UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO" FACULDADE DE CIÊNCIAS AGRONÔMICAS CAMPUS DE BOTUCATU-SP

AUMENTO DA DISPONIBILIDADE DE FÓSFORO EM SOLO CULTIVADO COM BRACHIARIA EM ROTAÇÃO COM SOJA

ALEXANDRE MERLIN

Tese apresentada à Faculdade de Ciências Agronômicas da Unesp - Campus de Botucatu, para obtenção do título de Doutor no Programa de Pós-Graduação em Agronomia – Agricultura.

BOTUCATU-SP Dezembro, 2011

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BOTUCATU-SP Dezembro, 2011 FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉCNICA DE AQUISIÇÃO E TRATAMENTO DA INFORMAÇÃO - SERVIÇO TÉCNICO DE BIBLIOTECA E DOCUMENTAÇÃO - UNESP - FCA - LAGEADO - BOTUCATU (SP)

Merlin, Alexandre, 1982M565a Aumento da disponibilidade de fósforo em solo cultivado com Brachiaria em rotação de soja / Alexandre Merlin. - Botucatu : [s.n.], 2011 viii, 53 f. : il., tabs.
Tese (Doutorado) - Universidade Estadual Paulista, Faculdade de Ciências Agronômicas, Botucatu, 2011
Orientador: Ciro Antonio Rosolem Inclui bibliografia
1. Capim braquiária. 2. Fosfatos. 3. Rotação de cultivos. 4. Solos - teor de fósforo. 5. Superfosfatos.
I. Rosolem, Ciro Antonio. II. Universidade Estadual Paulista "Júlio de Mesquita Filho" (Campus de Botucatu). Faculdade de Ciências Agronômicas. III. Título.

Palavras-chave: fosfato natural, superfosfato, frações de fósforo, disponibilidade no perfil, rotação de culturas.

UNIVERSIDADE ESTADUAL PAULISTA "JÜLIO DE MESQUITA FILHO" FACULDADE DE CIÊNCIAS AGRONÔMICAS CAMPUS DE BOTUCATU

CERTIFICADO DE APROVAÇÃO

TÍTULO: AUMENTO DA DISPONIBILIDADE DE FÓSFORO EM SOLO CULTIVADO COM BRAQUIÀRIA EM ROTAÇÃO COM SOJA

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Data da Realização: 07 de dezembro de 2011.

Dedicate

To my parents, Vitor Angelo Merlin e Ivete Maria Badin Merlin, my brother, Eduardo Merlin and my wife Tatiana Pires de Almeida Merlin and my daughter Julia Pires de Almeida e Silva, for support and confidence on me to conclude this new phase in my life.

ACKNOWLEDGMENTS

First of all, I would like to thank my esteemed advisor Dr. Ciro Antonio Rosolem, for his guidance and support over 10 years of study. Not only was he readily available for me providing comprehensive instructions in the lab and experiment phase, but also provide advice that I'll take for life.

My thanks go out to my American advisor Dr. Zhenli He and IRREC staff for the hospitality and support I had received during my one year period on USA. I am grateful for his advice, assistance and encouragement in every step towards my PhD degree.

I deeply express my appreciation to my colleges in Sao Paulo State University at Botucatu: Gustavo Spadotto (Spirro), Mariana Zampar Toledo (Tchutchuca), Rodrigo Arroyo Garcia (Bulbo) and Dorival Pires we studied and relaxed together. Without their help and friendship, the successful completion of my PhD study is impossible.

I gladly appreciate the people on the faculty and staff at Indian River Research Center of University of Florida for providing me wonderful environment for both studying and living for one year. Everyone from the center was so kind to me that I had the center as my second home. I am particularly thankful to Dr. Peter J. Stoffella, Brian M Cain, Rodrigo Diaz, Veronica Marinque, Dr. Yang, Jinghua Fan and Jordan Yancy. I am also willing to thank Ms. Jackie White for her help. It is lucky for me to have Eloise Mello and Thiago Nogueira, my Brazilian friends who helped my and hanged out sometimes.

Last but not least, I would like to thank my dearest English teacher Monica Rosolem who touch me and helped me to realize the biggest dream of my life, study in the USA.

I also want to thank Dr. Carlos Alexandre Costa Crusciol who can I consider as my second mentor and always helped me.

My deeply gratitude goes to my parents for their parts in making me who I am today. My enormous debt of gratitude can hardly be repaid to them for their unconditional love.

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AUMENTO DA DISPONIBILIDADE DE FÓSFORO EM SOLO CULTIVADO COM BRACHIARIA EM ROTAÇÃO COM SOJA Botucatu, 2011. 53 fl. Tese (Doutorado em Agronomia/Agricultura) - Faculdade de Ciências Agronômicas, Universidade Estadual Paulista. Author: ALEXANDRE MERLIN Adviser: CIRO ANTONIO ROSOLEM

1. RESUMO

O fornecimento de P para as culturas em solos tropicais é geralmente deficiente devido à sua natureza pouco solúvel no solo, e a adição de fertilizantes P tem sido obrigatória para se atingir rendimentos elevados. Uma abordagem possível é a adoção de sistemas de cultivo com plantas de alta eficiência no uso do P. O objetivo deste estudo foi determinar os mecanismos da braquiária (Brachiaria ruziziensis), cultivada em rotação, aumentando a disponibilidade de P no solo. Foram desenvolvidos dois experimentos: para o ensaio de campo os tratamentos foram doses de fosfato solúvel e natural aplicadas à lanço, com e sem cultivo de braquiária (Brachiaria ruziziensis). A gramínea foi cultivada por 289 dias e após esse período o solo foi amostrado de 0-5 e 5-10 cm de profundidade. O segundo experimento, em casa de vegetação, Brachiaria ruziziensis e Brachiaria brizantha foram cultivadas em solução nutritiva com complexos de P em óxidos de Ferro e Alumínio. À campo, os níveis de fósforo disponível foram superiores após o cultivo da gramínea. No experimento em casa de vegetação, o desenvolvimento das espécies de braquiária foi normal nos tratamentos onde o fósforo estava adsorvido pelos óxidos de Ferro e Alumínio, indicando a capacidade dessas duas espécies em acessar formas indisponíveis de P tornandoas disponíveis para outras culturas.

Palavras-chave: fosfato natural, superfosfato, frações de fósforo, disponibilidade no perfil, rotação de culturas.

MECHANISMS OF ENHANCED PHOSPHORUS AVAILABILITY BY *BRACHIARIA RUZIZIENSIS* IN OXISOLS Botucatu, 2011. 53 fl. Tese (Doutorado em Agronomia/Agricultura) - Faculdade de Ciências Agronômicas, Universidade Estadual Paulista. Author: ALEXANDRE MERLIN Adviser: CIRO ANTONIO ROSOLEM

2. SUMMARY

Phosphorus supply to crops in tropical soils is usually deficient due its sparingly soluble nature in soil, and the addition of P fertilizers has been mandatory in order to achieve high yields. One potential approach is to adopt cropping systems that could increase plant available P in soil. The objective of this study was to examine the mechanisms of cover crop (Congo grass - Brachiaria ruziziensis) rotation in enhancing soil and fertilizer P availability using long-term field trials and laboratory chemical fractionation approaches. The first experiment was a field cropped under no-till for five years. No P or 35 kg ha⁻¹ of P was applied on the soil surface as triple superphosphate or Arad rock phosphate and Congo grass was grown for 289 days. After this, soil samples were taken down to 10 cm and soil P was fractionated. The second experiment was carried out in a green house where the treatments were Brachiaria species (Brachiaria brizantha and Brachiaria ruziziensis) and two mineral-P complexes (goethite and amorphous Al-oxide) plus two controls with and without P as regular nutrient solution. In the first experiment, soil available P, as estimated by resin extraction was increased by congo grass down to10 cm when P fertilizers were applied. At the nutrient solution experiment, after 14 days of cultivation, the dry matter yields of both Brachiaria species were not affected by oxide-P (goethite-P or Al oxide-P) as sole P source, demonstrating that Brachiaria species could access non-labile adsorbed when P was supplied with Al oxide-P and goethite-P.

Key-words: natural phosphate, super phosphate, phosphorus fractionation, P availability in soil profile, crop rotation.

3. Literature Review

3.1. Phosphorus dynamics in soil as a function of management system

The maintenance of crop residues on the soil surface in reduced tillage systems change the nutrient cycling in the soil (Hedley et al., 1982). The changes in the management of crop residues resulting from the adoption of conservation tillage systems have the potential to alter the concentration and distribution of phosphorus (P) in the soil profile, especially in the upper layers. In general, the concentration of P in soils under no-tillage increases in the top 5 cm of soil surface, and decreases with depth, as compared to systems with conventional till (Bayer et al., 2000; Rehnheimer and Anghinoni, 2001; Almeida et al., 2005).

In long term experiments, the effect of no-tillage on the accumulation of nutrients in the soil surface layer is closely related to increased levels of organic matter (Amado and Mielniczuk, 2001). Among the macronutrients, P has the lowest mobility and has shown the largest increases, which may result in a level four to seven times greater in no-till than that of tilled soils, in the 0-5 cm layer (Neumann et al., 1999). On till, there is also a significant decrease in content of soil organic matter, with consequent change in the reactions of inorganic available P form in microbial biomass and available in organic forms (Rehnheimer and Anghinoni, 2001).

