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How does gypsum increase the organic carbon stock of an Oxisol profile under sugarcane?

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ABSTRACT

Gypsum is already a well-known soil conditioner, especially in regions with predominantly acidic soils. More recently gypsum has also been recognized as a strategy to increase carbon (C) accumulation in the soil, thus contributing to reduced C emissions agriculture. However, there are still doubts regarding how gypsum influences soil C accumulation and the role of the root system in this process. The objective of the present study was to evaluate the influence of gypsum on C accumulation and the relationships of C with soil chemical properties and development of the sugarcane root system to a depth of 2 m, evaluated in the field with an Oxisol. The experiment was arranged in a randomized complete block design, with four replications and two treatments: control (without gypsum) and gypsum application (5 t ha^{-1}). Sugarcane stalk biomass and straw production were evaluated in the first planted sugarcane and six ratoon crops. At 87 months after gypsum application, after the seventh cut, the soil was sampled in the depth of 0–200 cm, in 20 cm intervals, for evaluation of the root dry weight (RW) and soil chemical properties (S-SO_4^{2-}), calcium (Ca^{2+}), aluminum saturation (Al^{3+} saturation), pH, free fulvic acid carbon (FFAC), total C (TC) and particulate C (PC). Stocks of PC and TC were also estimated. The application of 5 t ha^{-1} gypsum favored the increase of S-SO_4^{2-} and Ca^{2+} contents and induced a reduction in Al^{3+} saturation in the soil profile. In association with these improvements in the chemical properties of the subsurface layers were increases in the RW, FFAC, TC and PC stocks. After 87 months of gypsum application, the TC and PC stocks increased by 12.3 and 2.2 t ha^{-1}, respectively, in the 0–200 cm layer, with the highest gains in the 40–200 cm layer. The multivariate analysis showed that in the 40–200 cm layer of this extremely weathered soil, the effect of gypsum on increasing TC and PC stocks, as well as FFAC, is a result of the reduced Al^{3+} saturation associated with the increase in nutrients such as Ca and S, stimulating greater root development.

1. Introduction

Soil is an important sink for C storage (Lal, 2018), particularly with regard to the subsurface layers, because approximately 37% of the organic C contained in tropical soils is stored in the 100–200 cm layer (Batjes, 2014). In Brazil, changes to land use and agriculture account for approximately 55% of CO_2 emitted into the atmosphere (MCTI, 2016), due to the interference of these activities on the soil C pool (Groppo et al., 2015). On the other hand, sequestration of atmospheric C in agriculture can be achieved by increasing the biomass input, not only from the above-ground plant parts but also the crop roots (Lal, 2018).

Sugarcane is extremely relevant in the Brazilian agricultural sector, with a cultivated area of 8.7 million hectares in the 2017/18 growing season (Conab, 2018). The main products derived from sugarcane are ethanol and sugar, as well as electricity as a by-product (Pippo and Luengo, 2013). In addition to being a C4 plant with high efficiency to transform solar radiation into biomass, sugarcane shows great potential for the fixation of atmospheric carbon. Within five years, sugarcane can accumulate an estimated 3.7 to 4 t C ha^{-1} in the soil (Silva-Olaya et al., 2017).

The input of plant biomass and the absence of soil disturbance induce C sequestration (Sá et al., 2014; Inagaki et al., 2016). Thus, production of both shoot and root biomass favors soil C accumulation. Approximately 13% of the sugarcane shoot biomass is transformed into soil organic C (Robertson and Thorburn, 2007). With regards to roots

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Table 1
Chemical characteristics of the soil prior to liming and fertilization of the experimental area[a].

