University of São Paulo
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Phosphorus dynamics in the rhizosphere of sugarcane under phosphate sources and filter cake

Bruna Arruda

Dissertation presented to obtain the degree of Master in Science. Area: Soil and Plant Nutrition

Piracicaba
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versão revisada de acordo com a resolução CoPGr-6018 de 2011

Advisor:
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DEDICATION

To my grandparents Celencina & Livino (in memoriam) and Hilda & Milton

To my parents, Catarina & Edu

To my brother Vitor

I dedicate

Aos meus avós Celencina & Livino (in memoriam) e Hilda & Milton

Aos meus pais, Catarina & Edu

Ao meu irmão, Vitor

Dedico

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HEADING

“Satisfaction lies in the effort, not in the attainment. Full effort is full victory.”
Mahatma Gandhi

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RESUMO

Dinâmica do fósforo na rizosfera de cana-de-açúcar sob fontes fosfatadas e torta de filtro

Em solos tropicais o fósforo (P) é adsorvido nas superfícies dos óxidos, reduzindo a disponibilidade às plantas. Assim, a solubilidade das fontes de P e as características particulares da planta que aumentam a eficiência de absorção tornam-se importantes na rizosfera, onde as raízes, microrganismos e solo interagem biologicamente de forma intensa. Os objetivos desta pesquisa foram avaliar cultivares de cana-de-açúcar quanto a mudanças na rizosfera e eficiência na absorção de P e avaliar a dinâmica do P na rizosfera de cana-de-açúcar sob a aplicação de torta de filtro juntamente com fertilizantes minerais. Foram conduzidos quatro experimentos em casa de vegetação. O solo utilizado foi um Latossolo Vermelho amarelo arenoso da região de Piracicaba – SP, com baixo teor de P, em delineamento em blocos ao acaso com quatro repetições para todos os experimentos. O experimento 1 foi conduzido em esquema fatorial 5x2, com 4 cultivares de cana-de-açúcar: RB92-579; RB85-5156; RB86-7515; RB86-6928 ou ausência de plantas, submetidos a presença ou ausência de adubação fosfatada (78.4 mg P kg^{-1}), e o solo aderido a raiz foi amostrado com sendo rizosférico. O experimento 2 avaliou cinco doses de adubação fosfatada: 0; 9.8; 19.6; 39.2 e 78.4 mg P kg^{-1} solo com o cultivar mais promissor para absorção de P do experimento 1, sendo o solo rizosférico coletado em cinco distâncias a partir do rizoplano (mm): *i)* 0-2; *ii)* 2-4; *iii)* 4-6; *iv)* 6-8; *v)* 8-10. O experimento 3 foi conduzido em esquema fatorial 2x3: ausência ou presença de torta de filtro (5 g MS kg^{-1}) com fosfato natural, fosfato solúvel (78.4 mg P solúvel kg^{-1} solo) ou ausência de fosfato, sendo o solo rizosférico amostrado a 2 mm do rizoplano. O experimento 4 envolveu cinco doses de torta de filtro: 0; 2.5; 5; 10 e 15 g MS kg^{-1} aplicados no volume total do solo ou no sulco de plantio. A cultivar RB96-6928 mostrou características de melhor adaptação sob baixa disponibilidade de P, com destacado desenvolvimento radicular e boa absorção de P. A dose 38.5 mg P kg^{-1} foi a mais apropriada para promover um adequado desenvolvimento das mudas. A torta de filtro foi eficiente para aumentar a absorção de P e a interação entre torta de filtro e fosfato natural foi eficiente para incrementar fatores de produção como o número de perfilhos por planta. A maior absorção de P se deu quando a torta de filtro foi aplicada no sulco de plantio na dose de 10 g MS kg^{-1}.

Palavras-chave: *Saccharum* spp.; Fosfato; Modificações na rizosfera de cana-de-açúcar; Sistema radicular

ABSTRACT

Phosphorus dynamics in the rhizosphere of sugarcane under phosphate sources and filter cake

In tropical soils, phosphorus (P) is adsorbed onto the oxide surface reducing the availability to plants. Thus, the solubility of the P sources and the particular plant characteristics which increases the absorption efficiency becomes important in the rhizosphere where roots and soil microorganisms interacts together. The objectives of this research were to evaluate sugarcane cultivars in relation to changes in the rhizosphere and the efficiency of P absorption and to evaluate the dynamics of P in the rhizosphere of sugarcane under the application of filter cake combined with mineral fertilizers. Four experiments were conducted in a greenhouse. The soil was a sandy clay loam Ferralsol from Piracicaba - SP, with low soil P, arranged in a randomized block design with four replications for each experiment. The experiment 1 was conducted in a 5x2 factorial arrangement with four sugarcane cultivars: RB92-579; RB85-5156; RB86-7515; RB86-6928 or no plants subjected to presence or absence of phosphate fertilizer (78.4 mg P kg^{-1}) and soil adhering to the root was considered rhizosphere. The experiment 2 evaluated five doses of phosphate fertilizers: 0; 9.8; 19.6; 39.2 and 78.4 mg P kg^{-1} soil with the most promising cultivar for P absorption from experiment 1, and the rhizosphere was collected in five distances from the rhizoplane (mm): i) 0-2; ii) 2-4; iii) 4-6; iv) 6-8; v) 8-10. Experiment 3 was conducted in a 2x3 factorial: absence or presence of filter cake (5 g DM kg^{-1}) with rock phosphate, soluble phosphate (78.4 mg soluble P kg^{-1} soil) or phosphate absence, and the rhizosphere soil was sampled 2 mm from the rhizoplane. Experiment 4 involved five filter cake rates: 0; 2.5; 5; 10 and 15 g DM kg^{-1} applied to the bulk soil or in the planting furrow. The cultivar RB96-6928 showed better adaptation characteristics under low availability of phosphorus, with good root development and P uptake. The rate 38.5 mg P kg^{-1} was the most suitable to promote optimal seedling development. The filter cake was efficient to increase P uptake and the interaction between filter cake and rock phosphate was efficient to increase production factors as the number of tillers per plant. The highest P uptake occurred when filter cake was applied to planting furrow at a rate of 10 g DM kg^{-1}.

Keywords: *Saccharum* spp.; Phosphate; Changes in the sugarcane rhizosphere; Root system

1 INTRODUCTION

"Decepar a cana
Recolher a garapa da cana
Roubar da cana a doçura do mel
Se lambuzar de mel

Afagar a terra
Conhecer os desejos da terra
Cio da terra, a propícia estação
E fecundar o chão"

Chico Buarque

Brazil is the largest sugarcane producer in the world. The cultivated area in the crop season 2015/16 is expected to be 8.95 million hectares with a production of 655.16 million tons of cane, increasing by 3.2% compared to the previous season. Sao Paulo State is expected to be the largest Brazilian producer, with 51.8% of the cultivated area, equivalent to 4.7 million hectares (COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2015).

In order to maintain high sugarcane productivity it is necessary that fertilizer inputs meet the nutritional demands of the crop, supplying the extra amounts needed over the soil supply. Among the macronutrients, phosphorus plays a key role as in plant structural compounds, such as, nucleic acids, phospholipids and also high energy compounds, as adenosine triphosphate (ATP).

In highly weathered tropical soils, phosphorus is strongly adsorbed onto the surfaces of sesquioxides, reducing its plant availability. Fertilizer supplementation is therefore necessary for adequate crop nutrition. Thus, plant and microbial activity in the rhizosphere is important to increase soil P availability and improve plant P uptake.

This issue has been discussed in recent national and international meetings of Soil Science converging that rhizosphere has been appointed as the new study frontier in nutrient dynamics and this has awakened great interest in the academic community. Locally, after six decades of studies on the basic questions of crop response to fertilizer application and dosage, nowadays soil fertility has achieved a new research level, where the studies are in the sense of refinement and adjustment of previously generated information.

In this context, enhancing the quality of fertilizer recommendations through the understanding of the dynamics of nutrients in the soil can help answer basic questions and provide useful technology for farmers.

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2 LITERATURE REVIEW

2.1 Scenario of Sugarcane

Sugarcane (*Saccharum* spp.) belongs to the family Poaceae and is a crop adapted to tropical climate regions. Brazil is the largest producer of sugarcane, followed by India and China (FAO, 2013), expanding in the last few years, mainly in the Southeast and Midwest of the country. The sugarcane cultivated area was 9 million hectares and the production of 634.8 million tons in the crop season 2014/15, with a yield average of 70.5 tons per hectare. Sao Paulo state is the largest Brazilian producer, with 52% of the cultivated area, equivalent to 4.7 million hectares and production of 379 million tons (CONAB, 2015).

Sugarcane represents a major supplier of raw materials for the food industry (sugar), paper and pulp (bagasse), transportation fuel (alcohol), power generation (bagasse burning), animal feed (fodder) and residual fertilizers for agriculture (vinasse and filter cake) (SOLOMON, 2011).

2.2 Breeding plant of Sugarcane

Brazil has a great network of sugarcane breeding programs, being formed by IAC (Agronomic Institute of Campinas), CTC (Sugarcane Research Center) and Ridesa-RB (Inter-University Network for the Development of the Sugar-Energy Industry - Brazil Republic). Thus, there is a huge number of sugarcane cultivars, which allows the farmers to choose the most adapted cultivar for specific soil conditions, harvest timing and agronomic management. In addition, the selection of cultivars is separated regionally and has some particular characteristics (DINARDO-MIRANDA; VASCONCELLOS; ANDRADE-LANDELL, 2008). The RB's varieties, developed by a network of ten federal universities, accounted for 69% of the cultivated area in the current crop season 2014/15, according to a data compilation by Sugarcane Breeding Program (PMGCA), and Ridesa considers RB92-579, RB86-5156, RB86-7515 as the best cultivars of their breeding program.

Each cultivar has a specific optimal environment, which is defined by the physical, hydric, morphological, chemical and mineralogical soil conditions, under adequate management, associated to the local climate (humidity, precipitation, temperature, solar radiation, evapotranspiration) (DINARDO-MIRANDA; VASCONCELLOS; ANDRADE-LANDELL, 2008). The classification of production environments follows: A; B; C; D and E.

The environments classified as A are in high soil fertility, high soil available water and high cation exchange capacity (CEC). The environments B; C; D and E are sequentially reducing these parameters, with the last one characterized by low fertility, extremely low available water; and low CEC.

2.2.1 Cultivar RB92-579

In northeastern of Brazil, approximately one million hectares is covered by sugarcane, which is the most important socio-economic crop in this region. Alagoas, Pernambuco and Paraíba are the main producer states. In these states, the rainfall distribution is concentrated between March and August, with lower light period, lower temperatures and longer nights. From September to February water stress occurs, with greater sunshine period, higher temperatures and long days. These factors have promoted less plant photosynthesis and reflect historically to lower sugarcane agricultural productivity (below 60 t ha^{-1}) compared to the central-southern region of the country. Thus, the most limiting factor for sugarcane cultivation in this region is climate irregularity. As consequence, there are some improvements in agricultural development in this region, as the use of drought-tolerant sugarcane cultivars.

In 2003, Federal University of Alagoas released the cultivar RB92-579. This cultivar is tolerant to drought and responsive to irrigation, presents high tillering per plant, covering entirely the crop field, and has high nutrient use efficiency. It is adapted to the production environments C and D. In the season 2008/09, this cultivar was used in 20% of the harvested sugarcane area in the northeastern region of Brazil, and accounted for more than 25% of sugar production, showing thus high efficiency in land use. Furthermore, in the south central region, cultivar RB92-579 has increased the planted area.

2.2.2 Cultivar RB86-5156

Increased industrial productivity via better quality of raw material is one of the most important goals of breeding programs of sugarcane. Therefore, the maturation precocity is a persecuted feature as it is harvested in the initial period with the greatest shortage of raw material and high sugar concentration. As a consequence of this characteristic and especially its precocity, cultivar RB85-5156 is currently the main variety for early harvest.

However, this cultivar may present flowering and low germination, a reason why it is suggested to plant it only in good environmental conditions. It is adequate to be used in the production environments A, B, C and D.

According to the Varietal Census conducted in 2009 by PMGCA - UFSCar, with data from 132 units in São Paulo, Mato Grosso and Mato Grosso do Sul, the cultivar RB85-5156 ranked seventh in acreage, accounting for 4.2% of the planted area. Considering only new areas, RB85-5156 is placed fourth with a share of 6.8%, in this way the contribution of RB85-5156 towards the Brazilian sugarcane industry is expected to further increase.

2.2.3 Cultivar RB86-7515

The RB86-7515 was officially released as a commercial variety in March 1997, by the Federal University of Viçosa, with an alliance that enabled the Ridesa, which facilitated the advancement the crops to low fertility soils, most of them sandy soils with water restrictions over the year. This cultivar normally presents the highest cane productivity, performing better in sandy soils of medium fertility and its adequate production environment is B, C and D.

2.2.4 Cultivar RB96-6928

Aiming to expand the harvest timing for the Paraná state, Federal University of Parana UFPR released the new cultivar RB96-6928 with early maturity cycle launching. The main feature of this cultivar is earliness emphasized together with high productivity. The cultivar RB96-6928 is relatively new in the breeding program, being a rustic and stable cultivar, more resistant to adverse conditions than Cultivar RB92-579 and Cultivar RB86-5156, with a capacity to produce in poor soil fertility. It has great ratoon development characteristics and has favorable performance for mechanic harvest. It is normally used in the production environments A, B, C and D.

2.3 Sugarcane response to phosphorus (P)

Although found in lesser amounts in sugarcane stalks compared to N and K, P plays a key role in plant metabolism, particularly in cell division process, photosynthesis, plant tillering, root and internode development and sucrose formation (KORNDÖRFER, 2004). It is a macronutrient present in structural compounds in the plant, as nucleic acids, phospholipids

of cell membrane and also in compounds of high energy molecules, as adenosine triphosphate ATP (MARSCHNER, 2012). Besides, P acts in enzymes regulation, phosphorylation and dephosphorylation (HAMMOND; WHITE, 2008), the highest action of this element is in plant activity centers, as growth and carbon assimilation tissue regions.

Consequently, P contributes to higher crop yields. Thus, one of the limiting factors for growth and crop productivity is P availability, mainly in tropical and weathered clayey soils, where this nutrient is removed from the soil solution into the surfaces of Fe and Al oxides to form compounds of low availability for plants (PARFITT, 1978).

The use of phosphate fertilizers is necessary to supplement the deficiency of available P in the soil. Fertilizer application must be based on the desired level of productivity and the recommended rate depends on the soil nutrient stocks and the amount exported by the crop, taking into account the loss into the soil (DEMATTÊ, 2005). Although the crop requires lower quantities of P, high doses are used, on the order of 90 mg kg^{-1} of P_2O_5, since the soil competes to the plant for the fertilizer applied (NOVAIS; SMYTH, 1999). Thus, the average plant P absorption during the crop cycle from the fertilizer applied is about 20%, in the year of application (RAGHOTHAMA; KARTHIKEYAN, 2005), resulting in a long term residual effect of P from mineral fertilizers (CIAMPITTI et al., 2011).

2.4 Phosphate fertilizers

2.4.1 Soluble P fertilizers

For providing the recommended sugarcane P amendments it is common to use high solubility mineral fertilizers because of their greater agronomic efficiency. Triple superphosphate (TSP) became widely used and technically is known as calcium dihydrogen phosphate and monocalcium phosphate [$Ca(H_2PO_4)_2.H_2O$] (SAMPLE; SOPER; RACZ, 1980).