In general, soils under conservation tillage have a higher concentration of available P in the surface layer and stratification in profile, with marked reduction with increasing depth (Santos et al., 2003). Lal et al. (1990), in an experiment lasting 12 years, found 2.42 times higher available P in no-till as compared to the till in the soil layer of 0-10 cm, but in the 10-50 cm layer the reverse is true. Phosphorus accumulation in the surface layer of soils under no-till results from the application of phosphate fertilizers, P released during plant and animal debris decomposition, and reduced P adsorption resulting form the slightest contact of P with soil constituents (Santos et al., 2003).

Cropping systems have several effects on soil P. According to Franchini et al. (2000), the continuous flow of different forms of carbon (C) from crop residue decomposition results in competition for sites of positively charged colloids by inorganic compounds, and complex formation between organic ligands with Al³⁺, Fe³⁺ and Mn²⁺. The occupation positively charged sites of on the surface of inorganic colloids reduces phosphate adsorption, thus increasing P availability to plant (Rehnheimer and Anghinoni, 2001).

Silveira and Stone (2001) evaluated six crop rotations and found no significant effect on the levels of available soil P, but in general the levels of P increased significantly with years of cultivation at all the rotations studied.

3.2. Importance of organic matter to soil

Soil organic matter (SOM) represents a large reservoir of plant nutrients, especially N, P and S. The elements in organic forms are not readily available to plants, but they become available upon mineralization of organic matter.

Indirectly SOM also plays an important role in mineral nutrition of plants through effects on the chemical, physical and biological soil properties. Therefore, SOM is essential to the quality of tropical soils. It plays an important role in crop productivity as an important reservoir of nutrients, including P, in addition to influencing pH, ion exchange capacity, microbial activity and soil structure (Balesdent et al. 2000; Glatzela et al., 2003). In addition, the SOM may contribute to the sequestration of atmospheric C (Glatzela et al., 2003, Bayer et al., 2000). Its dynamics in the soil is an aspect of great interest in studying, because of its importance both in terms of soil fertility and environmental health.

Soil OM consists of a mixture of compounds in various stages of decomposition, resulting from biological degradation of plant residues, and the activity of microorganisms. It can be grouped into humic and non humic substances. The non-humic substances are composed of substances with defined chemical characteristics, such as polysaccharides, amino acids, sugars, proteins and organic acids of low molecular weight. The humic substances are not well-defined chemically and physically, and can be divided into humic acid, fulvic acid, and humus, based on their solubility characteristics (Stevenson, 1994).

To maintain soil organic matter at a desired level, it is essential to adopt optimal management systems that enhance the input of organic carbon to the soil, subsequent soil quality and mitigation of CO_2 emissions.

3.3. Organic phosphorus

Organic P (Po) in soil can contribute 3% to 75% of the total content of P. Generally, between 1/2 and 2/3 of the total P contained in the surface horizon of the soil is organic. Most forms of Po that naturally occur in soil are esters of phosphoric acid, where we highlight the inositol phosphate group that can get up to 60% of the total Po (Dalal, 1977). The stability of these compounds in soil depends on its nature, and they are used as a source of carbon and electrons by microorganisms, which results in the release of P. Some compounds, such as phosphate diesters (phospholipids and nucleic acids) are rapidly released. Phosphate monoesters exhibit high binding energy to the inorganic soil colloids, thus difficult to release and less available to plants (Dalal, 1977; Stewart and Tiessen, 1987).

There is a definite proportion between the esters in soils with those present in living organisms from which they were derived. The cumulative amount of these compounds depends on its dynamics of production and decomposition. The development of microbially synthesized esters and other materials from which organic matter is formed, is affected by factors such as temperature, nutrients such as carbon, nitrogen and phosphorus, which may limit the production of SOM at low availability (Walker et al., 1958).

Organic P becomes available to plants after mineralization. Van Diest et al. (1959) showed that in an incubated soil, Po decreased concomitantly with the increase in available inorganic P (Pi). The mineralization of Po in the soil is mostly due to the combined action of soil microorganisms and free enzymes in the soil. Thus, the factors that regulate the activity of microorganisms govern the mineralization of Po in soil.

Thus, we can say that plants can use some Po sources efficiently due to their rapid hydrolysis to release Pi (Tarafdar and Claassen, 1988). The same authors reported that the limiting factor in the hydrolysis of Po to Pi as a supply to plants is not the availability of phosphatases, but hydrolysable Po, as they observed the activity of phosphatases that hydrolyze Po is capable of supplying 20 times more P than the amount absorbed by plants. Soil Po can act as a source or drain of available P, depending on soil management and fertilization (Novais and Smith, 1999). In natural ecosystems, where there is no addition of phosphorus, its plant availability is closely related to organic P cycling.

The increased P availability in the surface soil with the adoption of notill can result in greater transformation of Pi into Po. In a study conducted over a long period, Po in the surface soil layer increased with lower clay and iron oxides (Rehnheimer and Anghinoni, 2001). The same author mentioned that the inappropriate use of soils with these characteristics leads to high rates of decomposition of organic matter and drastically reduces the dust content of labile and moderately labile forms. This shows a low resilience, i.e., low capacity to continue operating without change for a disorder. However, when these soils are managed properly the levels of Po and organic matter recover quickly.

The presence of plant reduces Po content in the soil near the roots (Thompson et al. 1954; Jungk et al., 1993). Thompson et al. (1954) attributed this effect to the transfer of substances between the colloids in solution and roots.

The roots of cultivated plants have mechanisms that help in the mineralization of Po (Hinsinger, 1998, 2001; Raghothama, 1999), and a possible mechanism for this increase in efficiency is the acidification of the rhizosphere (Hinsinger et al. 2003, Neumann et al. 1999; Tang et al. 2004). Another mechanism studied is the exudation of carboxylic compounds (Neumann et al., 1999, Richardson et al. 2004; Wang et al. 2007). Moreover, the activity of enzymes in the mechanisms of dissolution of less soluble phosphates, for example, acid and alkaline phosphatases have been also evaluated and, for some species, the effects of these enzymes are evident

(Tabatabai et al. 1969; Rengel and Marschner, 2005). In a study of P fractionation in Oxisol, it was found that an increase of 33% labile P occurred in no-tillage as compared to conventional tillage and this result was attributed to the rise of Po and the absence of tilling (Selles et al. 1997). The same authors also found that, in tillage and no tillage, there could be more P available to plants due to the slow and continuous mineralization, held by the microbial population, resulting in the release of organic compounds that act by reducing the competition for adsorption sites.

The participation of inorganic fractions (phosphorus geochemistry) and organic (biological phosphorus) in maintaining the availability of P for plants was demonstrated by Tiessen et al. (1984) in soils with different degrees of development. These authors observed that in undeveloped land 86% of P estimated by anion exchange resins (RTA) came from inorganic forms, while in most weathered soils 80% was from organic fractions. Guerra et al. (1996) also observed that the labile Po (NaHCO₃ extractable) accounted for more than 60% of labile P in weathered soils in Central Brazil.

When the soil is not fertilized and there are additions of plant residues, the fraction of organic P buffers the soil solution. On the other hand, fertilization promotes the accumulation of P in inorganic forms, which buffer the solution, the organic fraction is used to a lesser extent, allowing its accumulation. When phosphate fertilizers are applied, P in all fractions is redistributed in soil, but the accumulation is more pronounced in the labile inorganic. Over time, adsorption energy is increased and P is gradually transformed into forms of higher stability, which characterizes the process of "P ageing" (Novais and Smith, 1999).

Araujo et al. (1997) reported that fertilization and cultivation of soil for 25 years led to increased levels of organic and inorganic P, compared to a similar soil under native forest. However, in relative terms, the organic P decreased from 55.6% of total P in the soil to 25.1% in the cultivated soil, showing that the accumulation of P is preferably added in inorganic forms.

Reports on the influence of soil organic matter in the availability of P have been inconsistent. There are claims that organic compounds in soil adsorb inorganic phosphate forming ternary complexes mediated by bridges of cations such as Fe and Al (Beldrok et al., 1997). Organic matter can increase soil phosphate adsorption by preventing oxides crystallization and increasing the Fe_o / Fe_d ratio, which results in greater adsorption surface (Schwertmann et al., 1986). Other reports claim that organic acids and other intermediate compounds of anionic character, coming from decomposing plant residues, can compete with phosphate for adsorption sites on soil colloids, thus reducing their adsorption (Mesquita Filho and Torrent, 1993; Iyamuremye et al., 1996).

3.4. Adsorption of P and organic matter

In soils with high content of oxides and clay, much of the P in soil undergoes the process of specific adsorption, i.e., strong bonds between the clay and / or oxides and phosphate ions. This specific adsorption of phosphate occurs by exchange of ligands with surface-OH groups of oxy-hydroxides of Fe and Al, edges of clay minerals and clays, which strongly influences the concentration of P in soil solution (Parfitt, 1978). The specific adsorption is also regulated by the presence of humic and fulvic acids and organic acids (OA) of low molecular weight that compete with P for adsorption sites (Fontes et al., 1992).

According to Rehnheimer and Anghinoni, (2001), the adsorption of phosphate in soil occurs in stages: in the first stage the neutralization of positive charges is dominant, and these sites are saturated when all the groups OH_2^+ are shared by phosphate ions; in the second stage there is ligands exchange; and links occur in the third stage of low interaction energy. Further additions of phosphate after the third stage is reached tend to decrease the curvature of the adsorption isotherms (Barrow et al. 1998; Rehnheimer and Anghinoni, 2001).

