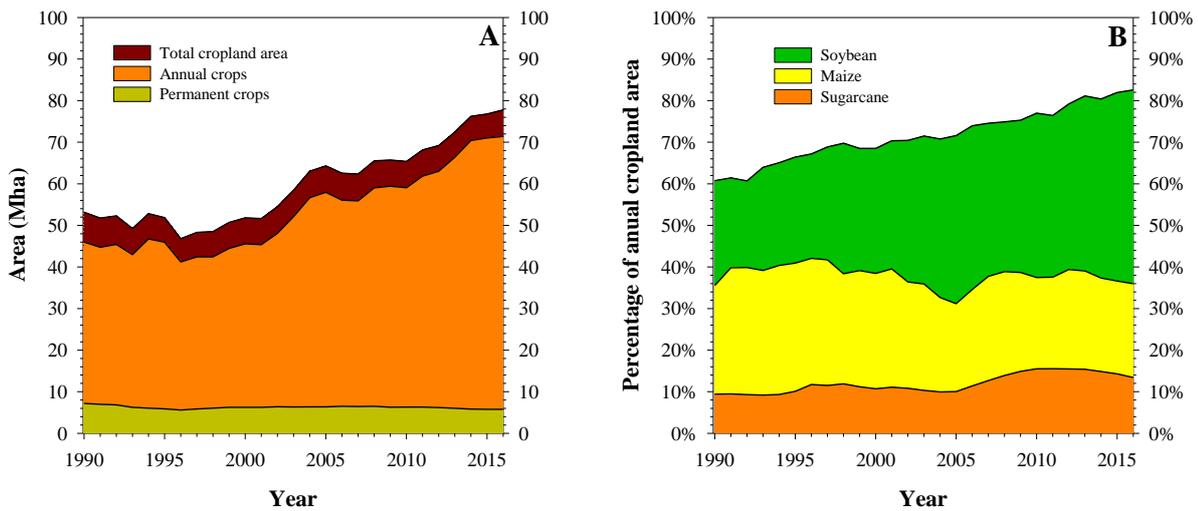


Figure S2. Trends in Brazil's cropland area from 1990 to 2015: **A)** Total, annual and permanent cropland area; **B)** Soybean, maize and sugarcane area as a percentage of the total annual cropland area.

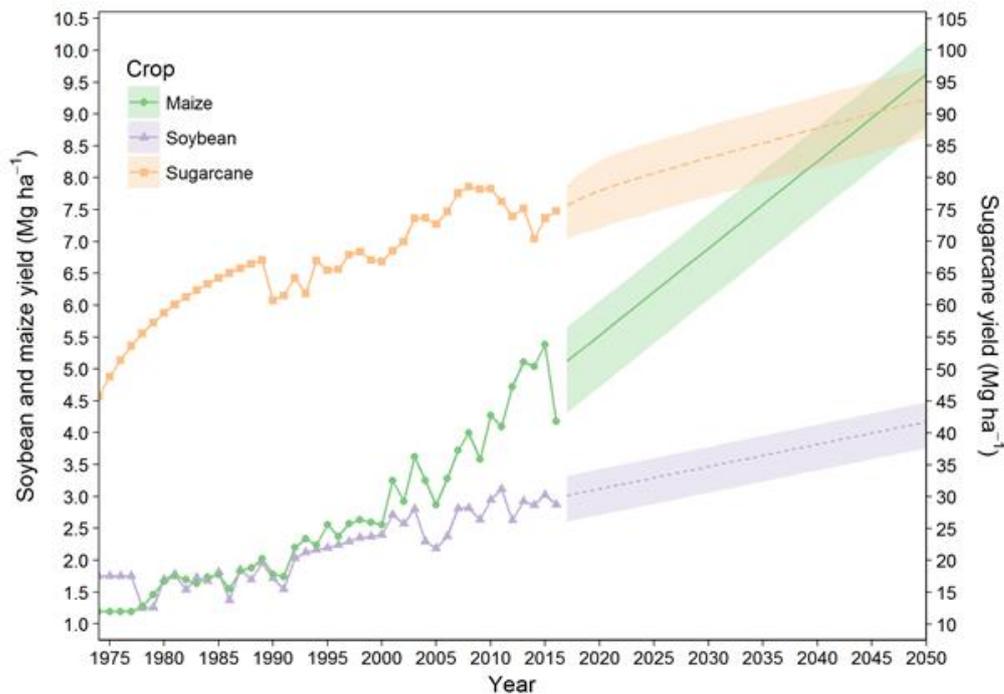


Figure S3. Current and future (2017-2050) trends in Brazil's soybean, maize and sugarcane yields. Coloured bands represent the uncertainty surrounding future predictions.

