

## Responses of microbial biomass, available phosphorus, and sugarcane yield after filter cake amendment in a tropical soil

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### Abstract

A field experiment was conducted from 2012 to 2015 aiming to evaluate two different P sources (TSP - triple superphosphate and NP - natural phosphate) associated with filter cake (FC) on soil microbial biomass, available P and sugarcane yield. The experiment design was a split-plot with four replications and consisted of different proportions of P sources: T1 - 100% TSP with FC; T2 - 100% TSP without FC; T3 - 66% TSP and 33% NP with FC; T4 - 66% TSP and 33% NP without FC; T5 - 33% TSP and 66% NP with FC; T6 - 33% TSP and 66% NP without FC; T7 - 100% NP with FC; T8 - 100% NP without FC. Application of filter cake increased soil microbial biomass and activity from the first to the third year. At the third year, the mixing of filter cake and TSP improved the status of microbial biomass and activity. Sugarcane yield did not show differences between treatments at the first and second years. However, at the third year, the application of FC associated with 33% of TSP and 66% of NP promoted highest sugarcane yield. In conclusion, the combination of both soluble and natural P sources associated with filter cake seems to be the best strategy for improving soil biological properties and increasing P availability and sugarcane yield in long-term.

**Keywords:** Soil microbial biomass; Respiration rate; *Saccharum* sp.

**Abbreviations:** FC\_Filter cake, TSP\_Triple superphosphate, NP\_Natural phosphate, SMB\_Soil microbial biomass, BR\_Basal respiration, SOC\_Soil organic C.

### Introduction

Brazil is the largest sugarcane producer with assets of 10 million hectares and an average of yield about 74.9 Mg ha<sup>-1</sup> (IBGE, 2016). In this crop system, fertilizers are important inputs for increasing sugarcane yield. However, the high costs of chemical fertilizers contribute to their reduced use by low-income farmers (Sousa et al., 2015). Phosphorus (P), is a limiting nutrient in tropical soils (Moda et al., 2015a). Its application favors the sugarcane establishment and yield (Caione et al., 2015). Although important for sugarcane production, P presents high fixation in tropical soils and it promotes high demand of P fertilization (Cheng et al., 2017). Alternatively, the use of P source with low solubility, such as natural or reactive phosphates, has increasing in sugarcane areas. The main limitation of using these P sources is their low reactivity that promotes a low release of P than soluble sources (Huang et al., 2014). On the one hand, P sources with low solubility can be mixed with soluble sources and, therefore, it increases P releasing for plant uptake (Stamford et al., 2004). On the other hand, organic wastes containing P, such as filter cake (FC), can release this nutrient gradually,

through microbial mineralization, to plants (Santos et al., 2014) and, therefore, P fixation in soil could be decreased.

FC is an industrial waste originated from the sugar clarification process (Santos et al., 2014). This waste is an important P source for sugarcane production (George et al., 2010) and could be used in association with inorganic P sources. This alternative could improve P releasing and consequently increase sugarcane yield. Previous studies have shown that the combination of FC and soluble P increases sugarcane yield (Santos et al., 2014; Moda et al., 2015a,b). Moda et al. (2015b) reported that sugarcane yield increased 19.6% with addition of inorganic P source (360 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>) associated with FC. According to Almeida Junior et al. (2011) the combination of FC with chemical fertilizers can maximize plant yield, reducing the costs of production. In addition, as organic source, FC can stimulate the soil microbial biomass (SMB) and activity. It is interesting since SMB is responsible for organic matter dynamic and nutrient cycling (Balota and Auler, 2011). Also, the improvement of SMB is important due to its influence on soil properties, and consequently, soil productivity (Kaschuk et al., 2010).

However, it is unclear how is the effect of FC associated with inorganic P sources on soil microbial biomass, P availability, and sugarcane yield in tropical soil. We hypothesized that the input of FC associated with inorganic P sources would increase P availability and, therefore, sugarcane yield due to the effect of organic residue on soil microbial biomass and activity. Thus, we evaluated the association of FC with two P sources (triple superphosphate, and natural phosphate) on soil microbial biomass, P availability, and sugarcane yield in a tropical soil.

## Results and Discussion

### Soil microbial biomass

The application of FC increased SMB at both first and third years of evaluation as compared with treatments without FC (Table 1). At the third year, the mixing of FC and soluble P improved the status of microbial biomass as compared with natural P that decreased SMB content. This result suggests that microbial biomass may be limited by P availability in soil (Liu et al., 2012) and the limitation of P for microbial biomass is higher when C inputs are given in great amount, such as FC (Hernández et al., 2015). Therefore, the use of available P, from soluble P sources, associated with FC favors the soil microbial biomass. On the other hand, the lower P availability in soil, promoted by natural P sources, does not release P, in short-term, to SMB.