The increase in organic matter content in the soil causes a significant increase in specific surface of clay minerals and oxides, especially in Oxisols (Almeida et al., 2003). Likewise, the increase in P adsorption capacity in depth is also directly related to the reduction of organic matter that occurs in this sense, because the locking mechanism appears to occur by coating the surface of the oxides of molecules of humic acids and other organic anions (Fontes et al., 1992).

Highly weathered tropical soils are known to have minerals such as goethite, hematite, gibbsite and kaolinite, which have surface Fe-OH or Al-OH on which the phosphate can be adsorbed, and this reduces the availability of the plant P (Iyamuremye al., 1996). However, the availability of P can be increased if there is an increase in organic matter in soil, such as the permanence of soybeans straw and other crops (Basamba et al., 2006). The adsorption sites of goethite can be blocked by organic matter fractions such as humic acids, thus decreasing the adsorption of P. Organic compounds of low molecular weight such as oxalate and malate can also act the same way, blocking the adsorption sites of oxy-hydroxides, but these effects have been transient, as reported by Afif et al. (1995).

In studies of soils from the Brazilian savannas (mostly Oxisols), Mesquita Filho and Torrent (1993) found that P adsorption increased dramatically after the removal of organic matter with hydrogen peroxide (H_2O_2). Although competitive adsorption is considered as the main mechanism of action of organic matter in the availability of P, the metal complexation reactions and the dissolution of Fe and Al oxides can potentially reduce the number of adsorption sites, also releasing P for absorption by plants (Guppy et al., 2005). The sorption of organic matter compounds can also increase the negative charge on the soil surface, or decrease the point of zero charge (PZC), making it harder to adsorption of P due to the presence of more negative charges in the adsorption sites.

In tilled soils, the low molecular mass organic acids (LMOA) from the decomposition of organic material, root exudation and microbial synthesis form complexes with Fe and Al and/or are adsorbed to the surface oxide by exchange of ligands and thus block the adsorption of P (Rehnheimer and Anghinoni, 2001).

Specifically adsorbed anions are desorbed by competitors, only when these may occupy the adsorption sites. This leads to increased negative charged surface, i.e., the power of competition depends on the ability to increase surface charge. The sorption of anions occurs when there is exchange between anions or when there are free sites to be occupied. With increasing amounts of adsorbed anions, surface coverage of colloids increases, arising nucleation or small aggregates and generating surface precipitates. According to Sparks (1995), there is a continuum between adsorption and precipitation in the soil so the soil will never be free from the action of adsorption, even in minimum quantities. In fertilized soils, the products of reaction with the soil persists for a period long enough to allow recrystallization to more stable forms of P, consistent with the presence in solution.

Ohno and Crannell (1996) suggest that organic acids affect the solubility of P in soil and that the molecular properties of OA, i.e. the functional groups involved in the reactions of coordination influence the magnitude of chemical reactions in the soil. The same authors also argue that the presence of acidic functional groups is one of the most important chemical characteristics of soluble carbon compounds, but their involvement in metal complexation reactions and mineralization depends on their specific chemical properties.

In soils with the presence of hydrolysable or exchangeable Al, the action of the OA would be more effective in Al complexed in the same soil (Fox et al., 1990). At low concentrations the presence of OA tends to increase the exchangeable Al in clays by inhibiting the hydrolysis of the same. In general, the formation of high stability complexes with Al tends to increase P availability and reduce P adsorption, while those with low stability complexes have less influence on the adsorption of P (Fox et al., 1990).

As for soil organic P, the monoester orthophosphates are the most stable and also the predominant form, being formed by inositol phosphates, including acid myoinositol hexaphosphate, which is phytic acid (Magid et al., 1996). Data presented by Condron et al. (1985) showed that there was accumulation of over 90% of organic P in this form after the application of three decades of superphosphate as a fertilizer in soils of New Zealand.

Extracellular enzymes have been used to characterize organic P in animal manure, soil extracts and solutions leachate (Dao, 2004), because the substrate specific phosphatases by these enzymes provides a good estimate of the analytical determination obtained. However, enzymatic methods have shown the difficulty of determining the relative low recovery of P in soils (Hayes et al., 2000).

Complexed inositol phosphates in the soil are relatively resistant to enzymatic hydrolysis, for complex polymer compounds are formed via intra and intermolecular bonds with polyvalent cations, limiting the availability of substrates for the phytases (Dao, 2004). It has been observed that organic anions facilitate the enzymatic release of phosphate in Ultisols, however, no difference in the efficiency of clearance by exchange of ligands between the organic anions, such as oxalate is more effective than the format (Fox et al. 1990). The forms of phosphate determined by enzymatic methods are called bioactive P soil (Dao, 2004).

It is clear from the dependence of organic matter accumulation on phosphate that the latter must be immobilized in some circumstances, but the literature on the immobilization of phosphate is not extensive. Part of the reason must be that mineralization and immobilization occur simultaneously and both show considerable spatial variability. Hedley et al. (1982) found evidence of immobilization when soil was incubated, both when cellulose and nitrogen were added and when they were not. Rewetting and incubating an air-dried Rhodesian (now Zimbabwean) soil also immobilized phosphate (Salmon, 1965). However, when Addiscott (1969) incubated a rewetted Tanzanian hill sand soil for 10 days, phosphate seemed to be immobilized during the first 4 days and then rereleased during the next 6 days, so that the final concentration of phosphate differed little from that at the start.

4. Introduction

The low phosphorus (P) use efficiency by crops in tropical soils is mostly due to soil P-fixation through adsorption or precipitation reactions (Tisdale et al., 1993). The sparingly soluble nature of soil P impairs crop yields and additional P sources must be applied as fertilizers in order achieve economic yields (Lindsay, 1979). One potential approach is to adopt cropping systems that could increase the level of plant available P in soil. Cropping systems that utilize green and animal manures have had positive impact crop yields and reduces dependency on chemical fertilizers (Karlen et al., 1994; Tisdale et al., 1993). Organic amendments can directly affect soil P availability through interaction with soil components (Iyamuremye and Dick, 1996).

Between 30 % to 50% of total soil P is organic P, appearing mainly as phytates, nucleic acids (and their derivatives) and phospholipids (Paul and Clark, 1989). Some of this organic P is contained in or originates from crop residues. Therefore, returning crop residues to the soil is important in P cycling. In a 16-week soil incubation study, Reddy et al. (2001) observed that soybean (*Glycine max*) and wheat residues favored build-up of labile inorganic and organic P at the expense of recalcitrant P. In field studies, crop residues applied together with rock phosphate resulted in increased soil P availability, cereal P uptake and yields more than application of rock phosphate alone (Sharma and Prasad, 2003; Waigwa et al., 2003).

Similarly, Essington and Howard (2000) reported that plots under no till had significantly higher values of organic P than those under conventional tillage. To become plant available, organic P must be mineralized (Frossard et al., 1995). Similarly, the availability of P held in the soil microbial biomass, or in plant debris, depends on the disruption of protecting cell structures. McGill and Cole (1981) defined biological mineralization as the release of inorganic P from organic materials during oxidation of C by soil organisms.

Whitbread et al. (2000) reported greater P content in wheat where legume residues had been retained as compared with plots where they had been removed. In a greenhouse experiment, Cavigelli and Thien (2003) reported a greater sorghum biomass following three out of four winter cover crops than biomass in the control treatment.

Congo grass has been widely used in crop rotation and crop-livestock integrated systems in Brazil because of its good adaptation to low fertility soils (Garcia et al., 2008), high yield potential, good forage quality, and ready desiccation (Klutchcouski et al., 2003). Besides, this tropical grass has been reported to increase soil P availability (Crusciol et al., 2009).

Mineralization of organic P is mediated by soil microorganisms but the rate and pattern is regulated by environmental conditions and residue quality. Changes in both soil moisture and temperature affect microbial activity and thereby P mineralization (Kabba and Aulakh, 2004). Net P mineralization is often positively correlated with residue P concentration (Kwabiah et al., 2003) and negatively correlated with C/P ratio (Hundal et al., 1987) and lignin concentration or lignin/P ratio (Lupwayi and Haque, 1999).

The objective of this study was to evaluate the effectiveness of congo grass (*Brachiaria ruziziensis*) grown as a cover crop in enhancing soil P availability and its relationship with carbon behavior using long-term field trials and laboratory chemical fractionation approaches.

Phosphorus (P) deficiency is a factor limiting crop production on tropical and sub-tropical soils (Fairhust et al., 1999; Mokwuny et al., 1986; Sanchez and Salinas, 1981). Correcting P deficiency by applications of P fertilizers is often too expensive for resource-poor farmers in these regions, especially for soils with high P-fixing capacity. Under such conditions, the integration of crop species and/or crop cultivars that can make efficient use of soil residual P, combined with application of maintenance fertilizers represents a key element of sustainable cropping systems (Ae et al., 1990; Lynch, 1998).

Previous studies indicated that plants most capable of surviving on acid soils with low P availability are likely those with inherently slow rates of growth as these plants are often adapted to survive rather than to be productive (Chapin 1983; Helyar 1994; Rorison 1986). Slow growers often have high tissue mass densities and longer tissue life spans which contribute to nutrient conservation, use efficiency and reduced losses (Vazquez de Aldana and Berendse 1997; Poorter and De Jong 1999; Ryser and Urbas 2000). Nutrient accumulation has also been suggested as an important ecological strategy of perennials (Chapin, 1980).