The TSP production for both non-granular and granular forms follows: Non-granular TSP is commonly produced by reacting finely ground rock phosphate with liquid phosphoric acid in a cone-type mixer. Granular TSP is made similarly, but the resulting slurry is sprayed as a coating onto small particles to build granules of the desired size. The product from both production methods is allowed to cure for several weeks as the chemical reactions are slowly completed. The chemistry and process of the reaction will vary somewhat depending on the properties of the rock phosphate.

However, when TSP is applied into the soil, the fast release of P can promote adsorption and precipitation of insoluble forms onto the components of the soil, forming low-labile compounds, decreasing its availability for plants (RANDHAWA et al., 2006).

2.4.2 Rock phosphate

Rock phosphate (RP) is a general term that describes naturally occurring mineral assemblages containing a high concentration of phosphate. The higher the phosphate (P_2O_5) content as apatite, the greater the economic potential of the rock. The term refers to both non beneficiated phosphate ores and concentrated products.

There are igneous and sedimentary ores. Igneous deposits have provided about 10-20% of world production in the last ten years. They are exploited in the Russian Federation, Canada, South Africa, Brazil, Finland and Zimbabwe but also occur in Uganda, Malawi, Sri Lanka and several other locations. These deposits usually contain fluorapatite that is relatively unreactive and is the least suitable for direct application. Sedimentary deposits occur in formations of widely varying geological ages, exhibit a range of chemical compositions and physical forms. The deposits that account for most of world RP production are in Morocco and other African countries, the United States of America, the Middle East and China. Most sedimentary deposits contain the carbonate-fluorapatite called francolite, which has high carbonate/phosphate substitution and are highly reactive and suitable for direct application as fertilizers or soil amendments.

Gafsa belongs to the francolite group. This rock phosphate, of high reactivity, has equivalent efficiency to high solubility sources when incorporated into the soil (CORRÊA et al., 2005). Its efficiency has being linked to the degree of match isomorphic substitution by other ions in the crystal lattice (HOROWITZ; MEURER, 2003). Gafsa is characterized by a chemical substitution that occurs naturally within the crystal structure of the phosphorite where some of carbonate groups (CO_3) have replaced a phosphate group (PO_4), which tends to make the RP more reactive when directly applied to the soil. According to standard solubility tests for reactivity assessment and various agronomic evaluations, Gafsa is classified by the international scientific community as one of the most reactive and most efficient rock phosphate fertilizer in the world.

However, natural rock phosphates have lower solubility compared to TSP, and for this reason, may not supply in the adequate levels for plants, because of its low initial dissolution rate, although increasing the residual effect over time (SHIGAKI; SHARPLEY, 2011).

2.5 Filter cake

Filter cake is a sugar mill byproduct, being an alternative fertilizer source for sugarcane, which can reduce the cost of mineral fertilizers. It comes from the broth clarification process. The sludge from decanters is filtered in a vacuum rotary filter, remaining the filter cake.

Some years ago (decades of 60's-70's), filter cake was applied in total area at cane planting or between rows of the ratoons. Nowadays, normally, the filter cake is applied: i) at planting in total area, with incorporation; ii) in ratoon, over the rows without incorporation; iii) at planting in the furrow; and iv) composting of filter cake.

The main benefits of filter cake as a source of organic matter are: the increase of CEC, helps in the water retention and is nutrient a source for P, Ca, N, Fe and even Mg and K in low amounts. In general, it is considered that 50% of the P from filter cake can be readily available to sugarcane (DINARDO-MIRANDA; VASCONCELLOS; ANDRADE-LANDELL, 2008).

During the mineralization of the filter cake in the soil, microorganisms release chelating and complexing compounds that may reduce the fixation of P in the soil and also may produce growth promoter substances. Despite the research evidence, the capacity of the filter cake to reduce the soil P adsorption is due to the release of low chain organic compounds. However, it is necessary more research relating the association of filter cake with mineral P sources to understand the influence of this management in improving soil fertility.

2.6 Strategies of the plants to uptake P from the soil

Total P content in the soil is around 0.2 to 5.0 g kg^{-1}, however, only a very small fraction of this is available for plants. The diffusion coefficient of P is very low (10^{-12} to 10^{-15} m^2 s^{-1}) and for this reason rapid plant P uptake creates a depletion zone around the roots and the ions diffuses in a negative chemical potential gradient towards the root surface (RAUSCH; BUCHER, 2002). After few days of absorption, P concentration in the rhizosphere can be reduced around 30-50% and the depletion zone extend until 2 mm from the root surface (JUNGK, 1987).

Furthermore, plant P acquisition is against a concentration gradient through a plasmatic membrane, because P concentration into plant cells in general are 1000 times (mmol L^{-1}) higher than in the soil solution (µmol L^{-1}) (RAGHOTHAMA, 2000). The energy source to transport it comes from the proton extrusion pump through the plasmalemma, where ATP_{ASE}

transports the H^+ out to the cells, generating both difference of electrical potential and difference of pH (GLASS, 1990). The rate of absorption is higher between pH 4.5 and 6.0, where the $H_2PO_4^-$ is predominant, indicating that this is the preferential form of P uptake (SENTENAC; GRIGNON, 1985).

When rock phosphate (RP) is applied, plants and microorganisms can effectively extract and/or solubilize P from this source in the rhizosphere. Thus, RP fertilizer can become a viable alternative for providing P to the plants (ARCAND; SCHNEIDER, 2006). This increment in solubility is associated with numerous changes occurring in the rhizosphere, especially the reduction of soil pH (JHA; SAXENA; SHARMA, 2013), absorption of Ca and P (PEREZ; SMYTH; ISRAEL, 2007) and root exudation of organic acids (LI et al., 2008).

When soil available P is lower than plant demand, the exploitation of a large volume of soil by the root system becomes important (NEUMANN; MARTINOIA, 2002), because this plant organs are capable to absorb more P through the root interception mechanism compared to the diffusion mechanism (MARTINEZ et al., 1993). Therefore, absorption of P by the roots is determined, among many factors, by the morphology of the root system (MACHADO; FURLANI, 2004), which are represented by length, volume, area and radius of the root and root hairs.

Additionally, in situations where the availability of inorganic P (Pi) is low in the soil, plants can use the organic P (Po). For this, it must be previously mineralized by phosphatase enzymes. The roots can induce high acid phosphatase activity in the rhizosphere, and thus increase the availability of P for plants (KANDELER et al., 2002; GAHOONIA; NIELSEN, 2004).

Microbial and plants metabolites can accumulate in the rhizosphere zone, including growth regulators, phytotoxins, soil stabilizers and antibiotics, and may affect microbial activity and the composition of the microflora in the rhizosphere, as well as the activity of the roots. Tawaraya et al. (2013), studying the composition of the rice plants exudates, observed a greater accumulation of metabolites under P deficiency compared to high P availability, suggesting that the release of many metabolites are a response to P deficiency.

Thus, studies of rhizosphere changes under P deficiency conditions are important to understand the interaction between plant and soil microorganisms, identifying specific compounds that might affect the solubilization of phosphates in the roots region. In this way, the soil fertility analysis and the interaction between plants, microorganisms and soil components can help us to understand the relationship between rhizosphere and alter into a

more efficient fertilizer recommendation, especially under the conditions operating in tropical soils of low P availability.

2.7 Rhizosphere

The term rhizosphere (from Greek), meaning the influence of a root on its surrounding, indicating the zone of soil where roots released from plants can stimulate, inhibit, or have no effect on soil microorganisms (PINTON; VARANINI; NANNIPIERI, 2001). The two areas, root and soil, are separated by the root surface, called rhizoplane.

The root growth uses the pre-existing macropores and explores between 1 and 3% of the soil, very little of the total volume. Thus, classically the soil analyzed does not correspond exactly to the ground which is exploited by roots, does not corresponding to the amount of nutrients potentially absorbed by plants and does not detect changes in the availability in the soil over the time.

The rhizosphere is where the roots and soil microorganisms interact intensely in chemical, physical and biological ways, including plant physiology and soil microbiology. The number of microorganisms around the root is much higher than in the bulk soil and the type of microorganisms in the rhizosphere also differ from the bulk soil. In this particular environment, exchange of energy, nutrients, and molecular signals take place, rendering the chemistry, biochemistry, and biology of this environment from the bulk soil (PINTON; VARANINI; NANNIPIERI, 2001).

2.8 Hypothesis

Root system improvement and root exudation, as particular characteristics of different sugarcane cultivars, would increase the soil exploration, alter the rhizosphere and hence enhance P uptake from the soil under low P availability conditions.

Filter cake improves the soil chemical and physical properties, what would promote changes in the rhizosphere and hence enhance P availability to the plants, especially when associated with mineral P fertilizers of low solubility.

2.9 General Objective

To assess P dynamics in the rhizosphere of four sugarcane cultivars under application of varied solubility sources in the presence or absence of filter cake.

2.10 Specific objectives

To evaluate the morphological root system of different sugarcane cultivars and the changes promoted by sugarcane roots and microbiological community in the rhizosphere, to assess the sugarcane efficiency to uptake P from the soil under low and high P conditions, and also determine the ideal rate for the initial development of the seedling.

To evaluate the filter cake associated to mineral phosphate fertilizer in improving the soil P availability by sugarcane and microbial community in the rhizosphere and to determine the filter cake rate for an adequate sugarcane seedling development.

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3 BIOLOGICAL AND MORPHOLOGICAL ROOT STRATEGIES OF SUGARCANE TO IMPROVE PHOSPHORUS UPTAKE IN THE RHIZOSPHERE

Abstract

Our study aimed to evaluate the dynamics of different cultivars of sugarcane in terms of morphological root and the microbiological community changes promoted in the rhizosphere and the sugarcane efficiency to uptake P from the soil/fertilizer under low and high P conditions, and also determine the best rate for an adequate initial seedling development. Two experiments were established in a greenhouse using a sandy clay loam Ferralsol in a randomized block design with four replications and harvested 45 days after seedlings planting. Experiment 1 was composed of sugarcane cultivars: RB92-579; RB85-5156; RB86-7515 and RB96-6928 without and with phosphate application (triple superphosphate – TSP at rate of 78.4 mg P kg^{-1}). At harvest, soil adhered to the root system was sampled as rhizosphere. Root system was quantified and microbial activity around the root system was assessed. And also the shoot dry matter and inorganic labile P fractions in the rhizosphere were evaluated. Experiment 2 evaluated P rates: 0; 9.8; 19.6; 39.2 and 78.4 mg kg^{-1}, applied in the most profitable cultivar for soil P exploitation from experiment 1. It was used a horizontal mesh to isolate the soil from root (rhizosphere). At harvest, soil over the mesh was sliced in 5 distances from the rhizoplane: 1; 3; 5; 7; and 9 mm. Cultivar RB96-6928 achieved the highest tillering snd area and volume of roots by producing more root dry matter compared to other cultivars, being a promising one under low P soil situations because of its higher soil volume exploration. However the microbial community was not changed by sugarcane cultivars. Under greenhouse pot evaluation, the rate of 38.5 mg P kg^{-1} promoted an adequate seedling development.

Keywords: *Saccharum* spp.; Phosphate efficiency; Plant root changes; Sugarcane rhizosphere modifications

3.1 Introduction

Brazil is the largest sugarcane producer in the world, followed by India and China (FAO, 2013), producing in the crop season 2014-2015 around 635 Mi tons in an area of 9.0 Mi ha (CONAB, 2015).

Phosphorus (P) plays as a key role in plant metabolism, particularly in the formation of proteins in cell division and photosynthesis and also is important for tillering, root development and formation of sucrose in sugarcane (KORNDÖRFER, 2004). Thus, P deficiency is one of the limiting factors for the growth and yield, mainly in tropical soils, where P is removed from the soil solution by precipitation with Fe or Al or adsorption on the surfaces of Fe and Al sesquioxides, forming compounds of low availability to plants, reducing the initial development of root system and compromising the absorption of other nutrients

(KHAN et al., 2010). For this reason, the efficiency of phosphate fertilizers applied at planting is about 20% in tropical soils (RAGHOTHAMA; KARTHIKEYAN, 2005).

The dynamics of phosphorus in the rhizosphere is determined by the interaction between plant, soil and microorganisms. Therefore, P uptake by roots is controlled by the spatial availability, bioavailability and P acquisition, among many factors (Figure 1) (SHEN et al., 2011). Some plant genetic characteristics as morphology of the root system (MACHADO; FURLANI, 2004) allow a better soil volume exploitation by the root system (NEUMANN; MARTINOIA, 2002) as well as the affinity to promotes mycorrhizal symbiosis. Also, particular characteristic as exudation of organic compounds, enzymes, acid phosphatases, become important plant tools in the rhizosphere to solubilize P for adequate plant nutrition.

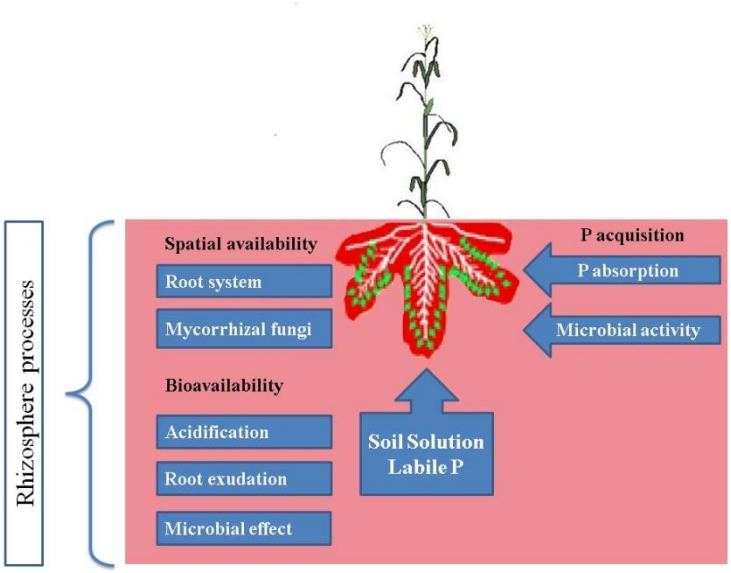

Figure 1 - Dynamics of phosphorus in the rhizosphere by the interaction between plant/soil/microorganisms. Adapted from Shen et al. (2011)

Brazil has a great network of sugarcane breeding programs, thus, there is a great number of sugarcane varieties or cultivars, which allows to the farmers find out the most adapted cultivar for specifics soil conditions, harvest timing and agronomic management.

Based on the necessity to improve the efficiency of sugarcane for P uptake from the soil/fertilizer, our study aimed to evaluate the dynamics of different cultivars of sugarcane in terms of morphological root and microbiological community changes promoted in the rhizosphere and the sugarcane efficiency to uptake P from the soil/fertilizer under low and high P conditions, and also determine the rate for an adequate initial development of the seedling.

3.2 Material and Methods

The study was carried on at "Luiz de Queiroz" College of Agriculture – University of Sao Paulo (ESALQ- USP), in Piracicaba-SP, Brazil, using a soil collected from the 0-20 cm layer of a grassland area. The chemical and textural composition and P fractions of the soil are presented in the Table 1. The soil is classified as sandy clay loam Ferralsol (FAO, 2013), with low levels of available nutrients (Ca, Mg, K and P_{AER}). Prior to experiments the soil was air-dried and sieved on 2 mm mesh, remoistened with deionized water until 80% of field capacity, and incubated for 15 days with lime to achieve a base saturation of 70% (RAIJ et al., 1997). Supplementation of nutrients followed the recommendation proposed by Raij et al. (1997) and was made using KCl (50 mg K kg^{-1} soil) for potassium and urea (30 mg N kg^{-1} soil) for nitrogen. Two nitrogen rates were also applied during plant growth (15 mg N kg^{-1} soil), at 15 and 30 days after transplanting.