3. Legacy Soil P

Six long term trials in Brazil's main cropland production areas were selected to examine the amounts and forms of legacy soil P that have accumulated since conversion of the native Cerrado vegetation. A summary of the background site details is given in Table S1. These sites are representative of a large area of cultivated cropland in the main Cerrado region of Brazil, with acid, clayey, high P-fixing soils and typical cropping systems that include soybean, maize and cotton. The sites provide a suitably large range in the legacy period over which a P input-output balance can be calculated (14-38 years), and were used to study the effects of either cultivation system (no-till vs conventional tillage), P input rates, P sources and/or P application methods in replicated trials starting from 1992 (Table S1).

Table S1. Site details, soil characteristics (0-20 cm) and cropping system for the six long-term experiments.

Parameter	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Location (City-State)	Costa Rica - Mato Grosso do Sul	Sapezal – Mato Grosso	Cristalina - Goiás	Tasso Fragoso – Maranhão	Planaltina - Distrito Federal	Botucatu - São Paulo
Biome	Cerrado	Cerrado	Cerrado	Cerrado	Cerrado	Atlantic Forest/ Cerrado
Rainfall ¹ (mm)	1937	2156	1606	1476	1570	1450
Altitude (m)	790	640	981	610	1014	840
Soil Order ²	Oxisol	Oxisol	Oxisol	Oxisol	Oxisol	Oxisol
Clay (%)	65.6	45.2	65.3	25.5	64.0	22.0
Silt (%)	24.3	36.3	22.3	4.6	9.0	11.0
Sand (%)	10.1	18.5	12.4	69.9	27.0	77.0
pH ³	5.4	4.8	5.1	4.6	4.7	4.9
Organic C (%)	1.91	1.84	1.50	1.05	1.62	1.42
Fe (g kg ⁻¹) ⁴	48.8	48.1	27.4	19.8	nd	17.9
Al (g kg ⁻¹) ⁴	10.7	13.1	12.5	5.8	nd	nd
Cultivation system	No-till and conventional	No-till				
Cropping rotation	Soybean, cotton, cover crops	Soybean, cotton, cover crops	Soybean, maize, cover crops	Soybean, maize, cotton, cover crops	Soybean, maize, cover crops	Soybean, brachiaria (cover crop)
Treatments used	Tillage management	Tillage management	Tillage management	Tillage management	Tillage management, P sources/ methods	P rates and sources
Date started	1974 (deforestation) 1994 (trial began)	1997 (deforestation) 2001 (trial began)	1977 (deforestation) 1992 (trial began)	1990 (deforestation) 2001 (trial began)	1976 (deforestation) 1994 (trial began)	2001 (trial began)
Legacy period (years)	38	15	36	23	17	14

¹Long-term (last 30-year) annual average; ²USDA soil classification system; ³CaCl₂; ⁴Ditionite-citrate-bicarbonate (DCB); nd: not determined.

For each site, a detailed P balance was estimated considering the total fertiliser P inputs and the total P offtake in the harvested crop since the area was converted from native vegetation to cropland (Table S2). Input was considered the amount of P added via fertiliser every year for crop production. Output was estimated by grain yield and the determined P level in harvested grains⁵. General input-output were measured for the period of the field trial evaluations and, when the areas were cultivated previously to trials establishment it was extrapolated those values considering the average input-output from the first three years of trial run (sites 1, 3 and 4). The annual mean P fertilizer addition over the period was 37.2 kg P ha⁻¹ at site 1, 44.8 kg P ha⁻¹ at site 2, 37.8 kg P ha⁻¹ at site 3, 44.0 kg P ha⁻¹ at site 4, 35.8 kg P ha⁻¹ at site 5 and 34.9 kg P ha⁻¹ at site 6 (HP). The annual mean P output at each site was 15.2, 15.6, 19.7, 20.0, 18.8 and 6.6 kg P ha⁻¹ respectively, which leaves a total surplus (legacy) P over the experimental period ranging from 240 to 836 kg ha⁻¹ (Table S2).