Plants can also activate a set of adaptive responses to enhance P acquisition and P recycling by reprogramming metabolism and restructuring root system architecture (Vance et al., 2003; Jain et al., 2007) to maintain their growth rate as high as possible (Gutschick and Kay 1995). Root attributes such as length, surface area, fineness, and root hair density influence plant adaptation to low-P soils (Rao et al., 1999b). A large root system is either a constitutive or an inducible trait (Marschner 1998) and is of great value for P uptake as diffusion to the root surface is the rate-limiting step, especially in high P-fixing tropical soils in which nutrient supply could be patchy (Rao et al., 1999b; Hodge, 2004). Not only do plant roots respond to P deficiency through greater root growth and lateral root formation (Hermans et al. 2006; Hammond and White 2008), but enhanced production and secretion of acid phosphatases (APases), release of H+ or OH-(Gahoonia et al., 1999), organic acid anions (Kirk et al., 1999; Gerke et al., 2000), the increase of reduction capacity (Holford and Patrick, 1979), will allow the plant to assess poorly available inorganic and organic soil P fractions and thus increase the pool of soil/fertilizer P which contributes to plant P nutrition. It is the suite of morphological and physiological attributes that determine resource uptake by plants (Jackson et al., 1999; Hammond et al., 2004; Lambers et al. 2006).

With this scenario the wide adoption of *Brachiaria brizantha* is attributed to its excellent adaptation to infertile acid soils (soil pH<5.5), which contributes to its superior persistence in long-term pastures (Rao et al., 1996a). Rao (2002) reported that P is often the most limiting nutrient for pasture establishment and production in highly weathered acid soils of tropical America. Amongst 55 Brachiaria genotypes, including apomictic signalgrass, subjected to field evaluation in low fertility

acid soils, a tetraploid sexual Brachiaria ruziziensis (congo grass) that facilitates Brachiaria breeding was found the least efficient in acquiring P and N and also, the least productive grass in the short-term (5.5 months after pasture establishment) (Rao et al., 1998). Other field studies indicated that the diploid sexual ruzigrass is better than tetraploid sexual ruzigrass during the first six months of pasture establishment; but even the diploid ruzigrass does not persist beyond 2 years in low P acid soils (CIAT, 1995; Rao et al., 1998; CIAT, 2007; Ricaurte et al., 2007).

The identification of morphological and physiological traits and mechanisms responsible for the high level of tolerance to low-P stress would help establish a promising agronomic approach for the integration into the cropping system of P mobilizing plant species as inter-crops or in rotation with Brachiaria genotypes. Thus, the main objective of the present study was to determine the physiological responses of signalgrass and ruzigrass that were grown in nutrient solution supplied with two mineraloxides bound P as a sole source, which is similar to chemically fixed P in highly weathered acidic soils.

5. Material and Methods

5.1. First Experiment

The long-term field trial site is located in Botucatu, São Paulo State (22°51' S, 48°26' W Grw and altitude 840 m). The soil is a Haplortox (Latossolo Vermelho distroférrico, according to EMBRAPA, 2006), sandy loam with 670 g kg⁻¹ of sand and 210 g kg⁻¹ of clay. Selected chemical characteristics of the soil are presented in Table 1.

The crop rotation system was first established in 1998 and consisted of triticale (X *Triticosecale Wittmack*) and black oat (*Avena stringosa*) grown in autumnwinter, pearl millet (*Pennisetum glaucum*) grown in the spring and soybean (*Glycine max*) in the summer. In 1998 and 2001 the experiment received 0 (control) and 35 kg P ha⁻¹ (total P) as triple superphosphate or Arad reactive rock phosphate.

Chemical characteristics								
Depth	pН	O.M	Р	H+A1	Κ	Ca	Mg	CEC
(cm)	CaCl ₂ (0.01 mol L ⁻¹)	g dm ⁻³	mg dm ⁻³		mn	ol _c dm ⁻³		
0-5	4.8	21	9.7	32	3.7	25.2	14.3	64
5-10	4.6	18	6.8	41	1.2	18.5	12.6	57
		Gran	ılometric	characte	ristics			
Depth	Sand	С	lay	(Silt		Texture	;
(cm)		g	g kg ⁻¹					
0-5	670	2	210		20		Sandy	
5-10	670	22	20		10		Sandy	

Table 1. Soil chemical and granulometric characteristics by the time the experiment was installed. (March/2008).

The impact of congo grass (*B. ruziziensis*) has been studied since 2006, when the rotation was changed from triticale, black oat, or pearl millet to *Congo grass*. In 2006 and 2009, the treatments consisted of 0 and 35 kg P ha⁻¹ which was applied as triple superphosphate (soluble) or natural Arad rock phosphate (reactive) to the same plots previously receiving the same fertilizers. The triple superphosphate had 180 g kg⁻¹ of P, 79 g kg⁻¹ of Ca and 12 g kg⁻¹ of S. The reactive Arad phosphate had 143 g kg⁻¹ of P, 269 g kg⁻¹ of Ca and 8.5 g kg⁻¹ of S. In February 2009, the P fertilizers were applied on the soil surface and *B. ruziziensis* was planted (without potassium or nitrogen fertilizer) in half of the plots at 30 kg ha⁻¹ of seeds (42% of viable seeds) and desiccated 289 days after emergence (DAE) using glyphosate at 2.88 kg ha⁻¹ (a.i.). In plots without Congo grass, glyphosate was applied twice to prevent weed grow.

In order to estimate *Congo grass* dry matter yields, plant residues were sampled at six randomized sites per plot using a 0.25 m² (0.5 x 0.5 m) wooden frame and dried in a forced air oven at 60° C for 72 hours. The plant samples were weighed and sub-samples were analyzed for N, P, K, Ca and Mg concentrations. The remaining material was returned to their original sites. Nitrogen in plant samples was determined by sulfuric acid digestion and steam distillation, and P, K, Ca, and Mg were determined using atomic absorption spectrometry (AA-7000, Shimadzu Scientific Instruments, Japan) after wet acid digestion as described by Malavolta et al. (1997).

In November 2009, six soil samples were randomly collected with an auger at two depths (0-5 and 5-10 cm) from each plot and combined into one composited

sample per depth for analysis. Soil pH was determined in 0.01 M $CaCl_2$ at 1:2.5 soil/solution (w/v) ratio using a pH meter (DM-22, Digimed, Brazil); and available P was determine by pearl resin methods as described by Raij et al. (2001).

Organic carbon concentration in soil samples was determined by dry combustion using a C/N Analyzer (Vario Max Macro Elemental Analyzer, Elemental Analyses System GmbH, Hanau, Germany), at the University of Florida Indian River Research and Education Center in Fort Pierce, FL. Soil organic P fractionation was conducted according to Bowman and Cole (1978), modified by Sharpley and Smith (1985) and Ivanoff et al. (1998). In order to estimate labile organic P, 0.5 g of soil was shaken in a water suspension for 16 h on a horizontal shaker (end-over-end) with 0.5 M NaHCO₃ (pH 8.5), inorganic P (Pi) and total P (Pt) were determined in the extract and labile organic P was calculated as the difference between them. Moderately labile organic P was determined by the difference between Pi and Pt extracted with 1.0 M HCl. Organic P bound to fulvic (Pf) and humic (Ph) substances was extracted with 0.5 M NaOH. To separate Pf from Ph, an aliquot of the 0.5 M NaOH extract was taken and acidified to pH 1.0 - 1.5 with concentrated HCl. At this pH, humic acids precipitate, and fulvic acids remain in the solution. Total P in the acidified sample is a measure of Pf. The Ph fraction was determined by subtracting Pf from the total P measured in the 0.5 M NaOH extract.

Phosphorus concentration in the solutions was determined by the ascorbic-reduction molybdate blue colorimetric method (Murphy and Riley 1962). All samples were analyzed in triplicate. Carbon concentration in the extracts containing fulvic and humic substances was determined by a Liquid Total Organic Carbon Analyzer (liquid TOC trace, Elemental Analyses System GmbH, Hanau, Germany). Carbon to P ratio was calculated using the values obtained from the analyses described above.

The experimental design was a 3 x 2 factorial with three P initial treatments with and without *Congo grass* and ten replications for each treatment. Data for each soil depth was analyzed separately. Plots were 5.0 x 8.0 m. Results were submitted to statistical analyses using the SAS - System for Windows 9.2 (SAS Inc. 2001) program, through the GLM procedure, and means were compared by LSD (P < 0.05).

5.2. Second Experiment

Seeds of *Brachiaria brizantha* and *Brachiaria ruziziensis* were surface sterilized and germinated in the dark (25 °C) for 7 days on a filter paper saturated with deionized water. The seedlings were grown for two weeks in sand culture in growth chambers with a day/night cycle of 12-h at 25 °C and 12-h at 18 °C, 60% relative humidity and a light intensity of 250 μ mol m⁻² s⁻¹. This step was included as *Brachiaria* grasses do not grow fast in the beginning due to their small seed size. At this point of time all seeds received the nutrient supply to sand cultures (mg kg⁻¹ sand) contained: 2.6 P, 2.5 N, 3.1 K, 1.0 Ca, 0.38 Mg, 0.38 S, 0.02 Zn, 0.03 Cu, 0.001 B and 0.001 Mo. After this period plants were transferred to plastic containers where P treatments were applied.