At the first year, soil respiration varied slightly according the treatments with and without FC (Table 1). However, soil respiration increased from the first to the third year with the application of FC associated with both soluble and natural P sources. The use of 100% of natural P with or without FC decreased soil respiration at the third year. Soil respiration is indicative of microbial activity and shows the ability of SMB in decompose the organic residues (Zhao et al., 2016). Thus, the increase in soil respiration over time may be explained due to the input of organic residues from the first sugarcane harvesting and it influenced SMB at the second and third years. Similarly, the soil microbial activity was driven by the P availability in soil that influenced the C-to-P ratio and, then, stimulated soil respiration (Griffiths et al., 2012). This finding agrees with Liu et al. (2012) that found an enhancement of soil respiration through P fertilization in an old-growth forest soil in China.  $qCO_2$  increased from the first to third year and in all treatments (Table 1). However, at the third year,  $qCO_2$  was higher in soil with soluble than natural P sources. The respiratory quotient measures the losses of carbon dioxide through respiration (Gama-Rodrigues and Gama-Rodrigues 2008) and can also indicate high microbial activity by organic matter decomposition (Tu et al., 2006) or soil disturbance (Totola and Chaer 2002). Since this system did not have disturbance, i.e. slash-and-burning or tillage, this result suggests that the highest respiratory quotient found in soil with soluble P and FC may be associated with organic residues decomposition and also higher P availability (Griffiths et al., 2012). Soil organic C did not show differences between treatments with and without FC (Table 2). However, there was a slight decrease in SOC over time. On the one hand, this result suggests that FC did not increase the organic C content, at least in short-term, due to

the highest biological activity found in these soils. On the other hand, the increase in SMB with application of FC may be important since SMB acts as a pool of nutrients. In fact, the application of FC increased the microbial quotient ( $qMic$ ), through the availability of organic C, since  $qMic$  indicates efficiency of SMB for using organic matter (Araujo et al., 2008).

### Soil available P

The use of P sources (soluble or natural) associated with FC provided more available P than without FC (Table 2). Markedly, soluble P associated with FC showed highest P releasing than natural P source. This result suggests the importance of using FC mixed with soluble P and also confirms the positive effect of P on SMB and activity. In addition, the use of organic sources can gradually release nutrients, such as P, and it can maintain plant growth. The addition of organic waste is an important strategy to protect agricultural soils from the over-exploitation and, also, to maintain soil fertility (Shukla et al. 2015).

### Sugarcane yield

Sugarcane yield did not show differences between treatments at the first and second years (Table 3). However, at the third year, the application of FC associated with natural P source promoted the highest sugarcane yield. This indicates that natural P sources seem to be most promising for increasing sugarcane yield in long-term. The results also show the importance of using different P sources in association with organic sources. Importantly, natural P sources can provide a better residual effect than soluble P sources and, thus, supply P to plants. Considering the average of the three years, we also found that FC associated with natural P source promoted the highest sugarcane yield than soluble P source. It suggests a higher residual effect from FC and natural P source. This finding agrees with Caione et al. (2012) who evaluated different P sources in sugarcane during 3 years and found that the soluble P source only provided significant increases in the first year, while the residual effect of insoluble P increased over time. In addition, the use of FC in sugarcane showed to be important for maintaining and increasing the sugarcane yield. It is a consequence of the highest microbial biomass and activity which improve the soil ability for storing and releasing nutrients, such as P. The results are in agreement with previous studies evaluating FC on sugarcane yield (Santos et al., 2010; Vasconcelos et al, 2015). These authors observed that sugarcane yield significantly increased about 10% with the application of FC as compared with soil without FC. In our study, the association between FC and natural P sources promoted an increase of 12% on sugarcane yield after three years.

## Materials and methods

### Location, climate and soil

A field experiment was conducted during three years at the University of West São Paulo, Presidente Prudente, SP, Brazil

**Table 1.** Effect of P fertilization with triple superphosphate (TSP) and/or natural phosphate (NP) associated with a filter cake on soil microbial biomass (SMBC), basal respiration (BR), and metabolic quotient ( $qCO_2$ ) during three years.