Table S2. Estimated P balance in six long-term sites representative of Brazil's crop production areas.

Phosphorus Balance	Sites											
	1		2		3		4		5		6	
	NT	CT	NT	CT	NT	CT	NT	CT	NT	CT	LP	HP
	----- kg P ha ⁻¹ -----											
Total P input	1412	1412	672	672	1359	1359	1013	1013	608	608	96	489
Total P output	576	577	227	240	706	713	460	460	368	272	49	92
Net P surplus	836	835	445	432	653	646	553	553	240	336	47	397
Annual P surplus	22	22	30	29	18	18	24	24	14	20	3	28

NT – No-tillage; CT – Conventional tillage; LP – Low P input via fertiliser; HP – High P input via fertiliser.

Soil sampling was performed using a shovel, collecting the surface layer of 0-20 cm in natural reference areas and in treated plots at each site described above, being collected in 2011 (site 5), 2012 (sites 1 and 2), 2013 (3 and 4) and 2015 (site 6). Changes in soil P forms were determined by the Hedley sequential P fractionation method⁶, with modifications by Condrón et al.⁷. This method uses chemical extractants to remove progressively from the same sample the most available to the most stable forms of inorganic (Pi) and organic P (Po). Sequentially-extracted P was grouped into three P fractions: a) labile P, corresponding to the inorganic P extracted by anion exchange resin and the inorganic and organic P extracted by 0.5 mol L⁻¹ NaHCO₃; b) moderately-labile P, corresponding to the inorganic and organic P extracted by 0.1 mol L⁻¹ NaOH, and inorganic P extracted by 1.0 mol L⁻¹ HCl; and c) non-labile P, corresponding to the inorganic and organic P extracted by 0.5 mol L⁻¹ NaOH, and the P in the remaining residue. The soluble reactive P concentration in each extract was measured according to colourimetry using the Murphy and Riley⁸ procedure for acid extracts, and Dick and Tabatabai⁹ procedure for alkaline extracts. Organic P in each alkaline extractant was determined by the difference between total P after and inorganic P. A summary of the amounts of P extracted in each sequential P fraction is given in Table S3.

Table S3 – Amounts of inorganic (Pi) and organic P (Po) in each soil P fraction (0-20 cm) at each site according to the Hedley sequential fractionation procedure.

Site	Tillage	Labile-P		Moderately-labile P		Non-labile P		Total P	
		Pi	Po	Pi	Po	Pi	Po	Pi	Po
----- mg kg ⁻¹ -----									
1	NT	58.3	42.2	114.8	79.9	406.1	39.2	579.2	161.3
	CT	52.1	33.6	114.8	58.1	359.9	48.1	526.8	139.8
	NV	18.6	19.5	55.2	28.5	249.4	12.7	323.2	60.7
2	NT	70.8	40.9	85.9	78.4	294.6	59.5	451.4	178.8
	CT	42.4	30.5	96.0	71.8	281.9	52.8	420.3	155.1
	NV	24.1	21.0	45.1	35.1	250.4	22.4	319.6	78.5
3	NT	52.9	17.3	79.6	112.4	337.0	74.8	469.6	204.4
	CT	50.7	28.1	77.3	132.0	323.8	65.5	451.8	225.6
	NV	18.9	13.3	32.5	59.5	234.7	26.9	286.1	99.8
4	NT	62.5	13.7	104.6	27.9	186.7	21.6	353.8	63.2
	CT	56.6	9.1	90.5	21.5	170.6	23.8	317.7	54.3
	NV	15.7	6.1	37.0	5.3	139.1	7.7	191.7	19.1
5	NT	12.6	9.7	92.5	54.6	206.9	23.3	312.0	87.6
	CT	13.8	9.6	84.5	77.8	205.4	21.3	303.7	108.7
	NV	1.8	7.5	34.6	53.4	163.7	22.1	200.1	83.0
6	LP	10.2	2.0	26.6	43.0	247.8	20.4	284.6	65.3
	HP	20.5	6.9	40.6	65.9	254.0	29.1	315.0	101.8

Pi – P inorganic; Po – P organic; NT – No-tillage; CT – Conventional tillage; LP – Low P input via fertiliser; HP – High P input via fertiliser.