Goethite and amorphous Al-oxide were artificially synthesized with methods proposed by Atkinson et al. (1972) and McLaughlin et al. (1981), respectively. Goethite was synthesized from freshly prepared 0.2 M Fe (NO₃)₃ after the pH solution was adjusted to 11.0 using 0.5 M NaOH. The resultant suspension was stored at room temperature (22 °C) for 2 days followed by heating to 90 °C for 16 h. The precipitates were thoroughly washed with deionized water. Amorphous Al-oxide was synthesized from freshly prepared 1.4 M AlCl₃ solution after the pH was adjusted to 4.7 using 0.2 M NaOH. The resultant suspension was heated at 80 °C for 4 h. After thorough washing, the suspension was dialyzed for 14 days at 20 °C. Some physico-chemical properties of the minerals were described by He et al. (1989, 1991). According to these properties mineral-P complexes were prepared by equilibrating variable P amounts (as KH₂PO₄) with goethite and amorphous Al-oxide in 10 mM CaCl₂ solution (pH adjusted to 7.0) for one month at 25 °C to saturate 85% of the maximum adsorption capacity. After this period the mineral-P complexes were dialyzed with deionized water four times until no P was detectable in the washing water to ensure that only non-labile P remained on the solid surface.

The experimental design was a randomized complete block with three replicates. The treatments were *Brachiaria* species (*Brachiaria brizantha* and *Brachiaria ruziziensis*) and two mineral-P complexes (goethite and amorphous Al-oxide) plus two

controls with and without P as regular nutrient solution, due to the evapotrasnpiration the nutrient solution was added to maintain the original volume (Table 1).

In this experiment each 5-L pot was divided into two separate compartments of equal volume, one compartment containing Hoagland nutrient solution without P and the other containing only goethite-P or amorphous Al-oxide-P. Pots containing Hoagland solution with and without soluble P were used for the controls in order to keep consistency, it was also placed some vials containing water and they were collected on each sampling time with the objective of detect any environment contamination.

Six plants of each specie were transplanted into each pot, *Brachiaria* roots were split in two parts, one being inserted in the P-free nutrient solution (Nutrient Solution "NS" compartment) and the other into the solution with the minerals-P complexes. The same split were done for the controls: Control without P starvation where one part of the roots was placed into a P-free nutrient solution (Nutrient Solution "NS" compartment) and the complete Hoagland nutrient solution; Control with P starvation where one part of the roots was placed into a P-free nutrient solution; Control with P starvation where one part of the roots was placed into a P-free nutrient solution; Control with P starvation where one part of the roots was placed into a P-free nutrient solution (Nutrient Solution "NS" compartment) and the other in deionized water.

Nutrient solution was sampled at the intervals of 24 h, 3, 7, 14 days after treatments were applied in the compartment.

Plants were harvested at 14 d and were dried in a forced air oven at 60 $^{\circ}$ C for 72 h. After dry weight was recorded, subsamples of the plant biomass were digested with concentrated HNO₃ and analyzed for P, K, Ca and Mg concentrations using inductively coupled plasma optical emission spectroscopy (ICP-OES, Ultima 2, JY Horiba Group, Edison, NJ, USA).

The concentration of inorganic P in nutrient solution was determined by the ascorbic-reduction molybdate blue colorimetric method (Murphy and Riley 1962). Total P was determined as described by Hedley et al. (1982). Organic P was determined by the difference between inorganic P and total P values. All samples were analyzed in triplicate. Dissolved organic carbon concentration in nutrient solution samples was determined using a liquid total organic carbon analyzer (liquid TOC Trace, Elemental Analyze system GmbH, Hanau, Germany). Results were submitted to statistical analyses using the SAS - System for Windows 9.2 (SAS Inc. 2001) program, through the GLM procedure, and means were compared by LSD (P < 0.05).

6. Results

6.1. First Experiment

6.1.1. Brachiaria ruziziensis

The average dry matter yield of *Congo grass* was significantly increased by P fertilizers (Table 2), irrespective of P sources. Phosphorus concentrations in plant tissue were also increased by P fertilization (Table 2), but were higher for the soluble source.

Table 2. Mean values of Brachiaria ruziziensis dry matter and phosphorus content under broadcast application of phosphorus sources and Brachiaria ruziziensis.

Treatments	Dry Matter	P content
kg of P ha ⁻¹	kg ha ⁻¹	g/kg
0	3142 b	0.73 c
35 Reactive phosphate	3524 a	1.15 b
35 Soluble phosphate	3855 a	1.58 a

Means followed by different letters within the same row indicate significant differences P<0.05

The average concentrations of N, K, Ca and Mg in plant tissue were 13.7, 20.1, 7.8 and 5.2 g kg⁻¹, respectively, without significant differences due to P fertilization. These nutrient concentrations are within the adequate range reported by Malavolta et al. (1997), showing that there was no nutrient deficiency other than P during the experiment.

6.1.2. Inorganic and Organic P

Soil inorganic available P (resin-P) was increased by Congo grass at both depths in P fertilized plots (Table 3), and no difference was observed between the two P sources. Moreover, *the cover crop* increased 0.5 M NaHCO₃ extractable organic P at the 0-5 cm depth of plots receiving soluble or reactive P fertilizer, but had no effect on organic P or resin-P for the plots without P fertilizers (Table 3). No differences in 1.0 *M* HCl extractable P was observed.

Humic substances bound organic P (Ph) contents in soils from *Congo grass* fertilized plots were significantly higher than those without the cover crop (Table 3). With the cover crop, P fertilization increased Ph at the 0-5 and 5-10 cm depth. Fulvic substances bound organic P (Pf) was not affected by P sources or the cover crop.

The P sources and the cover crop increased organic P pools and consequently affected total organic P content in the soil. The highest content was observed for soluble phosphate with *B. ruziziensis* cultivation at the 0-5 cm depth (Table 3).

6.1.3. Organic Carbon and C:P ratio

Cover crop treatment had the greatest influence on soil C fractions regardless of the rate or source of P fertilizers (Table 4). Growing Congo grass also increased the carbon content in the humic fraction at both depths (Table 4). The highest carbon content of the humic fraction was found in the soil receiving rock reactive phosphate at the 0-5 cm depth. It was observed an increase in C: P ratio (P=0.05) in plots under Congo grass (Table 5), regardless of soil depth. The humic fraction C:P ratio was also higher in plots cropped to Congo grass, regardless of soil depth.

Table 3. Mean values of available P (P-resin), labile organic P (0.5 M NaHCO3), moderately labile organic P (1.0 M HCl), humic substance bound organic P (Ph), fulvic substances bound organic P (Pf) and total organic P (Pt) levels in the soil at 0-5 and 5-10 cm and 5-10 cm depths under broadcast application of phosphorus sources and *Brachiaria ruziziensis* (presence; absence).

Treatments	P-re	esin	0.5 M N	NaHCO ₃	1.0 N	I HCl	F	'n	F	'n	I	Pt .
kg of P ha ⁻¹	With	Without	With	Without	With	Without	With	Without	With	Without	With	Without
Depth 0-5 cm												
	mg kg ⁻ⁱ											
0	30.1 b	34.9 a	22.6 a	13.1 b	25.6 a	28.0 a	36.8 b	49.2 a	15.1 a	14.4 a	111.5 a	119.8 a
35 Reactive phosphate	60.9 a	56.1 b	17.6 a	12.5 b	31.9 a	28.6 a	44.3 a	38.6 b	13.7 a	18.3 a	103.1 a	96.0 b
35 Soluble phosphate	54.4 a	47.1 b	21.0 a	15.2 b	21.6 a	26.5 a	46.3 a	34.3 b	17.8 a	16.7 a	109.2 a	96.1 b
						Depth 5	5-10 cm					
						mg	kg ⁻¹					
0	20.5 a	24.5 a	15.7 a	14.5 a	21.8 a	19.7 a	43.0 a	43.7 a	23.0 a	21.8 a	112.6 a	98.1 b
35 Reactive phosphate	48.8 a	34.9 b	18.7 a	16.4 b	28.5 a	26.7 a	42.1 a	37.3 b	22.9 a	21.7 a	106.8 a	99.5 b
35 Soluble phosphate	37.8 a	19.0 b	18.2 a	13.7 b	17.3 a	16.8 a	45.4 a	32.4 b	25.4 a	23.2 a	106.8 a	94.4 b

Table 4. Mean values of dissolved carbon in the extractant 0.5 M NaOH in the humic fraction (Humic - C), fulvic fraction (Fulvic - C) and Total (Total - C) in the soil at 0-5 and 5- 10 cm depths under broadcast application of phosphorus sources and *Brachiaria ruziziensis* (presence; absence).