Layer	pH_{H2O}	Al^{3+}	Ca^{2+}	Mg^{2+}	K^+	CEC[b]	m[c]	V[d]	P	$S\text{-}SO_4^{2-}$	OM[e]	Clay	Silt	Sand
cm		$cmol_c\ kg^{-1}$					%		$mg\ kg^{-1}$		$g\ kg^{-1}$			
0–20	4.9	1.16	0.19	0.06	0.09	8.6	77	4	0.8	4.4	24	620	80	300
20–40	5.0	0.93	0.27	0.02	0.05	6.9	73	5	0.6	3.4	18	650	65	285
40–60	5.0	0.61	0.16	0.01	0.03	5.5	75	4	0.5	2.8	14	660	65	275

[a] According to the methods described by Embrapa (1997), mean values, $n = 4$.
[b] Cation exchange capacity at pH 7.
[c] Aluminum saturation.
[d] Base saturation.
[e] Organic matter by the Walkley–Black procedure.

this percentage tends to be higher, as is the case with other grasses (Bolinder et al., 1999). Moreover, between 55% and 65% of the sugarcane roots die in each cultivation cycle (Otto et al., 2014), thus favoring C input into the soil (Silva-Olaya et al., 2017). However, to determine the contribution of sugarcane roots to the soil C pool the subsurface layers must be evaluated, because although most roots are concentrated in the top 40 cm of soil, studies report root growth to 2 m or even deeper (Ball-Coelho et al., 1992; Smith et al., 2005).

The important contribution of root growth to C storage in the soil profile may be limited by acidity (Briedis et al., 2012), which is related to calcium deficiency and aluminum toxicity. These are some of the main factors associated with the reduction of C input into highly weathered soils (Carmeis Filho et al., 2017; Ferreira et al., 2017). Acidic soils account for approximately 30% of the world's arable land (Von Uexküll and Mutert, 1995). In the case of the Cerrado under natural conditions, a very important region of Brazil for world agriculture, acidic soils are predominant at the surface as well as in subsurface layers (Lopes and Guilherme, 2016).

For the acidic soils of the Cerrado, acidity in the surface layers is mainly adjusted by applying agricultural lime, while for the subsurface layers gypsum is used (Goedert, 1983; de Sousa and Lobato, 2004). Research on gypsum application in the Cerrado began in the 1980s, mainly with the aim of reducing Al^{3+} saturation and providing calcium to the deeper soil layers so as to intensify root growth and increase agricultural crop yields (Ritchey et al., 1980). In this context, several studies have reported higher calcium contents and a reduction in acidity components of the soil subsurface layers (Caires et al., 2011; Dalla Nora et al., 2014), favoring intensified root growth (Farina and Channon, 1988; Caires et al., 2016) and increased agricultural crop yields.

Despite the known benefits of gypsum in relation to soil fertility, information regarding its influence on the soil C pool is still scarce (Inagaki et al., 2016). In our previous study (Araújo et al., 2016), it was demonstrated that gypsum could promote C sequestration in soil cultivated with sugarcane. In the present study, gypsum increased the soil C stock after 50 months of application, resulting in 5.4 and 4.4 t ha^{-1} in the layers of 0–100 and 40–100 cm, respectively. Despite the advances obtained by Araújo et al. (2016), doubts have remained with regards to the residual effect of gypsum for a longer period and its potential to increase C at depths below 1 m, which would represent an important strategy for a low C agriculture. Additionally, although the C content in the subsurface layers is lower than in the topsoil (Rumpel and Kögel-Knabner, 2011), the profile volume is more considerable. Thus, C stored in the subsoil may contribute considerably to the soil C stock (Angst et al., 2018). Additional research is also required to understand how gypsum increases C to a soil depth of 2 m and the role of roots in this process. Therefore, the objective of this study was to evaluate the influence of gypsum on the development of sugarcane roots and their relationship with C accumulation to a soil depth of 2 m.

2. Materials and methods

2.1. Description and management of the experimental area

The experiment was carried out in an experimental area of Embrapa Cerrados, in Planaltina – DF, Brazil (15° 36′ S; 47° 42′ W; 1014 m elevation). The climate was classified as Cwa according to Köppen (Alvares et al., 2013), with annual precipitation and temperature means of 1500 mm and 21.3 °C, respectively. The former, native vegetation was Cerrado woodlands, and the soil was classified as Oxisol (Rhodic; Soil Survey Staff, 1998) or Latossolo Vermelho distrófico, according to the Brazilian Soil Classification (Embrapa, 2013), or Rhodic Ferralsol (IUSS Working Group WRB, 2014).