P source	Filter cake					
	with		without		without	
	First year					
	SMBC ( $mg\ C\ kg^{-1}$ )		BR ( $mg\ CO_2\ kg^{-1}\ d^{-1}$ )		$qCO_2$ ( $mgCO_2g^{-1}\ BM\ h^{-1}$ )	
100% TSP	125 aA	121 aA	2.28 bA	3.84 aA	0.76 bcA	1.32 aA
66% TSP + 33% ARP	242 aA	116 aB	6.66 aA	7.19 aA	1.15 abB	2.58 aA
33% TSP + 66% ARP	151 aA	76 aA	6.34 aA	3.31 aA	1.74 aA	1.81 aA
100% ARP	241 aA	109 aB	2.86 bA	4.00 aA	0.49 cB	1.52 aA
	Second year					
100% TSP	286 aA	233 aA	5.28 aA	6.89 aA	0.76 bA	1.23 aA
66% TSP + 33% ARP	296 aA	215 aA	7.68 aA	5.88 aA	1.08 abA	1.13 aA
33% TSP + 66% ARP	314 aA	203 aA	6.93 aA	8.34 aA	0.91 abA	1.71 aA
100% ARP	197 aA	172 aA	9.20 aA	8.61 aA	1.94 aA	2.08 aA
	Thrid year					
100% TSP	272 aA	149 aB	20.6 aA	20.6 aA	3.16 abB	5.74 aA
66% TSP + 33% ARP	176 abA	103 aB	22.6 aA	11.5 abA	5.36 aA	4.66 aA
33% TSP + 66% ARP	143 bA	50 aB	16.8 abA	6.5 bA	3.74 abA	5.40 aA
100% ARP	95 bA	48 aA	6.30 bA	6.1 bA	2.76 bB	5.28 aA

Means followed by same letter, lowercase in the column and uppercase in the line, are not significantly different by Tukey test.

**Table 2.** Effect of P fertilization with triple superphosphate (TSP) and/or natural phosphate (NP) associated with a filter cake on soil organic C (SOC), microbial quotient (qMic), and available P during three years.

P source	Filter cake					
	with		without		without	
	First year					
	SOC org ( $g\ kg^{-1}$ )		qMic (%)		P ( $mg\ dm^{-3}$ )	
100% TSP	6.2 aA	6.1 aA	2.0 bA	2.0 aA	60.6 aA	43.7 aA
66% TSP + 33% NP	5.4 aA	5.7 aA	4.5 aA	2.1 aA	14.2 bA	18.1 bA
33% TSP + 66% NP	5.2 aA	5.0 aA	2.9 abA	1.5 aA	15.3 bA	21.6 bA
100% NP	6.0 aA	5.4 aA	4.0 aA	2.0 aA	20.1 bA	2.9 cA
	Second year					
100% TSP	3.7 aA	3.6 aA	7.7 aA	6.5 aA	46.6 aA	9.6 aA
66% TSP + 33% NP	4.2 aA	3.4 aA	7.1 aA	6.3 aA	20.9 abA	6.9 aA
33% TSP + 66% NP	4.5 aA	3.8 aA	6.9 aA	5.4 aA	6.6 bA	12.8 aA
100% NP	3.9 aA	3.7 aA	5.1 aA	4.6 aA	3.9 bA	5.9 aA
	Third year					
100% TSP	5.4 aA	4.4 aA	5.0 aA	3.4 aA	70.4 aA	7.1 aB
66% TSP + 33% NP	5.4 aA	4.7 aA	3.3 abA	2.2 abA	25.0 bA	9.3 aB
33% TSP + 66% NP	5.3 abA	4.2 aA	2.7 abA	1.2 bB	14.4 bcA	4.3 aA
100% NP	3.9 bA	3.3 aA	2.5 bA	1.5 bA	5.9 cA	3.8 aA

Means followed by same letter, lowercase in the column and uppercase in the line, are not statistically different by Tukey test.

**Table 3.** Effect of P fertilization with triple superphosphate (TSP) and/or natural phosphate (NP) associated with a filter cake on sugarcane yield during three years.

P source	Filter cake							
	With		Without		With		Without	
	Yield ( $t\ ha^{-1}$ )							
	First year		Second year		Thrid year		Mean	
100%TSP	155 aA	159 aA	125 aA	141 aA	84 bA	93 aA	121 bA	131 aA
66%TSP + 33%NP	150 aA	144 aA	146 aA	138 aA	78 bA	74 aA	124 abA	118 aA
33%TSP + 66%NP	159 aA	154 aA	154 aA	128 aA	98abA	78 aA	137 abA	120 aA
100%NP	157 aA	158 aA	153 aA	143 aA	107 aA	76 aB	139 aA	125 aA

Means followed by same letter, lowercase in the column and uppercase in the line, are not significantly different by Tukey test

(22°07'S, 51°26'W). The area has a tropical climate with well-defined rainy and dry seasons. According to Köppen, the regional climate is Aw with average rainfall and annual temperatures of 1800 mm and 25.5°C. The soil is a well-drained sandy loam (15% clay, 8% silt, and 77% sand). Before the experiment, four soil sub-samples (0–20 cm depth) were collected randomly in the field and pooled in a composite soil sample. Soil chemical properties were estimated according to Raij (2001). The content of total organic C, extractable P, K, and Ca were 11 g kg<sup>-1</sup>, 1.0 mg kg<sup>-1</sup>, 2.5 mmol kg<sup>-1</sup>, and 8.0 mmol kg<sup>-1</sup>, respectively.