Treatments	Hum	ic - C	Fulvi	ic - C	Total - C				
kg of P ha⁻¹	With	Without	With	Without	With	Without			
Depth 0-5 cm									
			g k	kg⁻¹					
0	0.967 a	0.857 b	0.208 a	0.195 a	1.175 a	1.052 b			
35 Reactive phosphate	1.001 a	0.797 b	0.186 a	0.194 a	1.196 a	0.983 b			
35 Soluble phosphate	0.884 a	0.759 b	0.209 a	0.171 b	1.093 a	0.930 b			
	•		Depth 5	5-10 cm					
			g k	kg⁻¹					
0	0.738 a	0.558 b	0.182 a	0.187 a	0.921 a	0.744 b			
35 Reactive phosphate	0.731 a	0.569 b	0.186 a	0.184 a	0.918 a	0.755 b			
35 Soluble phosphate	0.638 a	0.481 b	0.183 a	0.179 a	0.821 a	0.661 b			

Means followed by different letters within the same row indicate significant differences (P<0.05)

Table 5. Mean values of carbon and phosphorus (C:P) ratio in the soil at 0-5 and 5-10 cm depths under broadcast application of phosphorus sources and Brachiaria ruziziensis (presence; absence).

Treatments	C	:Р	Humic	c - C:P	Fulvic	: - C:P
kg of P ha ⁻¹	With	Without	With	Without	With	Without
Depth 0-5 cm						
0	94.8 a	75.9 b	133.5 a	92.4 b	40.6 a	42.7 a
35 Reactive phosphate	84.3 a	66.6 b	113.0 a	85.3 b	36.5 a	34.1 a
35 Soluble phosphate	79.3 a	72.5 a	103.3 a	104.6 a	39.3 a	38.7 a
			Depth 5	5-10 cm		
0	90.7 a	65.9 b	141.8 a	80.1 b	39.4 a	41.6 a
35 Reactive phosphate	81.1 a	65.1 b	118.0 a	89.3 b	36.0 a	36.9 a
35 Soluble phosphate	71.0 a	69.8 a	99.1 a	88.2 b	35.9 a	33.8 a

6.2. Second Experiment

5.2.1 Dry matter yields and P content

After 14 days of cultivation, the dry matter yields of both *Brachiaria* species were not affected by oxide-P (goethite-P or Al oxide-P) as sole P source (Table 6). Their growth was similar to those supplied with water soluble P. In both cases the shoot and root dry matter yield was higher when Brachiaria was cultivated in amorphous aluminum oxides treatment. There was a significant decrease on dry matter yield when P was not supplied (control), regardless of species.

There was no difference in plant P concentration for both root and shoot independent of Brachiaria specie. Plant P concentration for the treatment without P had the smallest observed values (Table 7).

Treatments	Shoot	Root				
	g pla	nt ⁻¹				
Bra	Brachiaria ruziziensis					
Without P	1.2 b	0,4 b				
Water soluble P	8.6 a	1.4 a				
Goethite-P	8.3 a	1.2 a				
Amorphous Al oxide-P	8.9 a	1.6 a				
Bra	achiaria brizantha					
Without P	1.8 b	0,7 b				
Water soluble P	9.2 a	2.1 a				
Goethite-P	8.9 a	1.9 a				
Amorphous Al oxide-P	9.6 a	2.3 a				

Table 6. Mean values of *Brachiaria ruziziensis* and *Brachiaria brizantha* shoot and rootdry matter.

Treatments	Shoot	Root				
	g kg	-1				
Bra	Brachiaria ruziziensis					
Without P	0.03 b	0.07 b				
Water soluble P	1.8 a	2.4 a				
Goethite-P	1.6 a	2.1 a				
Amorphous Al oxide-P	1.7 a	2.1 a				
Bra	achiaria brizantha					
Without P	0.05 b	0.09 b				
Water soluble P	2.1 a	2.7 a				
Goethite-P	1.9 a	2.3 a				
Amorphous Al oxide-P	2.0 a	2.5 a				

Table 7. Mean values of *Brachiaria ruziziensis* and *Brachiaria brizantha* shoot and rootP content on plant tissue.

Means followed by different letters within the same row indicate significant differences P<0.05

5.2.2 Inorganic and organic P in nutrient solution

No inorganic P was detected in P-free nutrient solution (NS) compartment throughout the entire experiment if no plant was grown (the control), while with the presence of plant, P started to appear in the NS after 7 d of culture, suggesting that some P was released from the plants (Table 7). However, when the plants were supplied with oxide-P (goethite-P or Al oxide-P) in a separate compartment using split root culture, inorganic P was detected within 24 h of plant growth, indicating that the plants were capable of using chemically adsorbed P on Fe, Al oxides, which are similar in nature to residual P in the highly weathered tropical soils such as Oxisols. The concentration of P in the NS compartment increased with the time of plant culture from approximately 0.5-0.9 mg L⁻¹ at the 24 h sampling event to 1-5 mg L⁻¹ after 14 d of plant growth. In comparison, Al oxide-P appeared to be more available to these plants than goethite-P as higher concentration of P was observed in the NS compartment when the plants were supplied with Al oxide-P and goethite-P (Table 8). Compared with *B. ruziziensis, B. brizantha* species seemed more efficient as evidenced by the higher P

concentration in the NS compartment regardless of the type of oxide-P. Higher P concentration was measured in the NS compartment when water soluble P was supplied as compared with oxide bound P, which is expected as plants can take up more P with a luxury supply (Table 8). It was not detected any P concentration in the vials containing water irrespective the sampling time.

Unlike inorganic P, organic P was detected only in NS samples collected on the 14th day after plant culture (Table 9). It is somewhat unexpected as we commonly believe plant roots excrete organic substances and organic P is among those. However, we did not detect any organic P in the 24 h, 3 d and 7 d sampling events. It is speculated that the organic P released at the early stage of plant growth might belong to simple organic molecules and readily mineralized through the action of enzymes on root surface. Similar trend was obtained for different treatments in this study, which may support the above assumption. More elaborate study is needed to prove this.

5.2.3 Dissolved Organic Carbon

Dissolved organic carbon (DOC) was detected in NS compartment for all the sampling dates with both *Brachiaria* species. The DOC concentration was higher when mineral-P was applied and its levels increased with time. The supply of water soluble P in nutrient solution inhibited the exudation of organic compounds in both compartments with this treatment and the same effect was observed with no P in nutrient solution.

There was no difference in DOC concentration for both *Brachiaria* species studied, and the highest DOC level occurred in nutrient solution 14 days after treatment for both genus on amorphous aluminum oxides (Table 10), indicating that presence of Al oxide might have stimulated the release of DOC by plants.

Tractmente	NS+G-P		NS+	NS+Al-P		NS + W-P		Control	
Treatments	C1	C2	C1	C2	C1	C2	C1	C2	
Sampling Time				mg	L-1				
				Bra	chiaria ruzizi	ensis			
24 h	n.d ⁶	0.55 c	n.d ⁶	0.63 c	5.92 b	8.28 a	n.d ⁶	n.d ⁶	
3 d	n.d ⁶	0.63 c	n.d ⁶	1.39 c	4.97 b	7.26 a	n.d ⁶	n.d ⁶	
7 d	0.44 d	2.07c	0.68 d	2.72c	3.76 b	5.68 a	n.d ⁶	n.d ⁶	
14 d	1.03 d	2.99 c	1.07 d	3.45 c	2.65 b	3.96 a	n.d ⁶	n.d ⁶	
		Brachiaria brizantha							
24 h	n.d ⁶	0.74 c	n.d ⁶	0.86	5.92	8.72 a	n.d ⁶	n.d ⁶	
3 d	n.d ⁶	1.63 c	n.d ⁶	1.83	4.27	6.19 a	n.d ⁶	n.d ⁶	
7 d	0.65 d	3.02 c	0.69 d	3.52 c	3.01 b	4.41 a	n.d ⁶	n.d ⁶	
14 d	1.27 d	4.07 c	1.38 d	5.08 c	2.35 b	2.89 a	n.d ⁶	n.d ⁶	

Table 8. Mean values of inorganic P in nutrient solution under P treatments with and without plants.

 $C1=P-free \ Hoagland \ nutrient \ solution; C2=(NS+G-P) = Goethite \ complex \ in \ suspension \ with \ P \ adsorbed; \ (NS+AI-P) = Amorphous \ aluminum \ complex \ in \ suspension \ with \ P \ adsorbed; \ (CHNS) = Complete \ Hoagland \ nutrient \ solution; \ (NS+W-P) = Water. \ Control = Vials \ containing \ P-free \ Hoagland \ nutrient \ solution \ without \ plant \ cultivation \ and \ vials \ containing \ water \ without \ plant \ cultivation.$

Tractments	NS+	NS+G-P		NS+Al-P		NS + W-P		Control	
Treatments	C1	C2	C1	C2	C1	C2	C1	C2	
Sampling Time	mg L ⁻¹								
				Brachiaria	ruziziensis				
24 hr	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	
3 d	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	
7 d	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	
14 d	0.54 d	1.67 c	0.62 d	1.89 c	2.26 b	3.75 a	n.d ⁶	n.d ⁶	
				Brachiaria	ı brizantha				
24 hr	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	
3 d	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	
7 d	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	n.d ⁶	
14 d	0.64 d	1.72 c	0.72 d	2.07 b	2.59 b	4.15 a	n.d ⁶	n.d ⁶	

Table 9. Mean values of organic P in nutrient solution under P treatments with and without plants.