At sites 1-4, the P efficiency index (P output/P input) over the experimental period was calculated both including and excluding years when in cotton (sites 1, 2 and 4). When cotton is excluded, the efficiency index gradually increased due to the increase in soil labile P and greater contribution of soil labile P to crop P uptake (Fig S4). The efficiency of P use by the cotton crop is very poor (Fig. S4D).

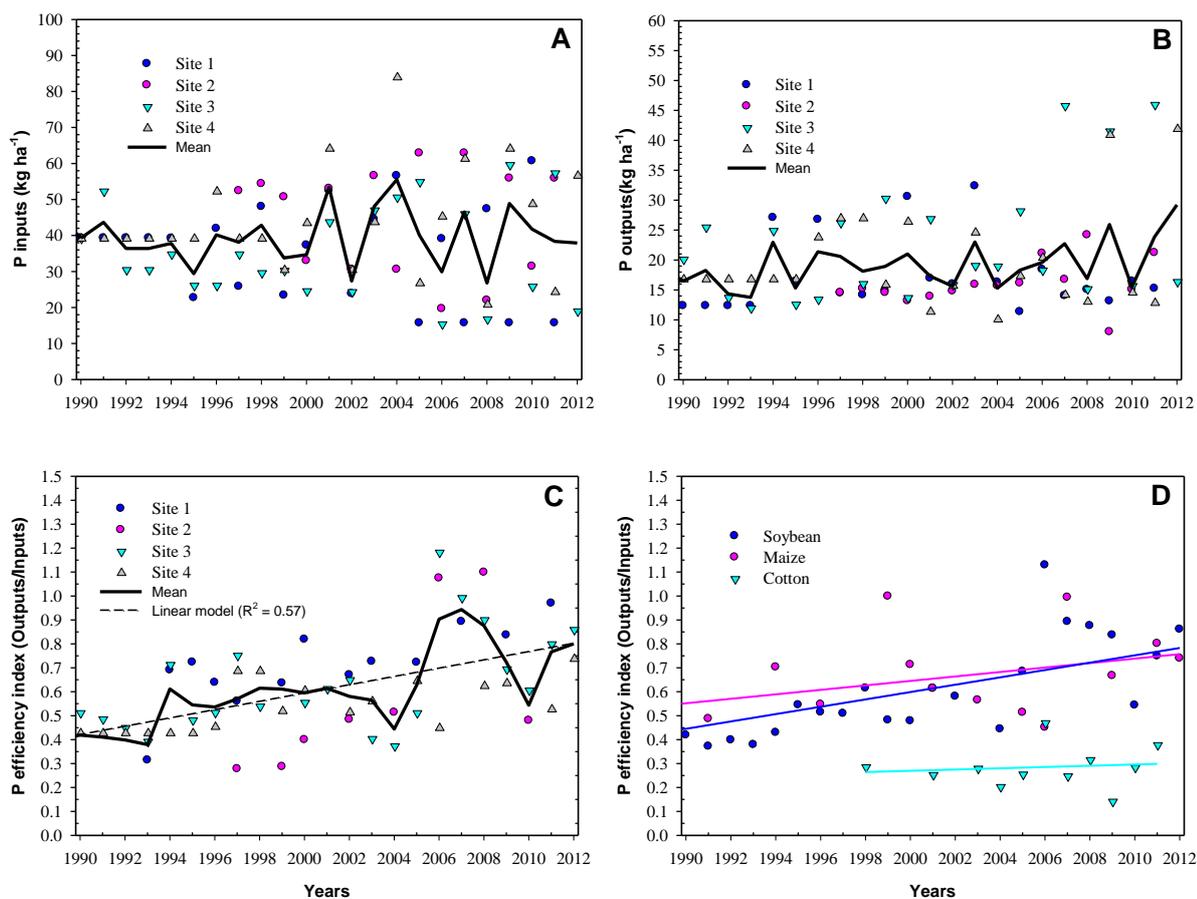


Figure S4. Phosphorus inputs (A), outputs (B) and P efficiency index (C) for soybean and maize crops at long-term sites 1-4 from 1990 to 2012, and the corresponding P efficiency index for each individual crop, including cotton, over the same period.