This study involves two experiments conducted in a greenhouse using a randomized block design with four replications. One was run to evaluate the capacity of the most cultivated cultivars of sugarcane in Brazil to adapt to soil P low availability and another was run to evaluate the most profitable phosphate rate, as TSP, for initial growth of sugarcane, using the most suitable cultivar selected in the previous experiment.

Table 1 - Chemical and textural analysis of a sandy clay loam Ferralsol collected from the 0-20 cm layer of a grassland area used for experiments

pH	K	Ca	Mg	EB	CEC	V	OM	Clay	Silt	Sand	P_{AER}*	Pi_{NaHCO3}
$_{CaCl2}$	----------$mmol_c$ dm^{-3}---------					-----%-----		---------g kg^{-1}-------			------mg kg^{-1}------	
4.3	1.4	8	5	14.4	45.4	31.7	2.2	201	11	788	5.4	2.8

EB: exchangeable base-forming; CEC: Cation exchange capacity; V: Base saturation; OM: organic matter.
*P_{AER}: P extracted by anion exchange resin; $Pi_{NaHCO3:}$ P extracted by sodium bicarbonate (HEDLEY; STEWART; CHAUHAN, 1982)

3.2.1 Plant growth for root system and rhizosphere analysis

This experiment was run with four sugarcane cultivars currently used in Brazil: RB92-579; RB85-5156; RB86-7515 and RB96-6928. Cultivar RB86-5156 is an early and stable cultivar, however may presents flowering and low germination, a reason why it is suggested to plant this cultivar only in good environmental conditions; Cultivar RB96-6928 presents an earlier growth cycle as well, however is a rustic and stable cultivar, with capacity to produce in poor soil fertility. Cultivar RB86-7515 normally presents the high cane productivity and as

RB96-6928 is adapted to poor soil fertility conditions. Cultivar RB92-579 is drought resistant and responsive to irrigation, presents high tillering per plant and has high nutrient use efficiency;

Following the current recommendation to Sao Paulo States (RAIJ et al, 1997), based on the soil analysis (Table 1), the rate would be 39.2 mg P kg^{-1}. For that reason, The treatments were without (control) and with phosphate fertilizer at rate of 78.4 mg kg^{-1} of soluble P to provide two times the plant demand as triple superphosphate (TSP) mixed uniformly with soil prior to planting.

Pots consisted of PVC tubes (0.15 m diameter and 0.45 m tall) with a removable plastic bag filled with soil and perforated at the base (Figure 2). Pots were packed with soil at a density at a density of ~1.2 kg dm^{-3} that similarly to that at the field where it was collected.

Sugarcane seedlings were germinated in sterile vermiculite for 22 days and then transplanted to the pots. Prior to harvest at 45 days after transplanting, plant height and number of tillers per plant were measured. Plant height was measured from the ground to the auricular region near the collar leaf (+1) which is present as the first fully visible collar (DILLEWIJN, 1952). Plant and soil were carefully removed from the PVC tube together for later separation of shoot, root and rhizosphere and non rhizosphere soil samples.

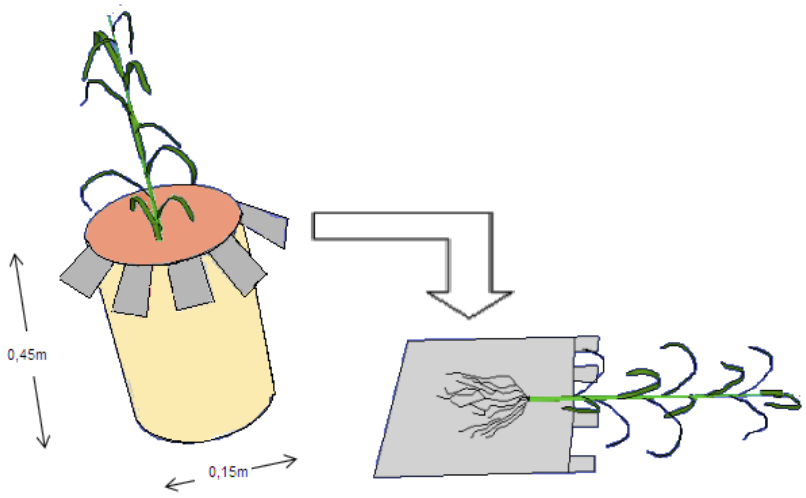

Figure 2 - Pots for sugarcane cultivars experiment at greenhouse without and with phosphate application at rate of 78.4 mg kg^{-1} of soluble P

Shoot was dried in an oven at 60°C until constant mass, weighed and milled though a 1 mm sieve. P content in the shoot was determined by vanadate-yellow color method, as described by Malavolta, Vitti and Oliveira (1997).

Soil that adhered to the root system after carefully hand agitation was considered as rhizosphere soil. Samples were taken and stored on ice immediately after sampling, a subsample was kept at 4°C for analysis of acid phosphatase activity (AP_{ASE}) and microbial biomass phosphorus (P_{MIC}). The remainder was stored at -80ºC freeze for community analysis by Denaturing Gradient Gel Electrophoresis (DGGE) analysis. Bulk soil was sampled from a set of unplanted controls plots. Activity of AP_{ASE} was determined using the methodology described by Tabatabai (1994). The P_{MIC} was estimated using the fumigation-extraction procedure as outlined by Brookes et al. (1985) and Vance, Brookes and Jenkinson (1987).

The determination of bacterial community structure was based on DGGE analysis. Total DNA was extracted from rhizosphere using the PowerSoil DNA isolation kit (MoBio, Carlsbad, EUA) according to the manufacturer instructions. Quality of extracted DNA was assessed in a 1% (w/v) agarose gel, followed by staining with ethidium bromide and photo documentation of ultra-violet light (transluminator, Storm 845 - GE Healthcare Life Sciences, Piscataway, NJ, USA). PCR amplification of the V6 region of the ribosomal gene 16S rDNA was subsequently performed with forward primer F27 (5'AGA GTT TGA TCM TGG CTC AG 3') and reverse primer R1387 (5’ CGG TGT GTA CAA GGC CCG GGA ACG 3′) using 2.50 µL 1X PCR buffer, 0.20 mM of each dNTP (2.0 µL), 3.75 mM $MgCl_2$ (3.75 µL), 0.20 pmol/µL of each oligonucleotide (0.05 µL), 0.05 U/µL-Taq DNA Polymerase (Fermentas, Burlington, Canada) (0.25 µL), 1 µL of DNA sample and sterile ultrapure water (Milli-Q) to a final volume of 25 µL. The PCR was performed in Veriti® thermal cycler (Applied Biosystems, Waltham, USA) using an initial denaturation step at 94 °C for 4 min, followed by 35 cycles of denaturation 94°C for 30 min, 63 °C for 1 min, extension at 72 °C for 1 min and a final extension step at 72 °C for 10 min. A nested PCR was then used to attach the GC clamp, which was performed by using 1 uL of the first reaction as template with forward primer F968/GC (5' AAC GCG AAG AAC CTT AC 3') and R1378 (5’ CGG TGT GTA CAA GGC CCG GGA ACG 3′). Reaction were in a final volume of 50 µL, containing 1X Taq Buffer, 2.50 mM $MgCl_2$, 0,20 mM of each dNTP, 0,4 mM of each primer, 80% formamide and 0.05 U/µL Taq DNA Polymerase (Fermentas, Burlington, Canada). The amplifications reaction was performed in the following conditions: denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, and elongation at 72 °C for 2 min. At the end of 30 cycles (94 °C for 4 min), the final extension step was at 72 °C for 10 min. DGGE was performed using the phorU2 systems (Ingeny International, Goes, The Netherlands). The PCR products were loaded onto 6 % (w/v) polyacrylamide gels with denaturing gradients of 45 to 65 % (urea 7M and formamide 40%). The gels were run for 16 h at 100 V and 60 ºC of temperature and

stained with SYBR Green I (Invitrogen, Breda, The Netherlands). DGGE gels were photo documented with Storm 845 (General Electric) and analyzed using the ImageQuant TL unidimensional (Amersham Biosciences, Amersham, UK, v.2003) (McCAIG; GLOVER; PROSSER, 2001), where band patterns were converted in matrices of presence/absence of bands.

At sampling, the root systems were carefully divided into two equal parts (I and II), based on pot volume. For part I: all the roots were collected by washing on a 2 mm mesh and stored in 70% ethanol. Root length, volume and surface area was then determined by scanning and analysis using WinRhizo software. 50% of the roots were then dried at 60 °C to obtain dry matter (estimated for the total roots in the pot). For part II: samples were similarly washed and stored in ethanol for analysis of percentage of mycorrhizal colonization (MC) and effective a determination of tissue P content. For MC, the roots were prepared according to Vierheilig et al. (1998) and dispersed in a petri dish with checkered background and scored using a stereomicroscope according to Giovannetti and Mosse (1980). P content in the root tissue was measured by the same methodology of shoot part, previously mentioned.

Inorganic labile P fractions were determined on representative 0.5 g subsamples of dried and sieved soil subjected to sequential extractions. Soils were first extracted by anion exchange resin (P_{AER}) with 2 cm^2 saturated with $NaHCO_3$ (0.5 mol L^{-1}) then immersed in 10 ml of H_2O with the soil, shaking for 16 hours in an orbital shaker. Inorganic P in the extract was quantified by colorimetric assay using the molybdate blue method (MURPHY; RILEY, 1962). The same soil sample was submitted to the second sequential extraction using 10 ml of $NaHCO_3$ (0.5 mol L^{-1}) and after 16 hour shaking, the extract was quantified by Dick and Tabatabai (1977) methodology.

3.2.2 Plant growth to rhizosphere response analysis to P rates

Experiment 2 assessed P acquisitions and rhizosphere P dynamics of one sugarcane cultivar (RB96-6928) selected from the first experiment as being the most P efficient based on the ratio P plant uptake/surface area of root dry matter and volume of the root. This cultivar was grown in the same soil but with five rates of soluble P (0; 9.8; 19.6; 39.2 and 78.4 mg kg^{-1}).

Plant growth conditions, sampling and processing were similar to experiment 1, with a more detailed sampling being used to collect rhizosphere soil. Rhizosphere and rhizoplane samples were obtained using PVC columns with a nylon mesh (25 microns) for root exclusion

(GAHOONIA; NIELSEN, 1991), whereby an upper PVC column (0.15 m tall) was fitted to a lower column (0.05 m) to provide a soil continuum. The nylon mesh at the base of the upper column allowed water movement and ions exchange between the root and the soil, growth of root hairs and mycorrhizas, but prevented root growth below it. At plant harvest, rhizosphere soil samples were taken from: 1 (0-2); 3 (2-4); 5 (4-6); 7 (6-8) and 9 (8-10) mm from rhizoplane (Figure 3). The rhizosphere was sampled through a screw mechanism which raised the soil above a level that controlled the distance of the sections and then the soil was shaved. The samples were similarly analyzed for immediately P_{MIC} and AP_{ASE}, as outlined for experiment 1, along with soil pH (0.01 M $CaCl_2$ 1:5 w:v) determination.

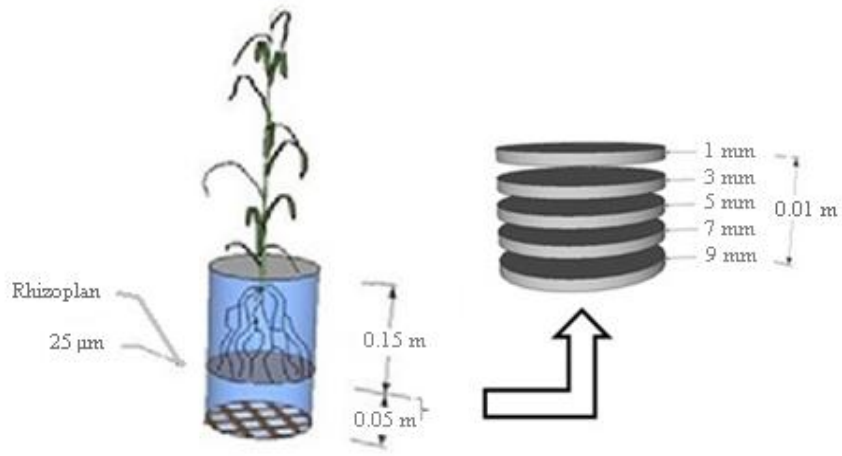

Figure 3 - Pots with sugarcane seedlings developing under P rates experiment at greenhouse detaching the nylon membrane with 0.25µm pore size forming the rhizoplane, isolating the root from the soil below

3.2.3 Statistical analysis

For the sugarcane cultivars (experiment 1) the data were subjected to two-way (cultivars and P application) analysis of variance (ANOVA) and significance (0.05 error probability) determined by F values. Data with significant effect had the means compared paired t test (LSD). When the interactions were significant, the unfolding of the interaction was conducted. For DGGE data, the statistical analyses were run in Past Statistics 1.90 program (HAMMER; HARPER; RYAN, 2001), using the principal coordinate analysis (PCoA) based in the Bray-Curtis algorithm. For the sugarcane response to phosphate (experiment 2) the data were subjected to one-way ANOVA (P rate) and data with significant effect were subjected to adjustment of the most significant regression degree.

3.3 Results

3.3.1 Characterization of root system of four sugarcane cultivars grown under low and high P

The total volume of roots was influenced by both P application and cultivar ($p < 0.05$), but no interaction was observed. The phosphate application (WP) increased the volume of roots compared to NP. The cultivar RB96-6928 exhibited the highest volume of roots with 35% of root mass fraction under NP and 21% under WP (Table 2), although this root mass fraction was not different ($p \geq 0.05$) across cultivars. Mycorrhizal root colonization showed interaction between P application and cultivars ($p < 0.05$). Under WP there was no difference between the cultivars. Under NP the cultivar RB86-7515 showed the lowest mycorrhizal colonization, being similar to the other cultivars under WP. Cultivars RB92-579, RB85-5156 and RB96-6928 all had higher colonization under NP compared to WP (Table 2).

Table 2 - Volume of roots; root mass fraction and mycorrhizal root colonization for four cultivars grown without phosphate application (NP) and with P (WP) supplied at rate of 78.4 mg kg^{-1} of soluble P

Cultivar	Volume of roots cm³						Root mass fraction † ----------%----------						Mycorrhizal colonization* ----------%----------					
	NP		WP		Mean		NP		WP		Mean		NP		WP		Mean	
RB92-579	15.62	ns	38.81		27.21	B	29.02	ns	16.37		22.69	ns	41	Aa	24	Ab	33	
RB85-5156	16.70		42.97		29.84	AB	29.77		14.34		20.99		41	Aa	24	Ab	33	
RB86-7515	18.80		30.95		24.88	B	28.94		13.95		21.45		29	Ba	26	Aa	27	
RB96-6928	35.34		55.91		45.62	A	35.02		21.00		28.01		35	ABa	25	Ab	30	
Mean	21.62	b	42.16	a			30.77	a	16.41	b			36		25			

Different capital letters within the same column show significant differences between cultivars by t test (LSD) ($p < 0.05$). Different small letters within the same row show significant difference between NP and WP by t test (LSD) ($p < 0.05$). ns Not significant by t test (LSD) ($p \geq 0.05$). † Root mass fraction = DM (root) / DM(shoot + root). *Transformed data to arc sen $\sqrt{P\%} / 100$, where P is the percentage of assessed mycorrhizal colonization

3.3.2 Shifts in the rhizosphere in soil P fractions and microbial structure for sugarcane cultivars grown under low and high P

Microbial biomass P (P_{MIC}) in the rhizosphere did not differ significantly across cultivars nor according to P treatment (Table 3). Acid phosphatase activity (AP_{ASE}) by comparison was affected by P treatment and also showed interaction with cultivar ($p < 0.05$). Under both NP and WP conditions there was no difference between cultivars in AP_{ASE}, although all cultivars differed to the no plants control under WP. The rhizosphere soil with cultivar RB96-6928 presented lower P_{ASE} under WP when compared to the other cultivars.