C1=P-free Hoagland nutrient solution; C2=(NS+G-P) = Goethite complex in suspension with P adsorbed; (NS+AI-P) = Amorphous aluminum complex in suspension with P adsorbed; (CHNS) = Complete Hoagland nutrient solution; (NS+W-P) = Water. Control = Vials containing P-free Hoagland nutrient solution without plant cultivation and vials containing water without plant cultivation. Means followed by different letters within the same row indicate significant differences P<0.05

NS+G-P	NS+	-G-P	NS+	Al-P	NS +	W-P	Cor	ntrol		
C1	C1	C2	C1	C2	C1	C2	C1	C2		
Sampling Time		mg L ⁻¹								
				Brachiaria	ruziziensis					
24 hr	21.4 c	52.7 a	32.4 b	62.7 a	42.9 b	12.5 d	n.d ⁶	n.d ⁶		
3 d	32.5 c	67.9 a	42.7 b	78.1 a	45.7 b	15.8 d	n.d ⁶	n.d ⁶		
7 d	45.9 c	92.5 a	65.3 b	103.5 a	48.9 c	21.4 d	n.d ⁶	n.d ⁶		
14 d	59.7 c	122.5 a	78.4 b	142.7 a	53.1 c	27.8 d	n.d ⁶	n.d ⁶		
				Brachiaria	ı Brizantha					
24 hr	28.6 c	63.5 a	36.7 b	65.9 a	38.3 b	9.7 d	n.d ⁶	n.d ⁶		
3 d	43.1 b	72.9 a	47.3 b	82.7 a	40.3 b	12.5 c	n.d ⁶	n.d ⁶		
7 d	52.1 c	108.2 a	71.7 b	111.3 a	50.9 c	23.7 d	n.d ⁶	n.d ⁶		
14 d	61.3 c	143.1 a	88.2 b	172.4 a	59.6 c	29.5 d	n.d ⁶	n.d ⁶		

Table 10. Mean values of dissolved organic carbon in nutrient solution under different P treatments with and without plants.

C1= P-free Hoagland nutrient solution; C2= (NS+G-P) = Goethite complex in suspension with P adsorbed; (NS+Al-P) = Amorphous aluminum complex in suspension with P adsorbed; (CHNS) = Complete Hoagland nutrient solution; (NS+W-P) = Water. Control = Vials containing P-free Hoagland nutrient solution without plant cultivation and vials containing water without plant cultivation. Means followed by different letters within the same row indicate significant differences P<0.05

7. Discussion

7.1. First Experiment

7.1.1. Brachiaria ruziziensis

The original soil P level (on average 9.9 mg dm⁻³ of P-resin) was inadequate for congo grass growth, since there was response in the average dry matter yield. Corrêa and Haag (1993) emphasized the importance of P supply for grasses in the first year of cropping in Brazilian soils. *Brachiaria brizantha* has been recommended for low P soils since this species is well adapted, and in some cases it can produce three times more dry matter than other *Brachiaria*. Hence, Congo grass *may* be not well adapted to low P availability soils. However, dry matter yield differences among *Brachiaria* species was not observed at high P supply (Corrêa and Haag, 1993). The same authors observed a decrease on P fertilization response after the second crop of *Brachiaria* species in the same area, probably due to a higher access to native soil P.

Phosphorus concentration in plant tissue was significantly higher due to P fertilization and an even higher concentration was observed with the soluble P source. Similar results were found by Corrêa and Haag (1993) studying critical levels of phosphorus for the establishment of pasture grass on a red yellow latosol. Phosphorus accumulation in plant tissue may vary by specie and on Corrêa and Haag (1993) experiment *Brachiaria* species had a greater capacity to accumulate P than *Panicum*. This characteristic is important on the decision of which crop to use as a cover crop since the ability to accumulate nutrient plus the capability of adaptation to low fertility can determine the success of P cycling in the system.

7.1.2. Inorganic and Organic P

Growing Congo grass increased soil available P as estimated by resin extraction at both soil depths when P fertilizers were applied. The buildup of available P in the topsoil by *B. ruziziensis* cultivation under no-till supports the findings of several other studies, such as Ellis and Howse, (1980); Resende et al. (2006); Merlin et al. (2007); Galvani et al. (2008). This effect may result from the influence of organic acids on P sorption (Iyamuremye et al., 1996), since *Brachiaria* can exude citrate or oxalate under low pH conditions (Wenzl et al., 2002; Wang et al., 2007; Louw-Gaume et al., 2010). In addition, the activities of root acid phosphatases and phytases of some grasses such as *Brachiaria brizantha* and Congo grass were higher under low P supply conditions (Rao et al., 1999;). Findings of Louw-Gaume et al. (2010) support the hypothesis that roots of *Brachiaria* can modify their P acquisition capacity by adjusting their physiological, morphological and architectural traits to meet changes in plant P demand (Hammond et al. 2004). These findings are supported by other authors as Duff et al. (1994) and Merlin et al., 2010 who reported that root tissue activity of alkaline phosphatases and phytases of *Brachiaria ruziziensis* and *Brachiaria brizantha* were higher with low P supply.

Soil organic P may be utilized by plants after mineralization and subsequent release of Pi. Some root exudates such as acid phosphatases, which catalyzes hydrolytic cleavage of the C-O-P ester bond of organic P present in soil (Eivazi and Tabatabai, 1977) and releases P as plant-available Pi forms ($H_2PO_4^-$, $HPO_4^{2^-}$), may originate from plant and soil microorganisms (Tarafdar and Jungk, 1987; Tarafdar 1989). In most agricultural soils, organic P forms comprise 20-85% of the total P, of which the largest fraction (50%) appears to be in the form of phytin and its derivatives (Dalal 1978). Organic P forms such as phytin, lecithin and glycophosphate may be important in the P nutrition of plants (Tarafdar and Claassen 1988). In this experiment, Congo grass increased some organic P forms. The higher increase was observed in 0.5 *M* NaHCO₃ fraction at the 0-5 cm depth of plots receiving soluble or reactive P fertilizer. This effect may be explained by root activity, since there is evidence that enhanced phosphatase activity in the rhizosphere is implicated in the depletion of organic P forms from P-deficient Oxisols (George et al., 2006).

The 0.5 M NaHCO₃ fraction, though not directly taken up by plants, is generally considered to be readily or potentially available because of its low molecular weight

and because it can be readily mineralized (Hedley et al., 1982; Gatiboni et al., 2007). The increase in 0.5 M NaHCO₃ fraction is in agreement with the increase in available P, indicating that Congo grass can enhance P availability in high P fixing soils. Tarafdar and Jungk (1987) reported a linear relationship between acid phosphatases activity and release of inorganic P from different organic P forms has been observed which could be a mechanism to explain this increase in the 0.5 M NaHCO₃ fraction. Some plants start to secret organic compounds as soon as their roots emerge and the maximum secretion occurs under P-deficient conditions, and the secretion stops after the soil inorganic P concentrations increase.

Interestingly Congo grass increased humic substances bound organic P (Ph) contents in soils from fertilized plots. The increase of Ph has a great effect on P availability since P in these humic fractions has been suggested to represent a moderately to highly resistant P pool in soils (Browman and Cole, 1978). Knowledge of its chemical nature seems essential for an understanding of its role in P cycling. Makarov et al. (1996) who investigated the P-species distribution in humic fractions from A horizons reported that the labile P in humic fractions indicate limited microbial activity, resulting in accumulation of organic P forms. Indirectly, the organic P accumulation observed in the same treatments could be a side effect of the higher content of P in humic substances. This great P pool can be the reservoir of organic P which supply the increase of inorganic P found after the cover crop cultivation.

In an agreement of this findings Hong and Yamane (1980) found that 60% of the organic P fractions in fulvic acid, generally considered a heavy fraction component, was found as inositol hexakisphosphate, and 40% was found as other forms of organic P which can be rapidly mineralized and become available to plants (Paing et al.,1999; Williams and Steinbergs, 1958).

7.1.3. Organic Carbon and C:P ratio

The carbon content in the humic fraction of the SOM was increased by Congo grass. No-till has been recommended as part of a strategy to reduce C loss from agricultural soils (Kern and Johnson, 1993). Dry matter decomposition rates are generally slower in no-till compared with conventional tillage, in which the decomposition of SOM is hastened by revolving the soil and alterations in the soil microclimate (Gatiboni et al., 2007). Holland and Coleman (1987) suggested that C sequestration is increased under no-till because the surface residue is primarily decomposed by fungi which have higher assimilation efficiency than the bacteria, which dominate the decomposition processes of residues mixed into the soil.

Five and ten year tillage with corn indicated that soil organic matter increased significantly in no-till compared to conventional till (Blevins et al., 1984). Tyler et al., (1983) showed, after 2 years in soybean plots, significantly higher levels of organic matter in no-till than in conventional till treatments.

Not only C and organic P were increases under Congo grass, but it was observed that C:P ratio was also increased. This effect brings extra benefits of growing grasses as cover crops. Soil microbial C:P ratios are highly variable. In the review paper by Manzoni et al. (2010) they reported mass C:P ratios for soil microbial biomass ranging from 23 to 333. According to Benintende et al. (2008) reported that soil microbial C:P is a sensitive variable to evaluate effects of crop rotation and other agricultural management practices. Growing forage species for grazing or mowing has shown benefits in terms of soil C accumulation (Cerri et al., 2004; Franzluebbers et al., 2010; Salton et al., 2008), although soils under hayed management are reported to accumulate less than those under grazed management (Franzluebbers et al., 2010). For integrated crop-livestock in the Brazilian Cerrado region (savannah), Salton et al.(2008) reported soil C accumulation rates of 0.44Mg ha⁻¹ year⁻¹ in a 9-year old integrated production system with soybean for two years followed by brachiaria (Brachiaria brizantha) pasture for another two years. Franzluebbers and Stuedemann (2008) did not observe a negative influence of integrated crop-livestock system on soil C and N fractions and thus recommended this system as a viable conservation approach for intensifying agricultural land use. Forages accumulate more C in soils, compared to grain crops, due to a higher root biomass production stimulated by grazing. Souza et al. (2008), in a study conducted in Southern Brazil, showed increases in oat plus ryegrass root biomass production due to grazing, but worldwide results of grazing effects on root biomass are not consistent (Milchunas and Lauenroth, 1993). For cash and cover crops, the role of shoots and roots as sources of soil organic matter has been the subject of several studies (Balesdent and Balabane, 1996; Gale and Cambardella, 2000). Results from these studies suggest that in croplands, root-derived C is the predominant contributor to soil organic C.