1. Bioavailability of moderately-labile P

Using a clayey soil under a no till cultivation system that received P fertiliser previously at rates twice the plant needs over 5 five years, Gatiboni et al.¹⁰ found that legacy soil P reserves provided sufficient P to give comparative crop yields over 6 years relative to crops which continued to receive P fertiliser (Table S4). Labile P extracted by anion exchange resin dropped from 30 mg kg⁻¹ at the start of the 6 year crop rotation to 21.2 mg kg⁻¹ at the end (20 mg kg⁻¹ is the critical level for this soil). Over the same 6-year period, the crops exported 18.7 mg kg⁻¹ of P. Soil P fractionation analysis^{6,7} showed that moderately labile P forms also reduced by 24.7 mg kg⁻¹ P¹¹, and additional enzyme assays showed an increase in acid phosphatase activity¹², which may have accelerated the utilization of moderately labile P. These data suggest that P legacy stored in less labile forms can be mobilised when P is withheld; for example due to a future P scarcity, or P price shock, as occurred in 2008.

Table S4. Phosphorus balance after six successive crops in a greenhouse experiment without P applied, using a clayey soil collected under no tillage system and previously fertilized during six years with 52 kg P ha⁻¹ yr⁻¹.

Parameter	Before crops	After six crops ¹	Relative change ²
Relative yield (%) ^{3,4}	-	99.3	- 0.7
Cumulative P removal (mg kg ⁻¹) ⁴	-	18.7	- 18.7
Labile P (mg kg ⁻¹) ⁵	30.0	21.2	- 8.8
Moderately labile P (mg kg ⁻¹) ⁶	393.4	370.9	- 24.7
Acid phosphatases activity (mg kg ⁻¹ hr ⁻¹) ⁷	419.2	618.1	+ 198.9

¹ Six successive crops in greenhouse (around 30 days each), being millet (crop 1), black oat (crops 2, 3, 4) corn (crop 5), soybeans (crop 6).

² Difference between values before and after six crops.

³ Yield as a percentage of plants which received P. Data from: Gatiboni¹¹.

⁴ Amount of soil P removed in total crop offtake over the 6-year crop rotation¹¹.

⁵ labile P extracted by anion exchange resin¹⁰, where 20 mg kg⁻¹ is considered the critical level for this soil.

⁶ sum of moderately-labile P fractions (extracted with 0.5 M NaHCO₃, 0.1M NaOH and 0.5 M NaOH)¹⁰.

⁷Data from Gatiboin et al¹²

4. Redesigning production systems through agro-engineering

Crop system engineering:

One crop engineering strategy is to grow more P efficient cultivars that store less phytate and total P in their tissues and grain, and therefore remove less P at harvest and require less P in fertiliser^{13,14}. The advantages of low P grain also extend into the livestock sector with reduced P excretion rates, and into the human health sector with improved utilization of trace elements within the body that would otherwise be immobilised by phytate. As nationally ca. 65% of P fertiliser in Brazil is currently applied to soybean, maize and sugarcane¹⁵, a reduction of 25% in the average seed P content (5 kg P/t on soybean and 3 kg P/t on maize) and a decrease of 25% in the average shoot P concentration of sugarcane (5 g/kg DM¹⁶) would reduce current P fertiliser requirements by 0.76 Tg annually (or 35% of current P fertiliser inputs).

An additional crop breeding strategy to enable a transition to lower P fertility soils is to improve soil P acquisition by plants¹⁷. Adaptation of crop varieties and/or crop rotations to include P-mobilising species has large potential to improve P acquisition in tropical soils with large reserves of non-labile P and limited reserves of plant available P. Exudation of carboxylates, modulation of acid and/or alkaline phosphatase activity, root morphology acclimation and proton release to decrease rhizosphere pH¹⁸ are mechanisms employed by plants adapting to low P availability. For example, in P-limiting conditions, sugarcane varieties can show variable adaptations to increase P uptake based on their root biomass¹⁹. Merlin et al.²⁰ recently showed that ruzigrass grown as a cover crop can take up P bound to Al and Fe oxides in tropical acidic soils and potentially make it

more available to succeeding crops. Sousa et al.²¹ showed that P critical level for soybean in an integrated crop-livestock system was half than that needed in annual cropping system. Pigeon pea increased P uptake of intercropped sorghum by exuding piscidic acid that chelates Fe, and subsequently releases P from iron phosphate (FePO_4)²².