Concentration of labile inorganic P in the rhizosphere, extracted by both resin (P_{AER}) and $NaHCO_3$ (Po_{NaHCO3}) is shown in Table 3. Cleary, when labile P fractions were compared between NP and WP, there was a large difference ($p < 0.05$) for both labile pools (P_{AER} and Pi_{NaHCO3}).

Table 3 - Microbial biomass P (P_{MIC}); acid phosphatase activity (P_{ASE}); Phosphorus extracted by anion exchange resin (P_{AER}); inorganic phosphorus extracted by $NaHCO_3$ (Pi_{NaHCO3}) for four cultivars grown without phosphate application (NP) and with P (WP) supplied at rate of 78.4 mg kg^{-1} of soluble P

Cultivar	P_{MIC} mg kg^{-1}			P_{ASE} mg kg^{-1} h^{-1}			P_{AER} ----------mg kg^{-1}----------			Pi_{NaHCO3} ----------mg kg^{-1}----------		
	NP	WP	Mean	NP	WP	Mean	NP	WP	Mean	NP	WP	Mean
No plant	1.83	0.78	1.30 ^{ns}	220 Aa	218 Ba	217 ^{ns}	2.2 ^{ns}	17.6	9.9	4.9	26.8	15.9
RB92-579	1.41	1.38	1.40	170 Ab	281 Aa	227	2.9	16.6	9.2	5.1	23.2	14.1
RB85-5156	1.32	1.67	1.49	217 Ab	284 Aa	250	2.3	16.5	9.4	4.8	20.0	12.4
RB86-7515	1.08	1.41	1.24	179 Ab	274 Aa	227	1.7	15.5	8.6	4.7	21.0	12.9
RB96-6928	1.04	1.33	1.19	188 Ab	245 ABa	221	2.2	16.7	9.5	4.7	22.9	13.8
Mean	1.33 ^{ns}	1.32		195	260		2.1 b	16.6 a		4.8 b	22.8 a	

Different capital letters within the same column show significant differences between cultivars by t test (LSD) ($p < 0.05$). Different small letters within the same row show significant difference between NP and WP by t test (LSD) ($p < 0.05$). ^{ns} Not significant by t test (LSD) ($p \geq 0.05$)

The microbiological community of organisms in the rhizosphere soil was changed by phosphate application, detaching two big groups (Figure 4). The cultivars are pretty much similar in the microbiological community of organisms in the soil, being the groups closer each other, only detaching from the soil without plant (no plant) in both conditions, NP and WP. Under NP the microbiological groups are much closer to each other and under WP the groups are more disperse, for all cultivars.

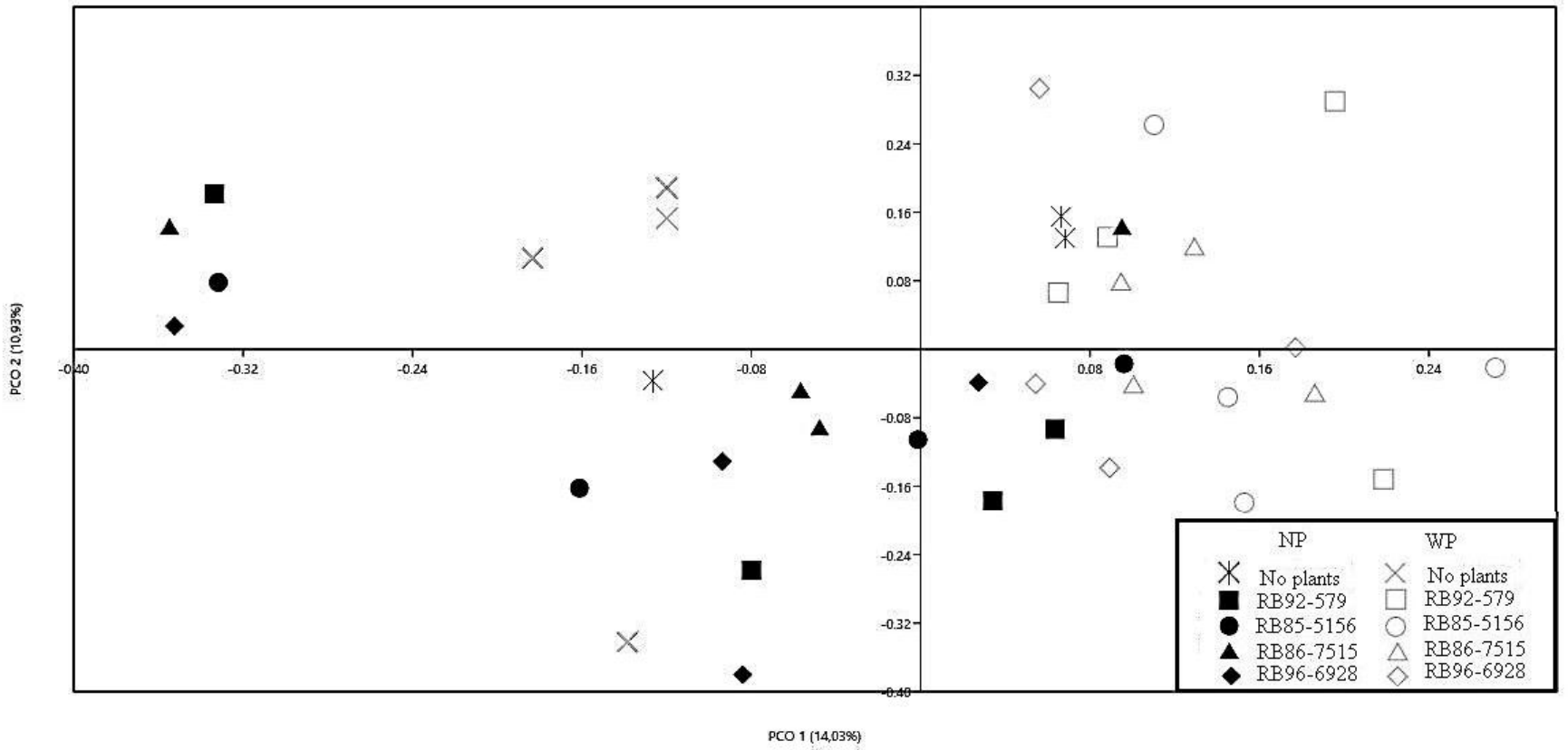

Figure 4 - Principal coordinate analyses (PCoA) based on DGGE for no phosphate application (NP); and phosphate application (WP) at rate of 78.4 mg kg^{-1} of soluble P and rhizosphere soil of four sugarcane cultivars: RB92-579; RB85-5156; RB86-7515; and RB96-6928 and a bulk soil under NP and WP

Both phosphate application (P) and cultivar (G) affected plant height ($p < 0.05$) with no significant interaction. Cultivar RB85-5156 had the highest average plant height compared to the other cultivars. The application of phosphate (WP) significantly increased plant height compared to no application (NP) (Table 4). The number of tillers per plant showed interaction between P application and RB92-579 presented only 0.25 tillers per plant in NP, whereas all 3 cultivars produced significant more tillers under WP. In contrast, cultivar RB96-6928 had the same number of tillers both in the absence (NP) and presence of P fertilizer (WP).

Table 4 - Sugarcane plant height and number of tillers per plant for four cultivars grown without phosphate application (NP) and with P (WP) supplied at rate of 78.4 mg kg^{-1} of soluble P

Cultivar	Plant height (mm)			Number of tillers per plant		
	NP	WP	Mean	NP	WP	Mean
RB92-579	206 ^{ns}	504	355 B	0.25 Bb	4.75 Aa	2.50
RB85-5156	236	591	414 A	0.00 Bb	2.25 Ba	1.13
RB86-7515	227	487	357 B	0.00 Bb	2.25 Ba	1.13
RB96-6928	220	480	350 B	3.75 Aa	3.75 ABa	3.75
Mean	222 b	516 a		1.00	3.25	

Different capital letters within the same column show significant differences between cultivars by t test (LSD) ($p < 0.05$). Different small letters within the same row shows significant difference between NP and WP by t test (LSD) ($p < 0.05$). ^{ns} Not significant by t test (LSD) ($p \geq 0.05$)

Shoot dry matter accumulation was increased significantly by P application across 4 cultivars (Figure 5A). Highest shoot DM under NP was observed for cultivar RB96-6928 and under WP was observed for cultivars RB92-579 and RB85-5156. For root dry matter under both NP and WP, the highest values were observed for cultivar RB96-6928. Cultivar RB86-7515 showed poor shoot and root development under WP.

The amount of P accumulated in shoot and root (P uptake) was higher for cultivar RB96-6928, both under NP and WP conditions (Figure 5B), which is consistent with the observed differences in shoot DM. However, under WP the cultivars RB92-579 and RB96-6928 contained more P stored in the root system than RB86-7515. For shoot P accumulation, cultivars RB92-579 and RB85-5156 had the highest P content, RB96-6928 was intermediate and RB86-7515 contained significantly less total P.

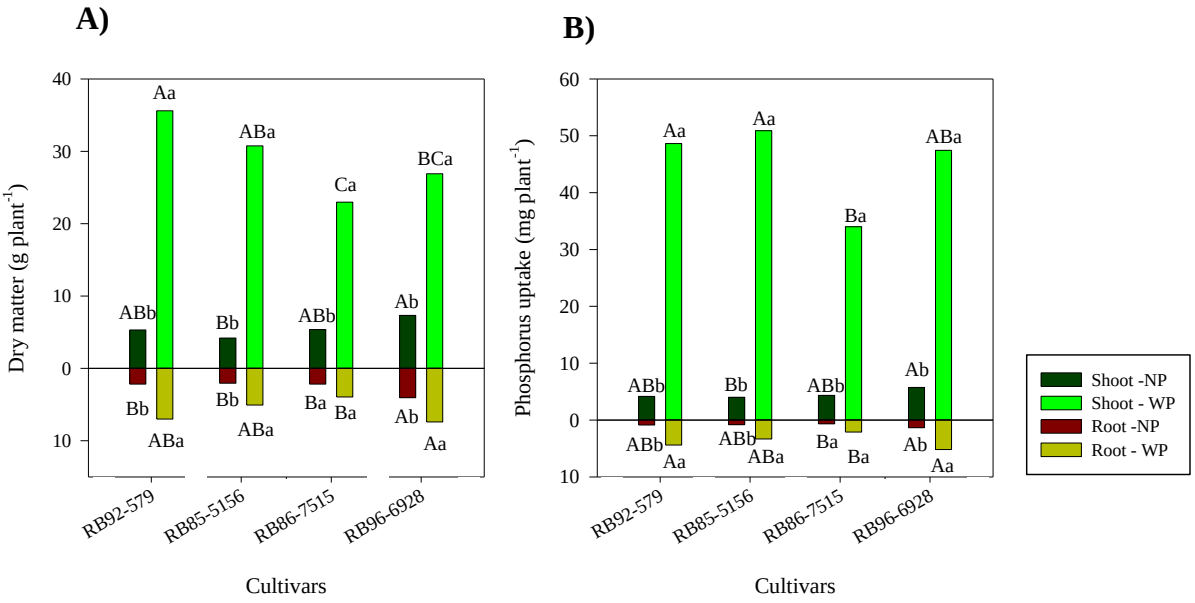

Figure 5 - A) Dry matter and B) Phosphorus uptake by sugarcane shoot and root for four cultivars grown without phosphate application (NP) and with P (WP) supplied at rate of 78.4 mg kg^{-1} of soluble P. Different capital letters show significant differences between cultivars by t test (LSD) ($p < 0.05$). Different small letters show significant difference between NP and WP by t test (LSD) ($p < 0.05$)

Considering the ratio between P uptake and surface area of root, the most responsive efficient cultivar was RB85-5156. RB92-579 was classified as responsive inefficient and both RB86-7515 and RB96-6928 were nonresponsive efficient (Figure 6).

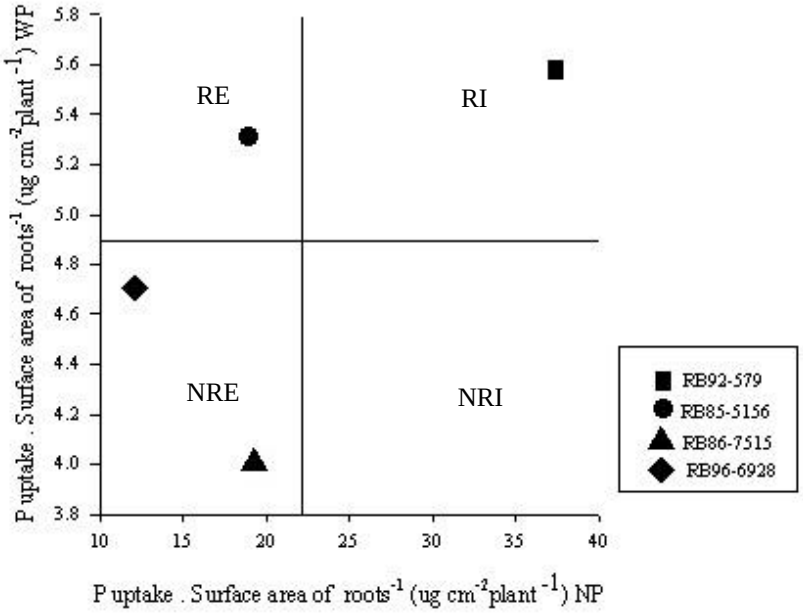

Figure 6 - Sugarcane cultivar classification based on the phosphorus uptake: Responsive efficient (RE); Responsive inefficient (RI): Nonresponsive efficient (NRE); Nonresponsive inefficient (NRI), for four cultivars grown without phosphate application (NP) and with P (WP) supplied at rate of 78.4 mg kg^{-1} of soluble P

To summarize, the selection of RB96-6928 is justified based on the root system, great root P uptake and high number of tillers per plant compared to other genotypes indicating a great volume exploration in the soil to P uptake. Moreover, RB96-6928 presented as an efficient and nonresponsive cultivar to plant P uptake and low and high P conditions, what justify a need to do a more detailed and fine scale sampling and analysis in the rhizosphere of this genotype under rates of P to determinate the amount of P for an adequate initial seedling development.

3.3.3 Responsiveness of sugarcane cultivars grown under low and high P

Changes in the rhizosphere properties were analyzed in thin layer soil sections at various distances from the rhizoplane. Lowest soil pH was observed in soil without P addition and was similar across all sections (Figure 7A). AP_{ASE} in the rhizosphere similarly varied in response to P fertilization and like pH, it has varied across soil sections (Figure 7B). High AP_{ASE} activity was observed nearest to the rhizoplane. The P_{MIC} just change by P rates ($p < 0.05$) only in the section 1 (Figure 7C), Where the control presented the lowest values of P_{MIC} in with higher values under 39.2 mg kg^{-1}. Far from the root, after 3 mm, there was no effect ($p \geq 0.05$) of fertilizer for P_{MIC}. P_{AER} showed difference between rates of P, but did not show any difference across the sections (Figure 7D).

The cultivar RB96-6928 showed a significant effect for shoot DM, P uptake and root mycorrhizal colonization across the rates of P applied (Figure 8). According to the quadratic regression for shoot DM accumulation (R^2 0.955), 90% of the maximum DM (12.1 g pl^{-1}) would be obtained at rate of 38.5 mg P kg^{-1} soil. P uptake by the plants increased linearly with P application (Figure 8A). Extrapolation of P uptake at a critical rate, would suggest a P content of 24 mg kg^{-1}, resulting in an internal critical P concentration of 0.198% (or 1.98 mg P g^{-1} DW) (Figure 8B).