Spontaneous vegetation consisting predominantly of grasses (*Brachiaria decumbens* and *Andropogon gayanus*) was left to grow in the experimental area, without animal grazing, for 35 years. In October 2008, soil samples were collected in the area from the layers 0–20, 20–40 and 40–60 cm to assess the soil chemical properties (Table 1) according to Embrapa (1997). Soil texture was assessed by the pipette method, and the methodologies for measuring the soil chemical properties are described in Section 2.4.

In February 2009, liming and fertilization rates were defined by de Sousa and Lobato (2004) as described below. Dolomitic limestone (CaO 36% and MgO 17%) was applied by hand at a rate of 7.08 t ha^{-1} (total relative neutralizing power 100%), which is required to raise soil saturation by 50% in the 0–40 layer cm, tilled into the soil to a depth of approximately 20 cm, followed by plowing with plow blades to a depth of approximately 40 cm. After these procedures, magnesium thermophosphate (240 kg ha^{-1} P_2O_5, 93 kg ha^{-1} Mg), potassium chloride (120 kg ha^{-1} K_2O), and as micronutrients 100 kg ha^{-1} fritted trace elements (FTE BR-10 powder, a commercial product containing all micronutrients in the form of oxysulfates and others), were broadcast and incorporated into the soil to a depth of 20 cm with a disk harrow.

In March 2009, *Crotalaria juncea* was sown in the study area (20 seeds m^{-1}; row spacing 45 cm), as widely used in the Center-South region of Brazil when replanting sugarcane plantations. The green manure was cut with chopping mowers when in the flowering stage.

The sugarcane variety RB867515 was planted during the dry season, in July 2009. The area was furrowed to a depth of approximately 40 cm and urea and triple superphosphate were applied in the furrows (Table 2). Three parallel stalk segments were then planted to ensure sprouting of at least 12 buds per meter.

Gypsum ($CaSO_4{\cdot}2H_2O$, with 21.3% Ca^{2+} and 17.2% $S\text{-}SO_4^{2-}$) was applied to the soil surface after sugarcane planting. The gypsum rate was calculated by the expression [Y = 75X], where Y is the gypsum rate (base 15% sulfur) expressed in kg ha^{-1} and X is the clay content (%) in the 40–60 cm layer, according to the official recommendation for perennial crops in the Cerrado region reported by de Sousa and Lobato (2004). Therefore, the gypsum treatments consisted of the control treatment (0 t ha^{-1}) and the recommended rate (5 t ha^{-1}). The experiment was arranged in a randomized complete block design, with

Table 2
Sources and rates of nutrients (N, K_2O, and P_2O_5) applied by fertilization, from the first to the seventh sugarcane cut.

Sugarcane cut	Source	Rate[a] – kg ha^{-1} (N, K_2O, and P_2O_5)	Dates of fertilization
1°	Urea	102[b]	July and November 2009
	Potassium chloride	150	November 2009
	Triple superphosphate	183[c]	November 2009
2°	Ammonium nitrate	120	November 2010
	Potassium chloride	150	November 2010
3°, 4° e 5°	Ammonium nitrate	120	September 2011, 2012, 2013
	Potassium chloride	150	September 2011, 2012, 2013
	Monoammonium phosphate	50	November 2011, 2012, 2013
6°	Ammonium nitrate	150	September 2014
	Potassium chloride	200	September 2014
	Monoammonium phosphate	50	November 2014
7°	Ammonium nitrate	225	September 2015
	Potassium chloride	400	September 2015
	Monoammonium phosphate	50	November 2015

[a] As proposed by de Sousa and Lobato (2004).
[b] The total amount applied represents the sum of fertilizer applied in furrow at planting (42 kg ha^{-1} N) and sidedress fertilizer (60 kg ha^{-1} N).
[c] Total application at planting.

four replications. The 60 m^2 experimental plots consisted of five 8-m rows, spaced 1.5 m apart. A total area of 22.5 m^2 was evaluated, comprising the three 5-m long central rows of each plot, after excluding 1.5 m at either end.