Microbial engineering:

Several groups of bacteria including the genera *Rhizobium*, *Enterobacter*, *Agrobacterium*, *Azotobacter* and *Erwinia*, and fungi affiliated to the genus *Aspergillus* and *Penicillium* have shown a capacity to mobilise soil organic and inorganic P from tropical soils via the release of enzymes (phytases and phosphatases), protons and organic acids (gluconic, citric, oxalic, succinic or tartaric), most notably in laboratory-based experiments^{23,24}. Uptake of mobilised P into microbial biomass can then be actively recycled to provide soluble P for plant uptake. For example, Mirza et al.²⁵ attributed up to 55% of in-vitro sugarcane growth to the P availability promoted by bacteria affiliated to the genus *Enterobacter*. Mycorrhizal fungi also play an important role in soil P acquisition through hyphal extension of plant roots²⁶.

Although the mechanisms of P mobilization by microorganisms are well known, microbial activity in field soils cannot currently be relied upon to sustain P supply for crops because of limited understanding of the microbial ecology of P-mobilizing genera. There are no data showing how abundant, or how diverse, the microbial communities involved in P cycling should be. Optimising P supply may require a large diversity of soil microbes and/or communities that exhibit functional redundancy²⁷, and microbial engineering offers the potential to prescribe microbial recipes for specific cropping systems, cultivation regimes, soil types and climatic regions. One can hypothesize that the greater the microbial diversity, the greater the microbial activity and the greater the likelihood of mobilizing moderately-labile or non-labile P in tropical soils^{28,29}. Research is only just beginning to explore how engineering of the soil microbiome can support the utilization of legacy soil P.

Fertiliser engineering:

Novel P fertilisers developed through various bio-technologies, and or P recovery strategies, have been evaluated, or used commercially in Brazil in order to increase the efficiency of P fertilisers³⁰. Key to the success of these technologies is producing fertilisers with a low water P solubility, and a slow pattern of P release to more accurately match crop P demand and reduce susceptibility to rapid immobilization (adsorption and precipitation) of P by Fe and Al oxides in the soil. For example, struvite recovered from wastewater, or from

livestock manures, has been shown to provide a slow-release and efficient P supply to crop without sacrificing productivity^{31,32}. However, it is noteworthy that while many novel fertilisers have been advocated for use on farms by the fertiliser industry in Brazil, there remains little scientific validation of improvements in P use efficiency in the field. Improved targeting of P through a better understanding of physiological demand through the growing season, and more innovative application technologies via seed dressings, placement and foliar applications may further enhance the role of precision farming in the P sustainability of Brazilian crop production systems³³.

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APPENDIX B. Supplementary material relative to chapter 3

Table S1. Summary of the analysis of variance (ANOVA) for phosphorus fractions after decades of tillage systems and cover crops cultivation in a Brazilian Cerrado Oxisol 1 (Ox-1).