Root mycorrhizal colonization was negatively affected by increasing P rate, being observed 38% of colonization of the roots in the control without P and the lowest colonization of 18% at 70 mg P kg^{-1}, according to the adjusted curve (Figure 8C). At the critical level of P supply the plants showed approximately 22.5% colonization.

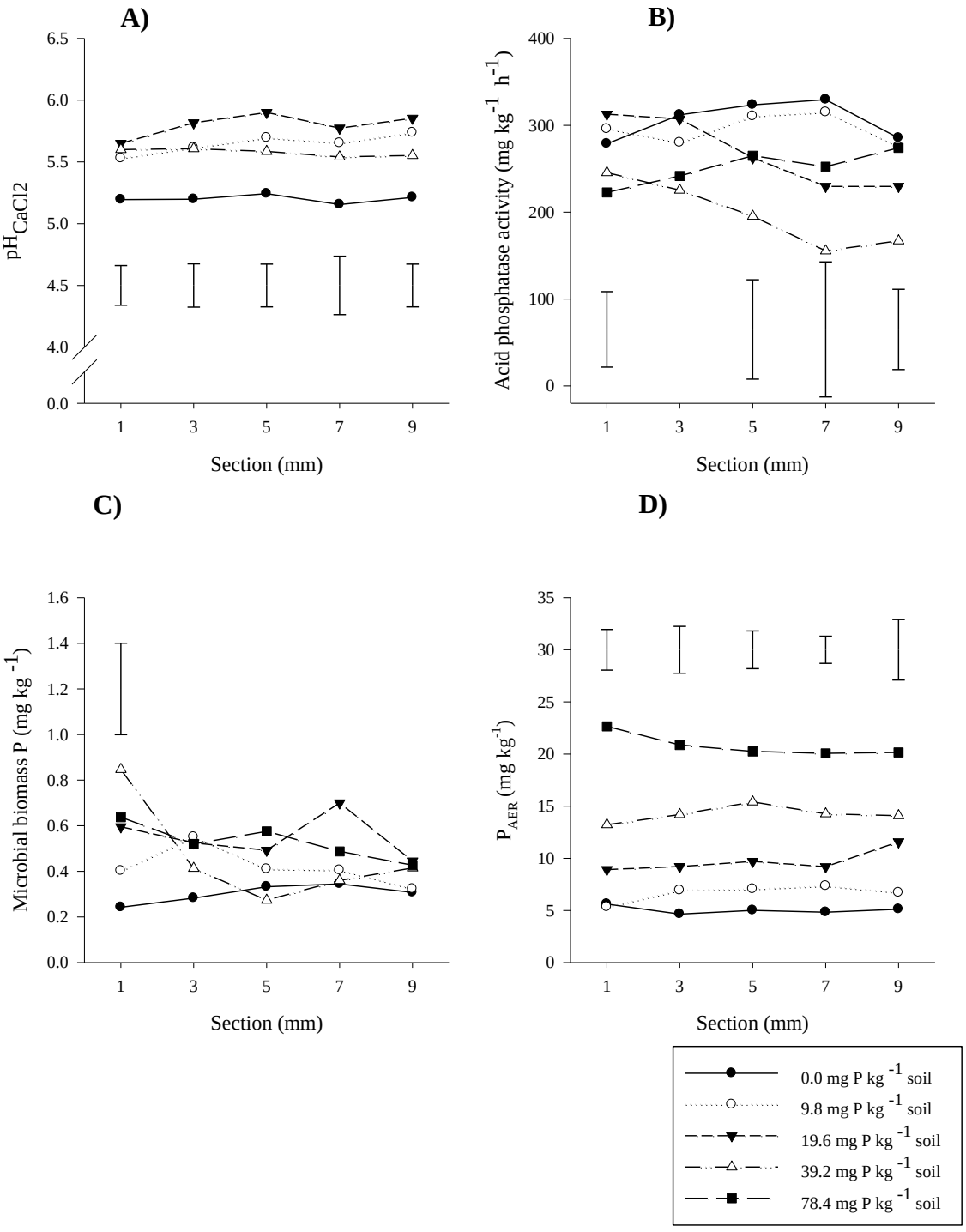

Figure 7 - A) pH_{CaCl2}; B) acid phosphatase activity; C) microbial biomass P; and D) inorganic labile P extracted by anion exchange resin by sections from the rhizoplane for sugarcane seedlings under five rates of P_2O_5 *significant by t test (LSD) ($p < 0.05$). ^{ns} Not significant by t test (LSD) ($p \geq 0.05$)

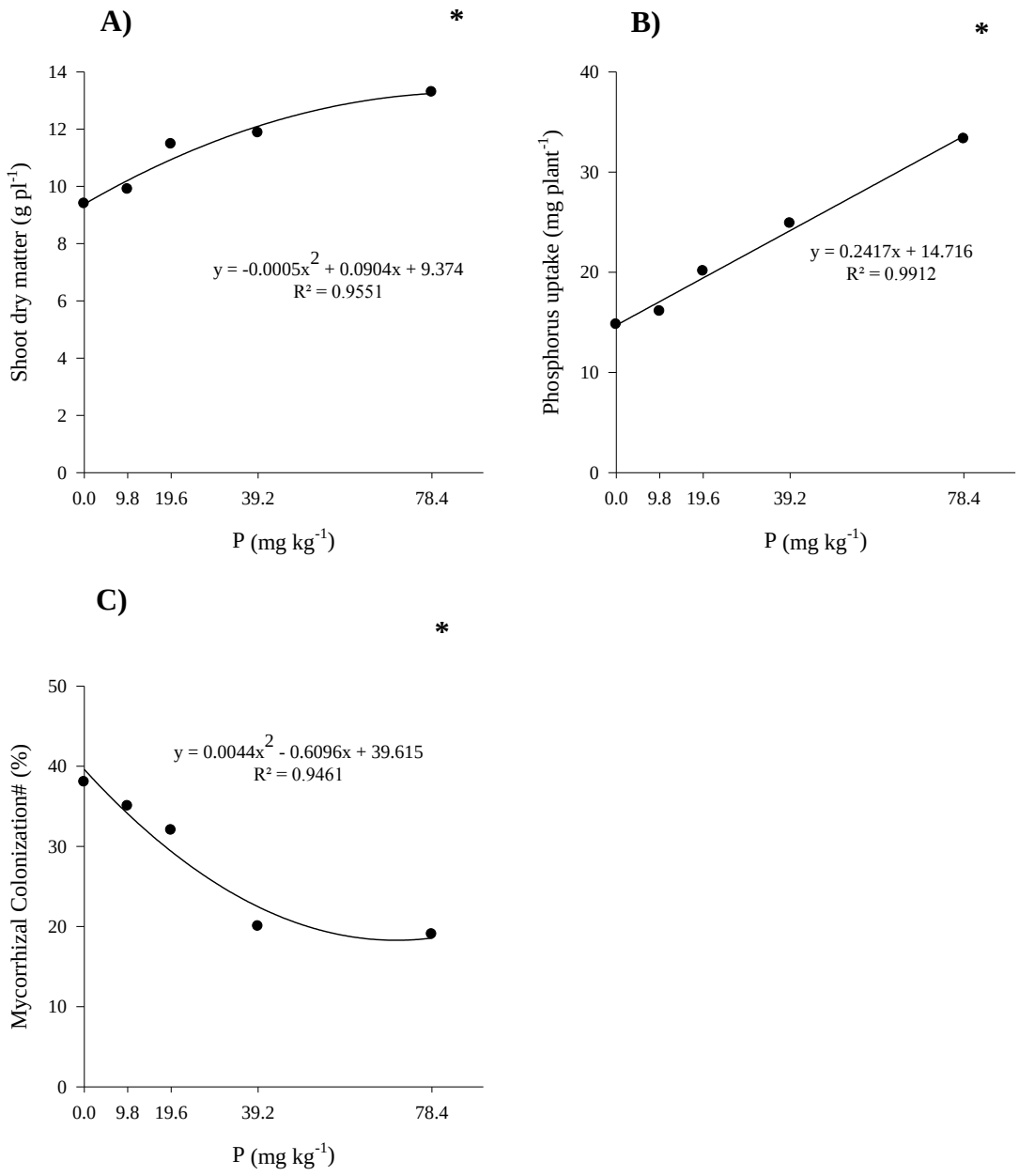

Figure 8 - A) Shoot dry matter; B) total phosphorus uptake; and C) root mycorrhizal colonization of sugarcane seedling under five rates of P_2O_5. *significant by t test (LSD) ($p < 0.05$). ^{ns} Not significant by t test (LSD) ($p \geq 0.05$). #Transformed data to arc sen $\sqrt{P\%}$ / 100, where P is the percentage of assessed mycorrhizal colonization

3.4 Discussion

Regarding to the root development, soil adequate P levels promotes the initial formation and development of the root system and increases the absorption and utilization of all other nutrients, mainly those with low mobility. Because of the direct relationship between production efficiency under low P conditions and large root system (TARIQ AZIZ; MAQSOOD; MANSOOR, 2005), the maintenance of root system under low nutrient condition is a mechanism used by several crops to adapt to nutritional stress, by more root mass production (MARSCHNER, 2011). On the other hand, in fertile soils, a relatively smaller amount of roots is required for shoot DM production. Changes in root architecture may characterize an adaptive measurement to increase the absorptive surface of the root system (LÓPEZ-BUCIO; CRUZ-RAMÍREZ; HERRERA-ESTRELLA, 2003). By that way, the cultivar RB96-6928 had the highest volume of root compared to others, mainly under no P application (NP), showing a potential of this cultivar to present great exploration of the volume of soil, important for P uptake.

Another way to improve the exploration of the volume of soil is the fungi association. To improve the uptake of soil relatively immobile nutrients, with low concentration in the soil solution, most plants can use specific mechanisms, like mycorrhizal symbiosis, providing about 10 times as much absorptive surface in the root system of an uninfected plant (BRADY; WEIL, 2010). Mycorrhizal symbiosis, an association between plant and fungus is an essential feature of the biology and ecology of the most terrestrial plants, since it influences nutrient absorption in the rhizosphere (BIANCIOTTO; BONFANTE, 2002). There was no difference between the cultivars for root mycorrhizal colonization under WP once the P presence discourages the root system to find out the symbiosis with the fungal, since the root by itself can get P from the soil. In P deficiency conditions this symbiosis is induced by root exudation of carbohydrates and amino acids, which increase the mycorrhizal colonization and also the microbial community close to the roots, showing an effect of the root to the microbiology, which was observed in this study. Under NP, some plants have more affinity to form a symbiosis. Cultivar RB86-7515 did not differ for mycorrhizal colonization under WP or NP, showing low affinity to a plant/microorganism relationship. Lery et al. (2011) reported a good interaction between Endophytic Plant-Growth-Promoting Bacterium and two sugarcane cultivars. However, some cultivars had higher colonization under NP compared to WP, showing bigger affinity to symbiosis relationship. The cultivar RB96-6928, may not showed

the highest mycorrhizal colonization in consequence of its big volume of root, reducing dependency of this association.

Another strategy of some plant under P deficiency is the proton and carboxyl extrusion, which leads to rhizosphere acidification and contributes to the solubilization of acid soluble Ca phosphate (HINSINGER; GILKES, 1997), P_{ASE} can improve the performance and solubilize more Ca bounded P. For acid phosphatase (P_{ASE}), a good activity in the soil without plant cultivation was observed, indicating that this enzyme is active even in the absence of plants, once the microorganisms present in the soil are the main responsible for exudation and activation of this enzyme (SATO et al., 2015). Microorganisms are an important part of the P cycle in the soil, and therefore act in mediating the availability of P for plants (RICHARDSON; SIMPSON, 2011). In the absence of plant there was no difference in P_{ASE} between NP and WP, showing that the presence of roots increased the P_{ASE}. On the other hand, P_{ASE} increased under WP in the presence of plants, which could be induced by priming effect, which is defined as the stimulation of soil organic matter (SOM) decomposition caused by the addition of labile substrates (CHENG, 1999; DALENBERG; JAGER, 1989), showing the significant impact of the root system in the performing of this group of enzymes. RB96-6928 presented the lowest activity comparing the cultivars. This cultivar showed the highest root system, which may have reduced the activity of this enzyme because the root interception mechanism allows greater exploitation and better P acquisition from the bulk soil.

The environmental conditions have influenced the dynamics of root development and the root system can also modify some soil characteristics. However, in our study $P_{MIC,}$ P_{AER} and Pi_{NaHCO3} did not show any cultivar effect. Perhaps the short duration of this study (45 days) can explain the lack of fertilizer effect to equilibrate with the soil in terms of inorganic labile P in the soil.

In addition, changes in the rhizosphere can be provided directly by the microorganisms present in the soil, because the presence of fertilizer has altered the microbial community composition compared to no fertilizer. Under NP the microbial groups are closer to each other, indicating low diversity, which may be due to the absence of available P, reducing the interaction between microorganisms and plant. Otherwise, under WP the microbial groups are disperse, indicating more diversity for all sugarcane cultivars, indicating that the presence of phosphate provided adequate environmental conditions for the development of a larger diversity of microorganisms that interact to each other and increase also the plant development.

The microbial activity and nutrient present in this microorganisms in the soil is normally affected by the crop and/or cultivar used, since the rhizosphere interaction is important to solubilize nutrients.

The final sugarcane yield in the field is defined by tons of tillers per hectare, which is characterized by the number of tillers and tiller weight. In our results, cultivars RB85-5156 and RB86-7515 showed a huge dependency on higher levels of soil labile P for tillering, since under NP these cultivars did not present tillers until 45 DAP. In contrast, cultivar RB96-6928 had higher number of tillers both under NP and WP because of its rusticity and ability to adapt under low soil P conditions, which characterize as a good cultivar under restricted field conditions. The tillering phase determines crop productivity, and a high number of tillers at the beginning of the cycle is an indicative of good crop establishment (DILLEWIJN, 1952). As a consequence of the sugarcane roots system development, the roots originated from new tillers have the function of supplying its own nutrition. In this way, the cultivar RB96-6928 presented many tillers and a great root system to supply its nutritional demand, indicating to be a good potential cultivar for crop establishment under restricted P availability environment. This cultivar had a high root DM, both under NP and WP, and was classified as nonresponsive considering the ratio between P uptake and surface area of root. These characteristics are important for the initial crop establishment, when considering the equilibrium between a poor soil condition (NP) and a fertilized condition (WP). Therefore, this cultivar had a higher root efficiency ratio then other cultivars. This is supported by the data presented by Silveira et al. (2014), which reported similar results when submitted 23 sugarcane cultivars to low and high P conditions.

Based on the results from a trial with different responses of sugarcane cultivar submitted to a restrictive condition of low P and a condition that provided the double of the required amount of P, it is important to know how much P would be necessary and used efficiently by the most profitable cultivar, un this case RB96-6928 for low P conditions, as in Brazilian tropical soils.

Under phosphate rates, the cultivar RB96-6928 showed, under no P fertilizer application, the lowest value of pH_{CaCl2} in the average for all the sections, showing as increment in the pH when P was applied from fertilizers. This pH conditions may altered the AP_{ASE} activity, which increased AP_{ASE} under acid conditions, when P was not applied via fertilizer. It worth to mention that when rhizosphere soil adhered to roots was sampled, P_{ASE} was lower under NP than WP. However, when rhizosphere soil was separated to the roots with a net, P_{ASE} was similar between control and lower P rates, decreasing under higher rates.

This may indicates that soil sampling methods can alter the enzyme dynamics and the microorganism's activity is dependent on the P available levels in the soil.