Four supplemental irrigations were applied to the crop after each planting. During each irrigation event, 25 mm of water were applied at intervals of 15 days between them. The first water application was performed in August in all years of the study period. This practice is common in the region for sugarcane planted in the dry season to induce bud sprouting after planting (Fig. 1). In November 2009, side-dressing was applied (Table 2). The planted sugarcane (first cut) was harvested by hand, without burning the leaves, in August 2010.

The second to seventh cuts were always carried out in August, with subsequent chopping of the sugarcane straw left on the soil, using a crushing-shredding device. This operation was applied to chop the sugarcane straw, simulating commercial mechanical harvesting. Seven to ten days after harvest, additional irrigations were carried out in all years to stimulate regrowth in the dry season (Fig. 1). Annual maintenance fertilization of the sugarcane ratoon was applied in November (Table 2).

2.2. Sugarcane stalk biomass and straw production

As described in our previous study (Araújo et al., 2016), the planted sugarcane and ratoons were cut by hand, in a green cane harvest system. The harvested and assessed area consisted of plots of three 5-m long central rows, with a total area of 22.5 m^2. Total stalk yield per hectare, industrial sugarcane fiber (%) estimated from the weight of the pressed wet bagasse and total reducing sugars were evaluated. The stalk biomass was determined as the sum of industrial fiber and total reducing sugars, both on a dry basis. The total stalk biomass was obtained by cumulative harvest along 7 years (planted sugarcane and six ratoons). In order to evaluate the straw quantity at each cut, all straw from the center row of the assessed area (4.5 m^2) was collected and weighed (leaves and leaf sheaths), withdrawing a sample for moisture determination.

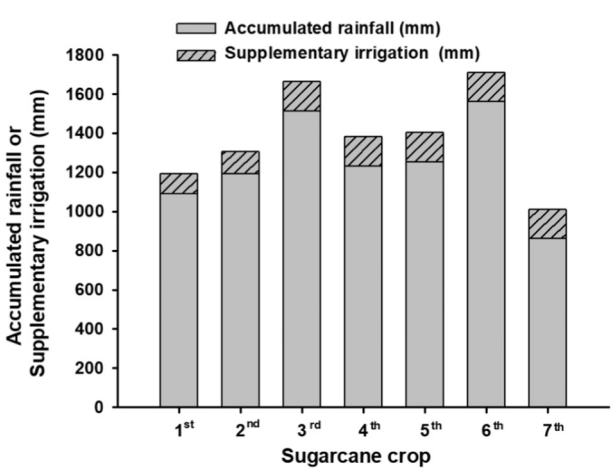

Fig. 1. Cumulative rainfall and supplementary irrigations of sugarcane from the first to the seventh cut.

2.3. Sampling and determination of the root dry weight

Sampling for determination of the root dry weight (RW) was performed in September 2016, one month after harvest of the seventh sugarcane cut, in 10 layers of 20 cm depth each, to a depth of 200 cm, using a hand auger (1571 cm^3). The evaluations were based on nine sub-samples obtained from the following positions: at distances of 20, 47.5 and 75 cm from the second, third and fourth plant rows as shown in the root sampling scheme (Fig. 2).

During field sampling the roots were pre-washed for soil separation, using a 0.5 mm mesh sieve. Thereafter, the roots were placed in plastic bags, stored in a refrigerator (4 °C) and later cleaned on a tray with water, removing impurities by hand. After root cleaning, the samples were oven-dried at 65 °C for subsequent RW determination.

2.4. Determination of soil chemical properties

The soil samples used to determine the chemical properties and soil C contents in the form of free fulvic acid (FFAC), particulate C (PC) and total C (TC) were obtained from soil sampled for the determination of the dry root weight (approximately 150 g soil) (Fig. 2). Thus, nine sub-samples were used to establish a composite sample of each experimental plot and depth.