Factor	Hedley phosphorus fractions											
	PAER	PiBic	POBic	PiHid0.1	POHid0.1	PHCl	PiHid0.5	POHid0.5	PResidual	PiTotal	POTotal	PTotal
	0 – 5 cm											
Tillage	ns	ns	*	ns	ns	#	ns	*	ns	ns	ns	ns
Crops	*	ns	*	*	*	#	ns	*	ns	*	*	*
Tillage*Crops	*	ns	ns	ns	*	#	ns	*	ns	ns	*	*
CV_a(%)	9.80	34.30	5.12	7.77	10.99	#	6.70	7.11	10.73	3.64	7.29	1.68
CV_b (%)	11.40	22.02	10.42	13.99	18.87	#	11.48	11.00	6.02	5.82	11.63	3.78
\bar{y}	5.498 / 7.444	29.485 / 12.149	1.658 / 6.861	234.65 / 760.27	214.2 / 631.5	#	9.175 / 27.218	31.69 / 75.85	1105.73 / 347.79	467.5 / 1191.4	299.6 / 763.1	194.8 / 987.5
	5-10 cm											
Tillage	*	ns	ns	ns	ns	#	ns	ns	ns	ns	*	*
Crops	ns	ns	ns	*	ns	#	ns	*	ns	*	ns	*
Tillage*Crops	ns	ns	ns	*	ns	#	ns	*	ns	ns	ns	ns
CV_a(%)	9.80	14.57	12.79	9.40	23.01	#	8.10	18.95	6.27	5.95	15.04	0.56
CV_b (%)	27.64	41.27	18.90	7.13	16.76	#	8.11	13.21	5.88	6.45	12.33	6.91
\bar{y}	7.204 / 58.035	12.726 / 102.178	7.536 / 16.457	324.9 / 187.1	1060.7 / 562.3	#	16.882 / 16.932	146.03 / 71.02	382.29 / 335.99	1308.6 / 1538.2	1163.5 / 782.6	22.0 / 3331
	10-20 cm											
Tillage	ns	ns	ns	ns	ns	#	ns	ns	ns	ns	*	ns
Crops	ns	ns	ns	ns	*	#	ns	ns	ns	ns	ns	ns
Tillage*Crops	ns	ns	ns	ns	ns	#	ns	ns	ns	ns	*	ns
CV_a(%)	9.37	40.33	3.48	9.31	12.57	#	9.93	16.36	3.78	3.93	6.19	4.10
CV_b (%)	24.97	59.22	16.08	17.37	15.32	#	7.73	24.71	7.94	7.61	11.86	7.79
\bar{y}	3.821 / 27.120	41.808 / 90.160	0.430 / 9.174	151.58 / 527.39	198.01 / 294.25	#	20.083 / 12.180	42.298 / 96.502	119.22 / 526.28	392.89 / 1473.13	1163.5 / 782.6	392.89 / 1473.13
	0-10 cm #											
Tillage	ns	ns	ns	ns	ns	#	ns	*	ns	ns	ns	*
Crops	*	ns	ns	*	*	#	ns	ns	ns	*	*	ns
Tillage*Crops	*	ns	ns	ns	ns	#	ns	ns	ns	ns	*	*
CV_a(%)	8.47	22.09	7.27	8.46	15.65	#	4.33	9.96	2.77	3.11	9.89	0.64
CV_b (%)	16.27	26.40	9.92	9.17	14.55	#	7.43	7.76	3.92	4.63	10.20	4.16
\bar{y}	4.753 / 17.554	19.823 / 28.325	2.867 / 5.340	270.83 / 318.17	462.21 / 399.51	#	4.341 / 12.762	50.70 / 30.79	74.21 / 148.30	350.50 / 775.80	527.4 / 560.4	194.8 / 987.5

* significant different detected at $p < 0.05$. ns: not significant difference observed.

obtained by average values from 0-5 and 5-10 cm results.

Table S2. Summary of the analysis of variance (ANOVA) for phosphorus fractions after decades of tillage systems and cover crops cultivation in a Brazilian Cerrado Oxisol 2 (Ox-2).