In our approach P_{MIC} did not showed little overall effect across treatments with either cultivar or rate of P. However, across the rhizosphere sections, the roots did show any effect on microbial P, where just near to the root surface lower P_{MIC} was observed. These differences may be indicative of intense competition for available P between roots and microorganism that is particularly evident under higher P stress. Increase in microbial biomass within the rhizosphere may also increase acid phosphatase activity, although the origin of such activity from either microorganisms or plant roots remains unclear. Many activities of microorganisms are regulated by the plant through the rhizosphere, as observed for the increased AP_{ASE}, only produced by microbial cells (SATO et al., 2015).

Considering the plant production, based on the 90% of the maximum shoot dry matter, the rate of P supply required for optimal growth for cultivar RB96-6928 was determined at 38.5 mg P kg^{-1} soil which provided sufficient P uptake to attain a critical internal P concentration to support maximum plant growth. Associated with its response to P application was observed a decrease in the extent of mycorrhizal colonization, confirming a lesser dependence on mycorrhizas at higher P availability.

3.5 Conclusion summary

The cultivar RB96-6928 was the most adapt under low soil P availability since it presented the largest volume of root and tillering. The microbial community of all cultivars was changed by phosphate fertilization. The rate of around 38.5 mg kg^{-1} of P as TSP was the most adequate to promote an adequate seedling development.

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4 PHOSPHORUS DYNAMICS AND EFFICIENCY OF FILTER CAKE IN SUGARCANE RHIZOSPHERE

Abstract

This study aimed to evaluate the dynamics of P in the rhizosphere of sugarcane under interaction between filter cake and phosphate fertilizers and filter cake rates. Two experiments were conducted in greenhouse in a sandy clay loam Ferralsol in a randomized block design with four replications. The experiment one followed a factorial design of 2x3: i) without and with filter cake (5 g kg^{-1} DM) and ii) non P application (NP); triple superphosphate (TSP) and rock phosphate (RP) (both at the rate of 78.4 mg kg^{-1} soluble P). Soil sampling was made by the separation of rhizosphere soil from the rhizoplane (root/soil separation) using PVC columns with a horizontal net for root exclusion. At harvest the rhizosphere soil was sampled 0-2 mm from the rhizoplane. The experiment two evaluated rates of filter cake: 0; 2.5; 5; 10 and 15 g DM kg^{-1} applied in a bulk soil and at planting furrow. Shoot dry matter, number of tillers per plant, mycorrhizal colonization, P uptake P fractionation and P budget were evaluated. The soil under non phosphate application reduced labile, moderately labile and non-labile P fractions as consequence of plant absorption. Filter cake improved P uptake and increased the availability of labile Pi in the rhizosphere and modified the fungal microbial community. In addition, the combination between filter cake and RP was efficient to improve sugarcane aspects, such as, shoot dry matter and number of tillers per plant. The maximum shoot DM and P uptake were observed at rate of 10 g DM kg^{-1} of filter cake in the planting furrow.

Keywords: *Saccharum* spp.; Rock phosphate; Mycorrhizal colonization; Fractionation of P

4.1 Introduction

Sugarcane is largely cultivated in Brazil, being used soil from poor to rich nutrient availability, depending on parental rock. To maintain the high productivity levels it is necessary the application of fertilizers to satisfy the nutritional crop requirements. Phosphorus (P) demands are not as high as nitrogen and potassium, however, P plays a key role in plant metabolism, particularly in the formation of proteins, in cell division process, photosynthesis, tillering, root and internode development and sucrose formation (KORNDÖRFER, 2004).

Water soluble fertilizers are recommended to provide rapid release of P in the soil. However, this event may favor the adsorption and precipitation of soluble forms to soil colloids, forming compounds of low lability and decreasing its availability to plants (RANDHAWA et al., 2006). On the other hand, rock phosphate with low solubility fails to maintain adequate levels of available P for plants, due to its low initial dissolution rate, although increasing residual effect over time (SHIGAKI; SHARPLEY, 2011).

An alternative slow release fertilizer to improve soil chemical and physical conditions is the filter cake, a byproduct of sugarcane, which may reduce the cost, i.e. substitute the mineral fertilizers. Despite the evidence through research (HU et al., 2001; RIGGLE; VON WANDRUSZKA, 2005), filter cake can decrease soil P adsorption by releasing organic radicals that should compete for adsorption sites in the soil. Also, there are studies relating to filter cake and mineral P sources, trying to detect the influence of this management in improving soil fertility (BOKHTIAR; PAUL; ALAM, 2008; GONZÁLEZ et al., 2014; SANTOS et al., 2010, 2014).

Based on the potential competition between organic compounds and P for adsorption sites in the soil, this study aimed to evaluate the dynamics of P in the rhizosphere of sugarcane under filter cake and phosphate fertilizers and filter cake rates for an adequate sugarcane seedling development.

4.2 Material and Methods

The soil used was collected from the layer 0-20 cm of a grassland area, located at "Luiz de Queiroz" College of Agriculture – University of Sao Paulo (ESALQ - USP), in Piracicaba-SP, Brazil, where the study was done. The chemical and textural composition and P fractions of the soil are presented in the Table 1. The soil is classified as sandy clay loam Ferralsol (FAO, 2013), with low levels of available nutrients (Ca, Mg, K and P_{AER}). Prior to experiments the soil was air-dried and sieved on 2 mm mesh, remoistened with deionized water until 80% of field capacity, and incubated for 15 days with lime to achieve a base saturation of 70% (RAIJ et al., 1997).

Two experiments evaluating sugarcane seedlings growth were conducted in greenhouse using a randomized block design with four replications. For both experiments, sugarcane seedlings were germinated in sterile vermiculite for 22 days and then transplanted to the pots. It was applied at transplanting 50 mg K kg^{-1} (KCl) for potassium and 30 mg N kg^{-1} soil (urea) for nitrogen. Also, two N rates were applied during plant growth (15 mg N kg^{-1} each), at 15 and 30 days after transplanting.

Table 1 - Chemical and textural analysis of a sandy clay loam Ferralsol collected from the 0-20 cm layer of a grassland area used for experiments

pH	K	Ca	Mg	EB	CEC	V	OM	Clay	Silt	Sand
$CaCl_2$	----------$mmol_c\ dm^{-3}$----------					-------%-------			------g kg^{-1}------	
4.3	1.4	8	5	14.4	45.4	31.7	2.2	201	11	788

P $_{AER}$†	Pi* $_{NaHCO_3}$	Po $_{NaHCO_3}$	Pi $_{NaOH0.1}$	Po $_{NaOH0.1}$	P $_{HCl}$	Pi $_{NaOH0.5}$	Po $_{NaOH0.5}$
----------mg kg^{-1}----------							
5.4	2.8	21.3	51.9	18.7	7.4	32.8	13.1

EB: exchangeable base-forming; CEC: Cation exchange capacity; V: Base saturation; OM: organic matter. † P fractionation extractors: AER: anion exchange resin; $NaHCO_3$: sodium bicarbonate; NaOH 0.1M: sodium hydroxide 0.1 M; HCl: hydrochloric acid; NaOH 0.5M: sodium hydroxide (HEDLEY; STEWART; CHAUHAN, 1982). *P fractions: Pi: inorganic phosphorus; Po: organic phosphorus

4.2.1 Sugarcane growth under filter cake and P fertilizers

The experiment was conducted in a 2x3 factorial: i) without and with filter cake (5 g DM kg^{-1}) (Table 2) and ii) no P fertilizer (NP); rock phosphate (RP) (Gafsa, 10% soluble and 29% total P_2O_5), and triple superphosphate (TSP 45% soluble P_2O_5), both at rate of 78.4 mg soluble P kg^{-1} soil.

Table 2 - Chemical analysis of filter cake used for sugarcane seedlings development

Determination	Dry base (110ºC)
Total organic matter (combustion) (%)	50.45
Organic carbon (%)	24.67
Total nitrogen (%)	2.17
Total phosphorus (P) (%)	0.70
Total potassium (K_2O) (%)	0.75
Total calcium (Ca) (%)	3.45
Total magnesium (Mg) (%)	0.40
Total sulphur (S) (%)	0.11
Total copper (Cu) (mg kg^{-1})	185
Total manganese (Mn) (mg kg^{-1})	684
Total zinc (Zn) (mg kg^{-1})	195
Total boron (B) (mg kg^{-1})	5
Total sodium (Na) (mg kg^{-1})	309

Prior to harvest, at 65 days after transplanting, number of tillers per plant was measured. After, the shoot was collected and dried in an oven at 60°C until constant mass, weighed and milled though a 1 mm sieve. P content in the shoot was determined as previously described by Malavolta, Vitti and Oliveira (1997). Root system was sampled, washed and stored in 70% ethanol for analysis of percentage of mycorrhizal colonization (MC). For MC, the roots were prepared according to Vierheilig et al. (1998) and dispersed in a petri dish with a checkered background and scored using a stereomicroscope according to Giovannetti and Mosse (1980).

Rhizosphere samples were obtained using polyvinyl chloride (PVC) columns with a horizontal nylon mesh (25 microns) for root exclusion (GAHOONIA; NIELSEN, 1991), whereby an upper column (0.15 m tall) was fitted to a lower column (0.05 m) to provide a soil continuum. The nylon mesh at the base of the upper column allowed movement of water and ion exchange between root and soil, growth of root hairs and mycorrhizas, but prevented root growth below it. At plant harvest, rhizosphere soil samples were taken from 0-2 mm from the rhizoplane, through a screw mechanism which raised the soil above a level that controlled the distance of the sections and then the soil was shaved. Samples were placed on ice immediately after sampling and a subsample was kept at -80°C for community analysis by Terminal Restriction Length Polymorphism (TRFLP) analysis.

The determination of bacterial community structure was based on TRFLP analysis. DNA from the microorganisms presented in the rhizosphere was extracted using the PowerMax® Soil DNA Isolation Kit (MoBio Laboratories) following the protocol. The primers used for bacterial (16S) PCR were 27F6Fam (5’ AGAGTTTGATCMTGGCT 3’) and 519R (5’ GWATTACCGCGGCKGCTG 3’) and for fungi (ITS) PCR the primers used were ITS1Fam (5’ CTTGCTCATTTAGAGGAAGTAA 3’) and IT4 (5’ TCCTCCGCTTATTGATATGC 3’). Each 50 µL PCR reaction consisted of 1 µL of full DNA, 10µL of My Taq Reaction Buffer (Bioline), 1 µL of each primer, 0.2 of My TaqTM DNA Polymerase (Bioline). Samples were amplified by use PCR machine GENE Amp® PCR System 9700 (Applied Biosystems) of an initial denaturation step at 95°C (2 min), followed by 30 cycles of denaturation at 95°C (30 sec), annealing at 53°C (30 sec), and an extension at 72°C (30 sec). Cycling was completed by a final extension at 72°C (10 min). The purification was done using Agentcourt® AMPure®XP (Beckman Coulter™) following the protocol. With this protocol, we removed all non-DNA fragments from PCR product, and keep only DNA. The restriction digestion was made by use the FAM-labeled PCR products, which was digested at 37°C for 3 hours. Each digest contained 30 ng of cleaned PCR product, 0.2µL of HinfI (Biolabs), and 2 µL of the recommended buffer (Buffer 2) (Biolab) (final reaction

volume 20µL). The product was centrifuged by use Centrifuge Allegra™ 25R centrifuge (Beckman Coulter™). The TRFLP was carried out at TRFLP machine 3130xl Genetic Analyser.

Soil P fractions were determined on representative 0.5 g subsamples of dried and sieved soil subjected to sequential extractions, according to Hedley, Stewart and Chauhan (1982), with modifications by Condron, Goh and Newman (1985). At each step, 10 mL of extractor was added to 0.5 g soil in a 15 mL centrifuge tubes (1:20 soil:solution ratio) and the tubes shaken end-over-end (orbital agitator, 30 rpm) for 16 h at 25 ºC. First, the P_{AER} extracts labile inorganic P readily diffusing into solution and sorbed into a resin membrane (2.0 cm^2 area). The 0.5 mol L^{-1} $NaHCO_3$ at pH 8.5 (P_{NaHCO3}) as second extracts labile inorganic P (Pi_{Bic}) weakly adsorbed on the surface of crystalline compounds and labile organic P compounds with low recalcitrance such as ribonucleic acid and glycerophosphate (TIESSEN; MOIR, 1993). The third extractor is 0.1 mol NaOH L^{-1} ($P_{NaOH\ 0.1}$) which removes moderately labile inorganic P strongly adsorbed onto Fe and Al and clay minerals (HEDLEY; STEWART; CHAUHAN, 1982), and moderately labile organic P mainly associated with fulvic and humic acids adsorbed onto mineral and SOM surfaces (LINQUIST et al., 1997). The HCl 1.0 mol L^{-1} is the fouth step, extracting moderately labile inorganic P associated with apatite, other sparingly-soluble Ca-P compounds or negatively charged oxide surfaces (GATIBONI et al., 2007). The fifth extract is 0.5 mol NaOH L^{-1} which removes more recalcitrant forms of inorganic P, associated with Fe and Al and clay minerals, and non-labile forms of organic P associated with fulvic and humic acids inside aggregates (CONDRON; GOH; NEWMAN, 1985). The residual P is obtained after the remaining soil is dried at 50 ºC, milled and digested with H_2SO_4 + H_2O_2 in the presence of saturated $MgCl_2$ (OLSEN; SOMMERS, 1982). After each step, soil suspensions were centrifuged at 4000 rpm (3278 g) for 20 min to collect clear supernatants. Total P (P_{total}) in the alkali extracts (P_{NaHCO3}, $P_{NaOH\ 0.1}$ and $P_{NaOH\ 0.5}$) was determined by sulphuric acid (H_2SO_4) and ammonium persulfate digestion in an autoclave at 121 ºC (UNITED STATES ENVIRONMENTAL PROTECTION AGENCY - USEPA, 1971). The Pi in alkali extracts was measured colorimetrically by the method of Dick and Tabatabai (1977). Pi in acid extracts (P_{AER} and P_{HCl}, and extracts after digestion of residual P, P_{NaHCO3}, $P_{NaOH\ 0.1}$ and $P_{NaOH\ 0.5}$) was measured by the colorimetric method of Murphy and Riley (1962). The Po in alkali extracts was obtained by difference between measured Pt and Pi.

4.2.2 Sugarcane growth under filter cake rates

This study assessed sugarcane response to five rates of filter cake (0; 2.5; 5; 10 and 15 g DM kg^{-1}), applied uniformly in the bulk soil (BS) or simulating a planting furrow (PF).

At harvest, 56 days (~8 weeks) after transplanting, the number of tillers per plant was estimated and shoot collected and dried in an oven at 60°C to determine total DM, then it was milled though a 1 mm sieve and submitted to the laboratory determination of P content as previously described in experiment 1. Soil samples were collected, air dried and sieved to determine labile P fractions, according to Hedley, Stewart and Chauhan (1982), as described in 4.2.1 section.

4.2.3 Statistical analysis

For sugarcane response to filter cake and P fertilizers the data were subjected to two-way (P application and filter cake application) analysis of variance (ANOVA) and significance (0.05 error probability) determined by F values. Data with significant effect had means compared paired t test (LSD). If there was significant interaction, unfolding of the interaction was conducted. For TRFLP data, the statistical analyses were run in Primer®. Peaks larger than 540 and 755 were removed from the data set and analysis of variance for bacterial and fungal community, respectively. TRFLP (relativized peak height values) data were transformed by square root and analyzed with non-metric multidimensional scaling (NMS). NMS was performed with Bray Curtis similarity distances. For the sugarcane response to filter cake the data were subjected to two-way (filter cake rates and application ways) and data with significant effect were subjected to the adjustment of more significant regression. When there was significant interaction, was made the adjustment of more significant regression for filter cake rates by application ways and the difference between application ways was present by mean square error.