Dos Santos et al. (2011) reported that forage-based rotations of semiperennial alfalfa and annual ryegrass for hay production contribute more to soil organic C sequestration than rotations based on cover crops (oat or vetch), although a large proportion of the net primary productivity is exported from the field by haying. The authors conclude that this can be explained by the roots, and either forage-based or cover crop-based rotations play a more relevant role in building up soil C stocks in this no-till Ferralsol than shoot residues.

6.2 Second Experiment

6.2.1 Dry matter yields and P content

The absence of difference in dry matter yields and plant P content between oxide-P and water soluble P supply indicates the great ability of *Brachiaria* species to access non labile P. This ability of the cover crop has been observed in fields by many authors (Lynch and Beede, 1995; Pelleta and El-Sharkawy, 1993; Rao et al., 1993), but the plant traits and mechanisms that contribute to the greater P acquisition and/or utilization in these genotypes are poorly understood. System could benefit from greater P acquisition by enhancing P cycling through residues. Following the concept of 'synchrony' of nutrient release and crop demand in systems (Swift, 1984), P cycling could be enhanced by intercepting P released from residues before it moves into less labile pools.

Understanding this mechanism and cycling would enable us to design better systems and management interventions which minimize undesirable P flows out of the cycle, through "fixation" reactions.

Phosphorus acquisition by plants depends on root system size and distribution, P uptake kinetics and P mobilizing capacity (Barber, 1984).

6.2.2 Inorganic and organic P in nutrient solution

The absence of detection in the first two sampling events may partly as attributed to the determination method, as the ascorbic-reduction molybdate blue colorimetric method has detection limit of 0.02 mg L^{-1} (Murphy and Riley 1962), which does not allow the detection of P concentration in sample solution below 0.03 mg L^{-1} considering the reagent needed for color development.

The presence of inorganic P in the P-free NS compartment when the plants were supplied with mineral-P complex indicates the utilization of oxide bound P by the cover crop. Similar phenomena may occur in fields when these plants grow in high P-fixation soils.

Several species has the ability to access non labile P forms. Some of them are well known in the scientific community, such as chickpea (*Cicer arietinum*), ground nut (*Arachis hypogea*), white lupin (*Lupinus albus*), Pigeon pea (*Cajanus cajan*), wheat (*Triticum aestivum*) and recently *Brachiaria* species.

White lupin (*Lupinus albus*) exuded organic acids to mobilize sparingly soluble phosphates which are made more available for wheat as compared to monoculture (Horst and Waschkies, 1987; Kamh et al., 1999). Pigeon pea increased P uptake of the intercropped sorghum by exuding piscidic acid that chelates Fe^{3+} and subsequently releases P from FePO₄ (Ae et al., 1990). In a field experiment, faba bean was found to facilitate P uptake by maize (Li et al., 1997, 2003). However, all these studies were focused on inorganic P in the soil.

Brachiaria species are the most widely planted tropical forage grasses in the world. *Brachiaria* species are well adapted to low-fertility acidic soils in the tropical and subtropical regions because they are highly tolerant to high aluminum, low P, and low calcium (Rao et al. 1996a, Rao et al. 1996b, Wenzl et al. 2003). Under P-deficient conditions, the grasses improve their P acquisition by enhancing root growth, uptake efficiency, and ability to use poorly available plant P (Rao et al., 2002). Although they have much lower internal requirements for P than do other grasses, they show interspecific differences (Rao et al. 1996b).

According to Nanamori et al. (2004) the tolerance of low P in the *Brachiaria* hybrid involved marked differences in P recycling and carbon metabolism. For the *Brachiaria* hybrid, low-P tolerance involves two major strategies: (1) increasing the ability to efficiently use P by inducing APase and RNase in shoots under P deficiency stress; and (2) enhancing sugar metabolism and subsequent synthesis of amino acids and organic acids in leaves under P deficiency.

The results from this experiment indicates that *Brachiaria* species can contribute to inorganic P pools in addition to the ability to solubilize various forms of insoluble P fractions (Richardson, 1994, 2001; Whitelaw et al., 1999).

This fact can be explained as a response to persistently low levels of available inorganic P in the rhizosphere, and some plant species, such as *Brachiaria* sp. have developed highly specialized physiological and biochemical mechanisms to acquire and utilize inorganic P from the environment. The ultimate consequences of these modifications are increased inorganic P availability in the rhizosphere and enhanced P uptake.

Modification of root growth and architecture is a well-documented response to inorganic P starvation (Lynch, 1995; Lynch, 1997). An increase in the root-shoot ratio under inorganic P starvation is a hallmark of plant response to inorganic P deficiency, enhancing the total surface area available for soil exploration and acquisition of nutrients for a particular species of plant. Plants with a more proliferated root system that is efficient in uptake are well suited to exploit soil inorganic P. In addition to increased root mass, root diameter decreases under inorganic P stress, while the amount of absorptive surface area relative to root volume increases.

The exudation of organic acids (OA), in dicots, particularly legumes, is more efficient than monocots in producing and excreting organic acids to the rhizosphere to enhance inorganic P solubilization under inorganic P deficiency. The root exudates of inorganic P-deficient plants contain a large number of organic acids (Grierson, 1992). The roots of rape excrete organic acids into the rhizosphere and solubilize inorganic P from rock phosphate (Hoffland et al., 1989).

6.2.3 Dissolved organic carbon

Dissolved organic carbon (DOC) detected in all samples indicates an intense and quick response of the root system to the treatments application. These index levels indicate, indirectly, the exudation of enzymes and OA as a strategy to access the non labile P forms on the mineral-P complexes.

This result supported the increase of inorganic and organic P in NS compartment when plants were cultivated. Since OA are low-molecular weight CHO containing compounds which are found in all organisms and which are characterized by the possession of one or more carboxyl groups. It is also well established that P deficiency significantly increases the leakiness of the root plasma membrane to solutes indicting that for some exudation studies the observed increases in organic acid release may be an indirect root

response of minimal importance (Ratnayake et al., 1978). This is especially true where only small increases (≤ 2 fold) in organic acid release and C diversion into root exudation (0.3%) are observed upon long-term P deficiency (Lipton et al., 1987), and where the calculation of results may cause significant biasing.

Malate and citrate appear to be the primary components released by roots under P deficiency. In Brassica napus the 4-fold increase in organic acid exudation is largely associated with the root apex, while smaller amounts are also released from mature root regions (Hoffland et al., 1989, 1992). In contrast, except under extremely high P levels, lupin and other species with cluster roots (e.g., Banksia) induce the development of short branched, tertiary lateral roots (proteoid or 'cluster' roots) (Dinkelaker et al., 1995; Keerthisinghe et al., 1998). These roots are directly responsible for the 13-40-fold increase in the citrate and malate excretion which constitutes >90% of the total root exudate under P deficiency and which commences 3 days after proteoid root development (Dinkelaker et al., 1989; Gardner et al., 1983; Grierson, 1992; John son et al., 1996a, b). This organic acid exudation under P deficiency constitutes a drain of 5-25% of the plant's photosynthetically fixed C, however, this does not appear to significantly affect dry matter production (Dinkelaker et al., 1989; Gardner et al., 1983; Johnson et al., 1996a, b; Keerthisinghe et al., 1998). To sustain this level of root exudation obviously requires a sustained production of organic acids as exudation under P stress can deplete the entire root organic acid reserves within hours (Johnson et al., 1996a). In lupins, it appears that C is mainly supplied in the form of phloem-translocated sugars (70%) whilst some is also supplied in the form of root-fixed inorganic C (30%) (Johnson et al., 1996a, b). The phloem-translocated sugars are subsequently converted to organic acids via the enzymes PEP carboxylase, malate dehydrogenase and citrate synthase at the site of release (Hoffland et al., 1992; Johnson et al., 1994, 1996a, b). The transport mechanisms controlling organic acid release and the number and regulation of genes determining this P deficiency trait, however, have still to be identified.

8. Conclusions

8.1. First Experiment

Congo grass is responsive to P fertilization and increased residual inorganic P in the soil in presence of P fertilizers.

Growing Congo grass as a cover crop increases soil C pools and C:P ratio, resulting in benefits on soil characteristics such as humic acid bound P fraction and organic P pools as sources of available P.

8.2. Second Experiment

Brachiaria specie could access non-labile adsorbed when P was supplied with Al oxide-P and goethite-P, indicating that the plants were capable of using chemically adsorbed P on Fe, Al oxides.

Brachiaria species used O.A. exudation to access the non-labile forms on Fe and Al oxides.

Brachiaria dry matter was not affected by the absence on available P in the nutrient solution.

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