Factor	Hedley phosphorus fractions											
	PAER	PiBic	POBic	PiHid0.1	POHid0.1	PHCl	PiHid0.5	POHid0.5	PResidual	PiTotal	POTotal	PTotal
	0 – 5 cm											
Tillage	ns	ns	ns	ns	*	#	*	ns	ns	ns	*	ns
Crops	*	ns	ns	ns	*	#	*	*	*	*	ns	ns
Tillage*Crops	*	ns	ns	ns	ns	#	*	*	ns	*	ns	*
CV_a(%)	22.82	8.18	18.68	7.80	15.55	#	0.54	23.62	4.00	2.56	7.02	5.75
CV_b (%)	13.52	17.51	10.76	19.16	32.19	#	5.36	16.16	3.51	4.33	13.23	5.94
	5-10 cm											
Tillage	ns	ns	ns	*	*	#	ns	ns	ns	ns	ns	*
Crops	*	*	ns	*	ns	#	*	ns	ns	*	ns	*
Tillage*Crops	*	*	ns	*	*	#	*	*	ns	*	*	ns
CV_a(%)	14.74	35.32	13.78	7.61	19.01	#	19.42	50.36	9.85	5.88	15.75	0.56
CV_b (%)	14.32	18.84	11.37	18.82	21.60	#	10.16	21.40	5.40	5.09	9.43	6.91
	10-20 cm											
Tillage	ns	*	ns	ns	*	#	ns	ns	ns	ns	ns	ns
Crops	ns	*	*	ns	*	#	ns	ns	ns	*	*	ns
Tillage*Crops	ns	*	*	ns	*	#	ns	ns	ns	*	ns	ns
CV_a(%)	23.21	15.90	23.75	28.33	19.63	#	11.40	28.57	8.15	9.87	5.90	6.72
CV_b (%)	21.50	18.14	12.62	16.16	18.69	#	10.05	22.66	4.92	5.32	12.09	4.91
	0-10 cm #											
Tillage	ns	ns	*	ns	ns	#	ns	ns	ns	ns	ns	ns
Crops	*	*	ns	ns	*	#	*	ns	ns	*	ns	*
Tillage*Crops	*	*	ns	*	ns	#	ns	ns	ns	*	ns	*
CV_a(%)	10.71	21.38	15.03	2.61	16.47	#	9.27	33.76	6.33	4.20	10.66	3.82
CV_b (%)	11.65	14.21	7.34	10.81	16.64	#	5.73	14.34	2.82	3.12	7.92	3.45

* significant difference detected at $p < 0.05$. ns: not significant difference observed.

obtained by average values from 0-5 and 5-10 cm results.

Table S3. Hedley's P fractions (mg kg⁻¹) in Brazilian Cerrado native soils (Ox-1 and Ox-2).

Soil	P	Pi	Po	Pi	Po	P	Pi	Po	P	P
	AER	Bic	Bic	Hid0.1	Hid0.1	HCl	Hid0.5	Hid0.5	Residual	Total
----- mg kg ⁻¹ -----										
0 – 5 cm										
	3.9	4.0	15.6	118.2	98.8	1.0	75.9	48.5	147.4	513.3
	(±0.2)	(±0.3)	(±2.3)	(±21)	(±17)	(±0.6)	(±7.1)	(±7.8)	(±10)	(±39)
5 – 10 cm										
Ox-1	3.2	2.5	16.1	106.5	90.9	0.7	62.0	42.2	135.6	459.6
	(±0.2)	(±0.4)	(±1.2)	(±8.6)	(±9.9)	(±0.1)	(±6.9)	(±7.6)	(±15)	(±17)
10 – 20 cm										
	3.1	2.2	16.7	74.4	29.9	0.9	66.3	25.0	135.0	353.6
	(±0.1)	(±0.4)	(±2.2)	(±10)	(±8.4)	(±0.4)	(±3.9)	(±7.2)	(±7.6)	(±11)
0 – 20 cm (weighted mean)										
	3.3	2.7	16.3	93.4	62.4	0.8	67.7	35.2	138.2	420.0
	(±0.1)	(±0.4)	(±1.9)	(±13)	(±11)	(±0.3)	(±5.5)	(±7.5)	(±10)	(±20)
0 – 5 cm										
	15.7	23.0	51.7	56.5	6.9	0.4	82.2	26.3	178.7	441.3
	(±4.3)	(±3.3)	(±4.1)	(±6.3)	(±2.6)	(±0.1)	(±8.9)	(±6.6)	(±2.7)	(±11)
5 – 10 cm										
Ox-2	11.0	13.6	62.2	55.2	5.5	0.4	68.4	8.8	180.9	406.0
	(±3.8)	(±1.1)	(±10)	(±9.7)	(±2.8)	(±0.1)	(±9.8)	(±2.1)	(±3.1)	(±13)
10 – 20 cm										
	9.7	9.7	42.8	51.8	6.8	0.4	60.4	12.3	175.3	369.3
	(±2)	(±0.8)	(±2.1)	(±4.6)	(±1.6)	(±0.2)	(±7.9)	(±4.9)	(±11)	(±17)
0 – 20 cm (weighted mean)										
	11.5	14.0	49.9	53.8	6.5	0.4	67.8	14.9	177.5	396.5
	(±3)	(±1.5)	(±4.8)	(±6.3)	(±2.1)	(±0.1)	(±8.6)	(±4.6)	(±7.4)	(±15)