4.3 Results

4.3.1 Sugarcane response to filter cake and P fertilizers

For all P fractions there was no interaction between filter cake and phosphate application and filter cake only affected positively the inorganic labile P fraction. Both Pi_{AER} and Pi_{NaHCO3}, showed significant effect under filter cake and phosphate sources. Both Pi_{AER} and Pi_{NaHCO3} increased 7.4 mg kg^{-1} comparing NFC to WFC ($p < 0.05$). Regards to P sources, TSP had the highest average for both, Pi_{AER} and Pi_{NaHCO3} compared to NP and RP, also increasing organic moderately labile P ($Po_{NaOH\ 0.1}$). RP increased P_{HCl} and $P_{iNaOH\ 0.5}$ in average, compared to NP and TSP (Table 3).

Comparing initial soil-test P (Table 1) and the rhizosphere after the experiment with plant (Table 3), in NP treatments P uptake from rhizosphere decreased in all fractions, even in no labile fractions, resulting in a reduction of 29 mg P_{Total} kg^{-1} soil (Table 4). When RP was applied (210 mg P kg^{-1} soil), labile fractions reduced, and P_{HCl} increased, which in the total balance augmented the P_{Total} in according to P fractionation analysis. Furthermore, the difference between P applied and P uptake was higher, showing that fractionation analysis did not detected 36 mg P_{Total} kg^{-1}. When TSP was applied (78 mg P kg^{-1} soil), it was not observed any reduction in the P fractions in the rhizosphere and the P_{Total} was increased, which was also observed in P_{Total} estimated by fractionation analysis.

Regarding to soil bacterial community, it was observed a strong positive effect ($p < 0.05$) from both phosphate application and filter cake (Figure 1A). This shows that both phosphate and filter cake promoted changes in bacterial community, however, these changes were independent. The effect of filter cake was also detected ($p < 0.05$) in fungal community, but phosphate source did not affect fungal community. Two fungal groups were formed (Figure 1B) with a large separation between NFC and WFC, presenting more than 50% of similarity.

Table 3 - Soil P fractions after sugarcane seedlings cultivation under no phosphate application (NP) and with phosphate application (Rock phosphate- RP and Triple superphosphate – TSP) at rate of 78.4 mg kg^{-1} of soluble P, submitted to no filter cake application (NFC) and with filter cake application (WFC) at rate of 5 g DM kg^{-1}

Sources of P	NFC		WFC		Mean		NFC		WFC		Mean		NFC		WFC		Mean	
	P_{RTA}						Pi_{NaHCO_3}						Po_{BIC}					
	mg kg^{-1}																	
NP	3.2	ns	6.6		4.9	B	11.6	ns	13.4		12.5	B	4.9	ns	8.0		6.5	
RP	5.1		9.3		7.2	B	11.1		13.8		12.4	B	4.4		5.3		4.9	
TSP	17.3		31.9		24.6	A	24.6		42.2		33.4	A	4.6		3.2		4.0	
Mean	8.5	b	15.9	a			15.7	b	23.1	a			4.6		5.5			
	$Pi_{NaOH\ 0.1}$						$Po_{NaOH\ 0.1}$						P_{HCl}					
	mg kg^{-1}																	
NP	31.9	ns	36.8		34.4		52.9	ns	54.4		53.5	B	3.8	ns	5.3		4.6	B
RP	26.1		40.7		34.4		63.1		60.5		62.0	B	144.7		208.6		181.2	A
TSP	40.7		38.6		39.8		68.0		83.8		75.9	A	5.5		7.4		6.5	B
Mean	33.5		38.7				61.3	ns	68.0				42.9	ns	73.8			
	$Pi_{NaOH\ 0.5}$						$Po_{NaOH\ 0.5}$						P_{Res}					
	mg kg^{-1}																	
NP	23.4	ns	18.8		21.1	B	11.7	ns	17.2		14.4		60.7	ns	67.8		64.2	
RP	33.5		44.7		39.9	A	15.1		21.6		18.4		47.7		89.6		68.6	
TSP	26.2		25.0		25.6	B	11.3		18.4		14.9		67.5		65.8		66.7	
Mean	27.3	ns	29.9				12.6		18.9				58.6		74.4			

Different capital letters within the same column show significant differences between cultivars by t test (LSD) ($p < 0.05$). Different small letters within the same row show significant difference between NP and WP by t test (LSD) ($p < 0.05$). ^{ns} Not significant by t test (LSD) ($p \geq 0.05$).

P fractionation extractors: AER: anion exchange resin; $NaHCO_3$: sodium bicarbonate; NaOH 0.1M: sodium hydroxide 0.1 M; HCl: hydrochloric acid; NaOH 0.5M: sodium hydroxide (HEDLEY; STEWART; CHAUHAN, 1982). *P fractions: Pi: inorganic phosphorus; Po: organic phosphorus

Table 4 – Soil P fractions balance after sugarcane seedlings cultivation under no phosphate application (NP) and with phosphate application (Rock phosphate- RP and Triple superphosphate – TSP) at rate of 78.4 mg kg^{-1} of soluble P, submitted to no filter cake (NFC) and with filter cake application (WFC) at rate of 10 g DM kg^{-1}

Balance	P Applied	Shoot P Uptake	Balance	P_{AER}*	Pi_{NaHCO3}	Po_{NaHCO3}	$Pi_{NaOH0.1}$	$Po_{NaOH0.1}$	P_{HCl}	$Pi_{NaOH0.5}$	$Po_{NaOH0.5}$	P_{Res}	P_{Total}
	mg kg^{-1}	mg $plant^{-1}$	----------mg kg^{-1}----------										
(NP final) – (NP initial)	-	22	-22†	-2	9	-16	-20	34	-4	-9	-11	-9	-29
(RP*NFC)- (NP*NFC)	210	27	183	2	-1	-1	-6	10	141	10	3	-13	147
(TSP*NFC) – (NP*NFC)	78	31	47	14	13	0	9	15	2	3	0	7	62

†Negative numbers means P increase; positive numbers means P reduction.
P fractionation extractors: AER: anion exchange resin; $NaHCO_3$: sodium bicarbonate; NaOH 0.1M: sodium hydroxide 0.1 M; HCl: hydrochloric acid; NaOH 0.5M: sodium hydroxide (HEDLEY; STEWART; CHAUHAN, 1982). *P fractions: Pi: inorganic phosphorus; Po: organic phosphorus

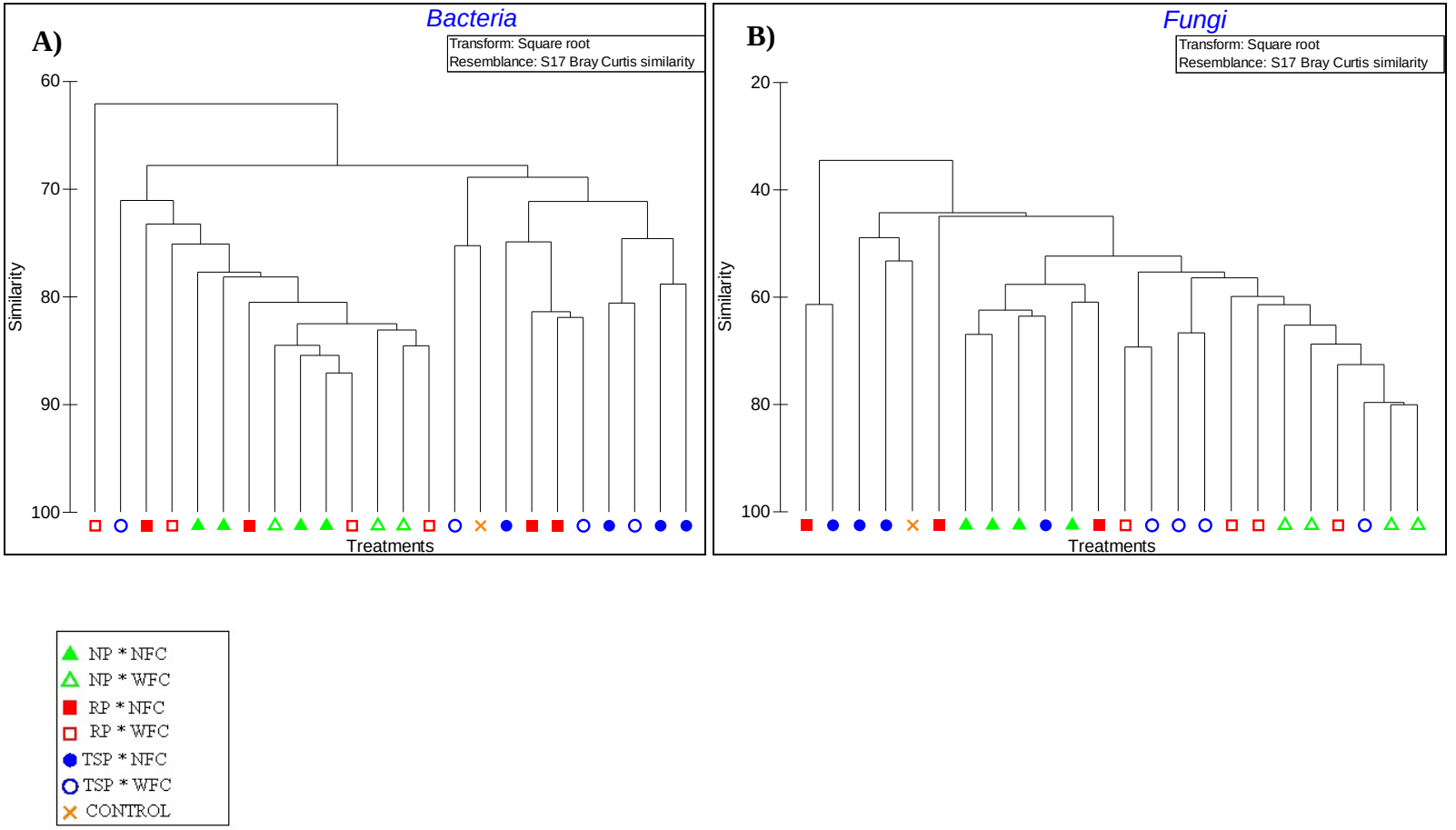

Figure 1 - A) NMS (Bray Curtis similarity) plots from bacterial (16S) TRFLP results and B) clusters from fungal (ITS) TRFLP results. Points represent samples. NP – no phosphate; RP – rock phosphate; TSP – triple superphosphate (Both at rate of 78.4 mg soluble P kg^{-1}); NFC – no filter cake; and WFC – with filter cake at rate of 5 g DM kg^{-1}

The filter cake affected shoot DM yield (Table 5), showing an interaction between filter cake application and sources of P fertilizers. Under NFC, TSP showed the highest average dry matter. However, there was no difference in dry matter between P sources under WFC, but both were increased compared to NFC treatments. The number of tillers per plant also showed an interaction between filter cake and P sources. Filter cake increased the number of tillers per plant when associated only with RP. Under NFC combined with TSP, it was observed the highest average tillers per plant. However, under WFC, RP did not differ from TSP. For mycorrhizal colonization an interaction between filter cake and mineral P sources was observed. Filter cake did not increase mycorrhizal colonization under NP and TSP (Table 5). The highest colonization was observed under NFC combined with the presence of RP. The levels of P uptake showed interaction between P sources and filter cake. Filter cake increased P uptake when associated to low solubility sources (RP) or NP, and the highest P uptake was observed under TSP.

Table 5 - Sugarcane shoot dry matter (DM); number of tillers per plant; mycorrhizal colonization; and P uptake under: no phosphate application (NP); rock phosphate (RP); and triple superphosphate (TSP) at rate of 78.4 mg kg^{-1} of soluble P, submitted to no filter cake application (NFC) and with filter cake application (WFC) at rate of 5 g DM kg^{-1}

Sources of P	NFC		WFC		Mean	NFC		WFC		Mean	NFC		WFC		Mean	NFC		WFC		Mean
	Shoot dry matter g pl^{-1}					Number of tillers per plant					Mycorrhizal colonization* %					P uptake mg $plant^{-1}$				
NP	21.43	Cb	25.28	Aa	23.36	0.25	Ba	1.00	Ba	0.63	45	Ba	47	Aa	46	8	Bb	17	Ba	13
RP	23.80	Bb	26.02	Aa	24.91	0.50	Bb	1.75	Aa	1.19	51	Aa	41	ABb	46	12	Bb	17	Ba	15
TSP	27.28	Aa	27.34	Aa	27.31	2.25	Aa	2.00	Aa	2.13	35	Ca	36	Ba	36	32	Aa	30	Aa	31
Mean	24.17		26.21			1.00		1.63			44		41			17		21		

Different capital letters within the same column show significant differences sources of P by t test (LSD) ($p < 0.05$). Different small letters within the same row show significant difference between NFC and WFC by t test (LSD) ($p < 0.05$). ^{ns} Not significant by t test (LSD) ($p \geq 0.05$). *Transformed data to arc sen $\sqrt{P\% / 100}$, where P is the percentage of assessed mycorrhizal colonization

4.3.2 Sugarcane response to filter cake

The fractions P_{AER} and P_{HCl} were affected by the interaction between application way and filter cake rates ($p < 0.05$). Both P_{AER} and P_{HCl} presented increases with rates of filter cake and the application at PF showed higher values compared to BS. In average P_{AER} showed 51.4 and 24.7 mg kg^{-1} at rate of 15 g DM kg^{-1} for PF and BS, respectively, and P_{HCl} presented 32.3 and 23.4 mg kg^{-1} when applied in PF and BS, respectively (Figure 2A and D).

Alkaline-extracted fractions P_{NaHCO3}, $P_{NaOH0.1}$, and $P_{NaOH0.5}$, both organic and inorganic, were not affected by application ways. However, these fractions presented effect by rates of filter cake, also increasing Pi for all these fractions and consequently decreasing Po (Figure 2B, C and E). For labile fraction (P_{NaHCO3}), there was an increase promoted by filter cake, showing average Pi variation between 6.4 mg kg^{-1} in control and 20.1 mg kg^{-1} in the highest rate applied, increasing in average 13.7 mg kg^{-1}. On the other hand, organic P decreased from 23.7 to 17.9 mg kg^{-1} (8.8 mg kg^{-1} in average) (Figure 2B). Regards to $P_{NaOH0.1}$, inorganic P increased from 65.3 mg kg^{-1} under NFC to 85.6 mg kg^{-1} at rate of 15 g DM kg^{-1} (20.3 mg kg^{-1} of increase). Also, organic P decreased from 20.9 mg kg^{-1} under NFC application to 3.3 mg kg^{-1} at rate of 15 g DM kg^{-1} (17.6 mg kg^{-1} of decrease) (Figure 2C). In contrast, non-labile fraction ($P_{NaOH0.5}$) showed low values of Po (12.6 mg kg^{-1}) under NFC but increased with filter cake rates (Figure 2E). For inorganic P, it was observed a reverse behavior, decreasing $P_{NaOH0.5}$ values with the increase of filter cake rates.

The application way did not affect shoot dry matter, but there was a significant effect of filter cake rates (Figure 3A), where the maximum DM following the regression should be observed at rate of 10 g DM kg^{-1}, showing an average of 22 g $plant^{-1}$. However, there was no significant effect of the treatments in number of tillers per plant (Figure 3B). Relating to P uptake, it was observed an interaction effect between filter cake rates and application way ($p < 0.05$). When filter cake was applied in bulk soil (BS), P uptake was higher than planting furrow (PF) observing the maximum P uptake values at rates of 34 and 10 g DM kg^{-1}, showing an average of 51 and 37 mg $plant^{-1}$ for BS and PF, respectively (Figure 3C). These DM results affected directly labile P in the soil (P_{AER}) (Figure 2A).