### **Experimental design**

The experimental design was a randomized complete block design with four replications in a split-plot design. In addition, the plots received an application of 150 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> using triple superphosphate (TSP), and natural phosphate (NP) in different mixtures. The subplots either contained or did not contain an FC (at a dose of 20 Mg ha<sup>-1</sup>) in the planting furrow. Thus, the treatments at planting were as follows: T1 - 100% TSP with FC; T2 - 100% TSP without FC; T3 - 66% TSP and 33% NP with FC; T4 - 66% TSP and 33% NP without FC; T5 - 33% TSP and 66% NP with FC; T6 - 33% TSP and 66% NP without FC; T7 - 100% NP with FC; T8 - 100% NP without FC.

### **Filter cake chemical characteristics**

The chemical analysis of the filter cake was performed according to Malavolta et al. (1997) with the following results (%): 0.9 of N, 0.94 of P, 0.14 of K, 3.41 of Ca, and C/N ratio of 28 (dry weight).

### **Management in the experimental areas**

The area was plowed and limed (500 kg ha<sup>-1</sup>) two months before the planting. Sugarcane was planted, in the first year, spaced 150 cm row intervals using sets of five-bud cane. Before placing the sets into the furrows, the soil was fertilized with 30 kg ha<sup>-1</sup> N, using ammonium nitrate as source, 100 kg ha<sup>-1</sup> K<sub>2</sub>O, using potassium chloride, and 150 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, using triple superphosphate. FC was applied associated with fertilizers before planting. The remaining 100 kg ha<sup>-1</sup> N and 100 kg ha<sup>-1</sup> K were applied at the beginning of sugarcane ratoon development.

### **Analysis of soil microbial activity**

Soil microbial biomass (SMB), activity, organic C, and available P were evaluated three times during the sugarcane cycle, the first evaluation was carried out in October 2013 (first year), the second evaluation in October 2014 (second year), and the third evaluation October 2015 (third year). Three soil sub-samples of 0–20 cm depth were collected from each subplot and placed in plastic bags. They were subsequently homogenized to obtain a sample for chemical and microbial analyses.

The samples were sent to the laboratory where they were dried and sieved and analyzed for soil organic carbon (SOC), determined by oxidation of organic matter with 0.2 M potassium dichromate in an acidic medium, and available P using the resin method (Raij, 2001)

Microbial biomass C (SMB) was estimated by irradiation (Ferreira et al., 1999) and extraction methods (Vance et al. 1987) with a correction factor of 0.21 (Frighetto and Valarin 2000). To determine basal respiration (BR), 50g of soil was incubated in 1-L air-tight sealed jars along with a small flask containing 10 mL of 0,025M NaOH. Evolved CO<sub>2</sub>-C trapped in NaOH was measured after 5 d by the conductometric method of reading the conductivity in an NaOH solution (Rodella and Saboya 1999).

The qCO<sub>2</sub> was obtained from the relationship between respiration and soil microbial biomass and was expressed in mg CO<sub>2</sub> g<sup>-1</sup> SMBC h<sup>-1</sup>. qMIC was calculated by the ratio of SMB and SOC and expressed as a percentage (%).

### **Sugarcane yield**

Annually, sugarcane was harvested to evaluate stalk production. Therefore, samples were taken within 3 m (linear) in two central rows of each subplot and weighed with the aid of an electronic balance to estimate the sugarcane yield per area.

### **Statistical analysis**

Data were analyzed by an analysis of variance (p < 0.05) by test F and mean comparisons were done by a Tukey test at 5% probability, according to the procedures proposed by Pimentel-Gomes (2000).

### **Conclusion**

The application of FC associated with soluble P source improved the status of soil microbial biomass and activity during three years as direct response of P availability in soil. This improvement of soil microbial biomass increased the P availability in the soil over time. However, the combination of soluble and natural P sources associated with FC is the best strategy for improving soil biological properties and increasing P availability and sugarcane yield.

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