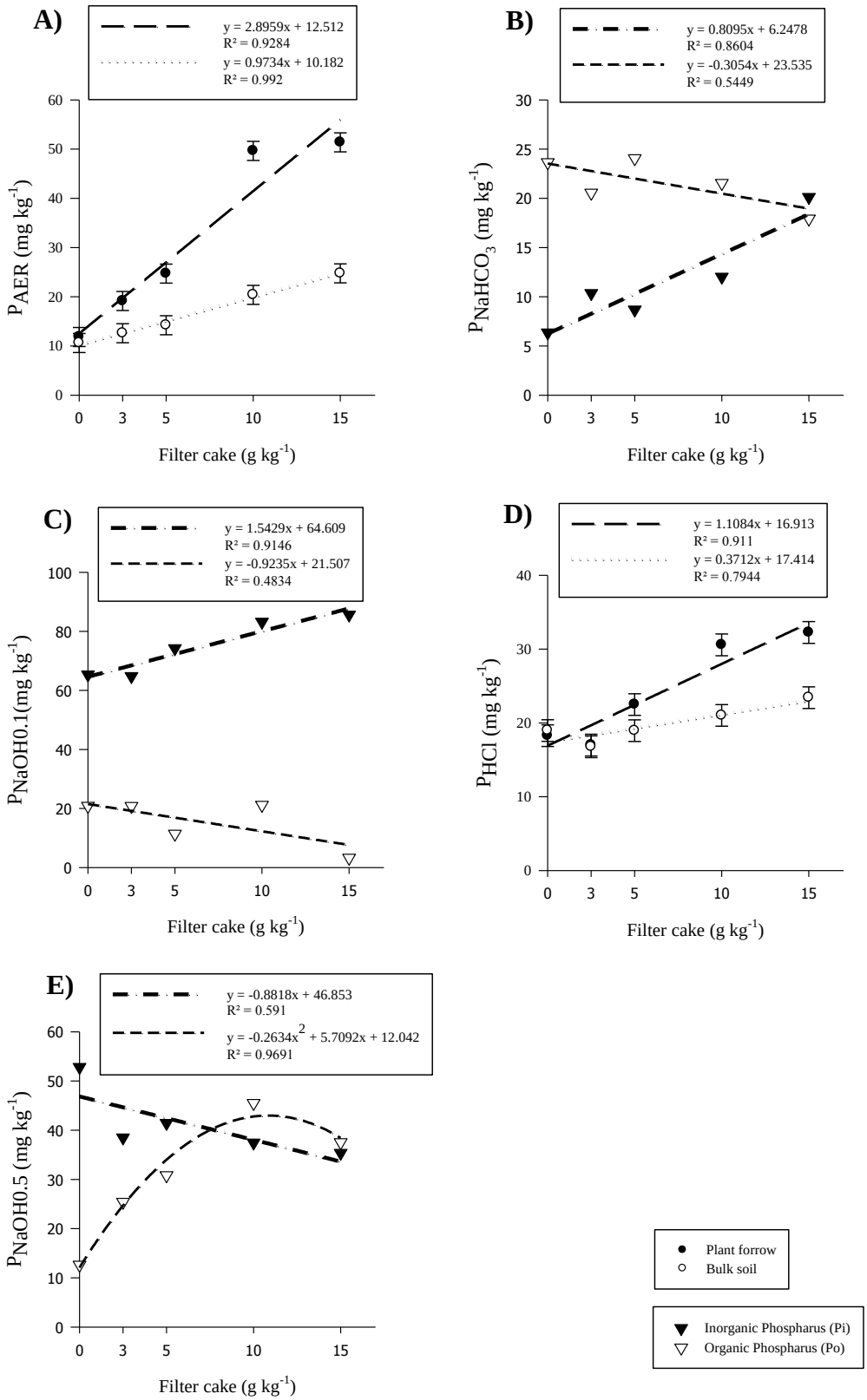

Figure 2 - Soil P fractions after cultivation of sugarcane seedlings under five rates of filter cake applied uniformly in the bulk soil (BS) or at planting furrow (PF): A) P_{AER} in the bulk soil (BS) or at planting furrow (PF).; B) Pi_{NaHCO3} and Po_{NaHCO3}; C) $Pi_{NaOH0.1}$ and $Pi_{NaOH0.1}$ D) P_{HCl} in the BS or PF; E) $Pi_{NaOH0.5}$ and $Pi_{NaOH0.5}$. P fractionation extractors: AER: anion exchange resin; $NaHCO_3$: sodium bicarbonate; NaOH 0.1M: sodium hydroxide 0.1 M; HCl: hydrochloric acid; NaOH 0.5M: sodium hydroxide (HEDLEY; STEWART; CHAUHAN, 1982). *P fractions: Pi: inorganic phosphorus; Po: organic phosphorus.

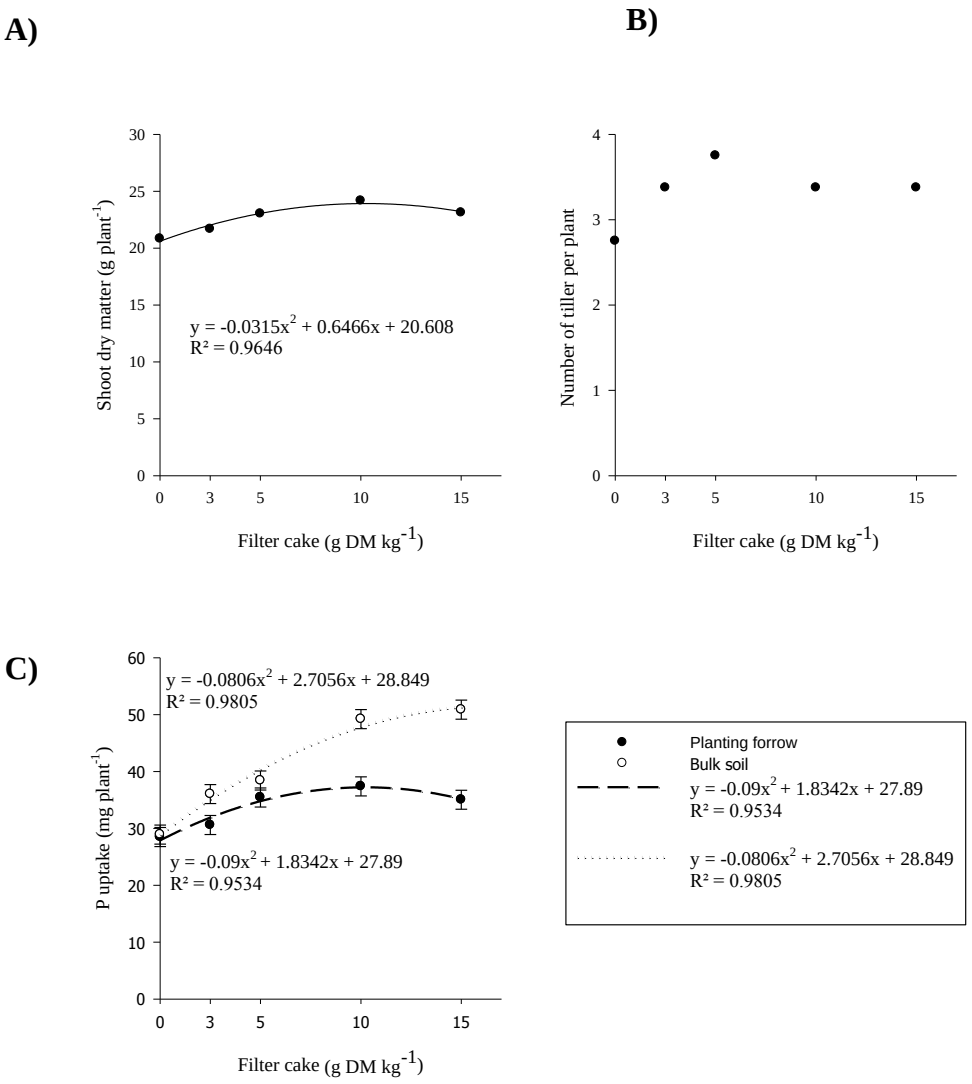

Figure 3 – A) Shoot dry matter; B) number of tillers per plant; and C) P uptake of sugarcane seedling under five rates of filter cake applied uniformly in the bulk soil (BS) or at planting furrow (PF).

4.4 Discussion

Under no P fertilizer application (NP) the sugarcane plants were able to take up P from non-available fractions in the rhizosphere, however it was not enough to guarantee plant development. These results are supported by Gatiboni et al. (2007), who reported that all fractions of soil P can act on the bioavailability, but its release from recalcitrant fractions is lower than necessary to plant absorption for a good crop production. Furthermore, P fractionation detected lower P_{total} compared to the estimation by P balance. This difference

could be explained by root P uptake, which was not evaluated in this experiment because the methodology used to evaluate rhizoplane results in a strong spatial stress in the root system, which could influence the treatment effect and does not represent the normal root growth condition.

RP increased P_{HCl} and also $Pi_{NaOH0.5}$, as expected, since P extracted by HCl is bond to Ca (CROSS; SCHLESINGER, 1995). The interaction between P and Ca can be observed in non-acidified fertilizers, as rock phosphate (GATIBONI et al., 2007). P_{HCl} is a moderately labile P fraction and is necessary for dissolution of RP in the soil to occur and enable uptake of P and Ca by plants (FREITAS; BANERJEE; GERMIDA, 1997). Hanafi, Syers and Bolan (1992) observed a greater dissolution of two RPs, Gafsa and Christmas Island, under open-leaching than under closed-incubation, which they attributed mainly to the removal of Ca released from the dissolution of RP through leaching. The P residual extracts highly recalcitrant P forms. Transformations in these recalcitrant P forms are related in long time soil P fertilization conditions (GATIBONI et al., 2007) and in general is not observed in short time studies, as the present study (56 and 65 days after transplanting). However, when RP was applied the fractionation of P was not able to detect all the P in the rhizosphere. This could be occurred as a fact that P_{total} is estimated by the sum of all determined Hedley's fractions, which underestimate P_{total} if there were refractory fractions in the insoluble residue, as related by Roberts et al. (2015).

TSP increased P_{Total} but in contrast showed the lowest mycorrhizal colonization, with a high labile P fraction P_{AER} and Pi_{NaHC03} remaining in the soil after cultivation, and excessively available in the soil, more than necessary by the crop. It is well known that interplay of signals coordinates the symbiosis between host plant and fungal (GIOVANNETTI et al., 1980), and that high P levels in soil inhibit mycorrhizal development and root colonization (ABBOTT; ROBSON, 1984). These results are supported by the P balance, which shows that the P applied was sufficient to provide plant P uptake. In other hand, when RP was combined with filter cake, the mycorrhizal colonization was decreased. Filter cake is a source of organic matter which promotes changes in soil chemical properties, as N, P, and Ca availability increments, increasing cation exchange capacity (CEC), and reducing concentration of exchangeable aluminum (KORNDÖRFER; ANDERSON, 1997). The increase of P availability may have promoted a reduction of the symbiosis with fungal communities under filter cake in the presence of RP. These results were verified by molecular analysis, where two fungal groups were formed, with a large separation between with and without filter cake, presenting more than 50% of similarity, which shows that absence of organic matter alters the

fungal composition. Fungi decompose complex organic material and assimilate into their tissues a larger proportion of the organic materials (BRADY; WEIL, 2010). Beneficial effects of filter cake on physical and biological soil properties were also observed by Prado, Caione and Campos, (2013).

Bacteria community is very sensitive to environmental conditions, and phosphate solubilizing bacteria group are an important key in the system which has a broad range of enzymatic capacities (BRADY; WEIL, 2010). Indeed, different fertilizer conditions, either mineral fertilizer or filter cake application, are likely to change the bacterial community. A group of bacteria was formed under NP and RP fertilization, showing a similarity between bacterial communities under these conditions. However, the bacterial community under RP had a disperse behavior, what may have promoted bacterial community variability to increase solubilisation, which did not occur under NP. Under TSP application, the bacterial community formed another distinct group, showing about 70% of similarity. As observed, the activity of P-solubilisation by microorganisms is affected by the presence of soluble P (MIKANOVA; NOVAKOVA, 2002), what has changed the communities in our study.

Number of tillers per plant was affected by filter cake application only when associated to rock phosphate (RP). This is supported by Basha (2011), who showed that filter cake use, enriched by RP in the presence or absence of a biofertilizer, in organic onion culture in Egypt, resulted in improved plant nutrition, growth and crop production, in addition to better export quality.

When only filter cake was applied under rates, remobilization of P could be taking place from moderately labile fractions to labile fractions. Moderately labile fraction P_{HCl} showed higher means when filter cake was applied in PF, than applied in BS. Filter cake is a source of Ca and P, providing both nutrients and increasing P_{HCl}. The non-labile $P_{NaOH0.5}$ decreased Pi, which may be remobilized to moderately and labile fractions promoting the equilibrium. On the other hand, organic $P_{NaOH0.5}$ increased as a fact of filter cake contains around 50% of P as not readily available to plants (DINARDO-MIRANDA; VASCONCELLOS; ANDRADE-LANDELL, 2008) thus, this P composes non-labile Po.

As a consequence, it has increased shoot P uptake, shoot DM and also soil inorganic labile P. Similarly, Almeida Júnior et al. (2011) reported a positive response on sugarcane plants to the addition of filter cake, increasing the levels of P, K, and Cu in the shoot. In general, the highest DM production was observed at FC rate of 10 g DM kg^{-1} and the highest P uptake value was observed at rate of 17 g and 10 g DM kg^{-1}, when applied to bulk soil and planting furrow, respectively, and with higher values of P_{AER} when applied at PF.

Considering filter cake as an alternative source involving high transportation costs and application with incorporation expenses, the ideal option is to increase sugarcane yield at low rates of FC combined with low costs, reflecting in satisfactory P tissue content, between 0.2 and 0.4%. When only filter cake was added, increased Pi and consequently decreased Po, for all fractions analyzed, indicating Po mineralization during the crop cycle. The fraction P_{NaHCO3} showed high organic P when FC was not applied and at high rates of filter cake the amount of Po was almost the same to Pi, indicating high mineralization when organic matter was applied, which increased considerably the Pi_{NaHCO3}. Such increased of Pi_{NaHCO3} could be induced by priming effect, which was defined as the stimulation of soil organic matter (SOM) decomposition caused by the addition of labile substrates (CHENG, 1999; DALENBERG; JAGER, 1989).

4.5 Conclusion summary

A cultivated soil under non phosphate application reduced labile, moderately labile and non-labile fractions of P. Filter cake improved P uptake and increased the availability of labile Pi in the rhizosphere and modified the fungal microbial community. In addition, the combination filter cake with rock phosphate was efficient to improve sugarcane aspects, such as shoot dry matter and number of tillers per plant.

The maximum shoot dry matter and P uptake were observed at rate of 10 g DM kg^{-1} of filter cake when applied in the planting furrow.

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5 FINAL CONSIDERATIONS

Experiments at greenhouse are very suitable to a more precise study of the rhizosphere compared to the field, once the conditions are controlled and the samples are easily taken.

To study rhizosphere, we used a methodology with pots to restrict the root system, but this method did not allow us to use a bigger pot, restricting the time of the experiments to 45 days. This short period may have interfered in P dynamics. However, this was enough to compare the treatments regards to initial sugarcane seedling phase.

We were interested to answer issues regarding to the rhizosphere involving sugarcane cultivars, phosphate sources and filter cake rates and its interaction. For that reason, we carried out four individual experiments, which allowed us to get more conclusive results in each experiment.

The microbiological analyses are very important approaches for helping us to understand the soil fertility and what is happening in the soil phosphorus fractions.