The samples were homogenized, air dried and passed through a 2 mm mesh sieve to perform soil chemical analyses. The soil chemical properties were determined according to Embrapa (1997). In summary, the pH was determined in water using a 1:2.5 (w/v) soil:solution ratio suspension; 0.5 mol L^{-1} potassium chloride was used as the extractor in the determination of calcium (Ca^{2+}), magnesium (Mg^{2+}), and exchangeable aluminum (Al^{3+}) contents. This solution was left for overnight equilibration. Subsequently, Ca^{2+} and Mg^{2+} were determined by atomic absorption spectrometry (Shimadzu AA-6300) and Al^{3+} by titration with a 0.01 mol L^{-1} sodium hydroxide solution; sulfur (S-

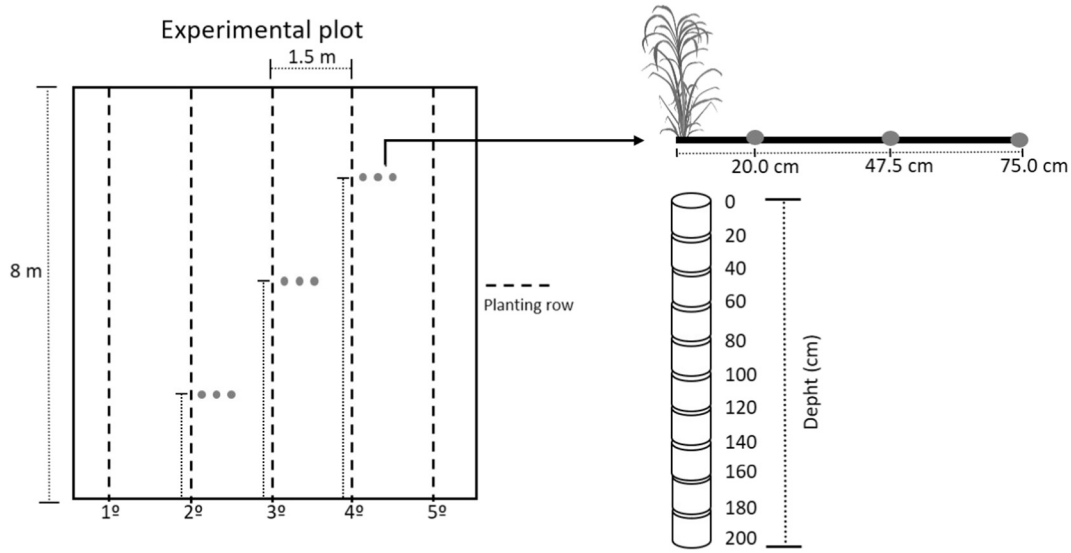

Fig. 2. Diagram of the nine sampling points in an experimental plot, to determine the sugarcane root dry weight and illustration of three sampling points using a hand auger.

SO_4^{2-}) was extracted with 0.01 mol L^{-1} calcium phosphate monobasic monohydrate at the soil:solution ratio of 1:5, and quantified by inductively coupled argon plasma atomic emission spectroscopy (Thermo Scientific iCAP 6000). Potassium and phosphorus contents were determined using the Mehlich-1 extractor and analyzed by flame spectrophotometry (K^+). Aluminum saturation was calculated as follows: m, % = $(Ca^{2+} + Mg^{2+} + K^+) / Al^{3+}$.

2.5. Determination of free fulvic acid, total and particulate carbon

The differential solubility technique was used (Swift, 1996) to obtain the FFAC. Air-dried soil samples were treated with 2 mol L^{-1} phosphoric acid solution to solubilize the free fulvic acid. In the extract obtained from FFAC, the C content was determined by dichromatometry.

The soil was crushed, and approximately 100 mg soil sample were ground and fully sieved (0.149 mm) to determine TC by dry combustion in an elemental analyzer (Vario MACRO cube, Elementar).

Particulate C was determined from the physical particle-size fractionation as described by Cambardella and Elliott (1992), using a sodium hexametaphosphate solution as a soil dispersion agent. The air-dried samples were sieved to 2 mm. Thereafter, 20 g of the air-dried samples were placed in plastic bottles (volume of 500 mL), and 80 mL of sodium hexametaphosphate was added at a concentration of 5.0 g L^{-1}. This mixture was shaken for 16 h in a horizontal shaker at 150 oscillations min^{-1}. After this process, the entire content of the bottle was placed in a 53 μm sieve and washed with a weak jet of distilled water. The material retained on the sieve, defined as particulate fraction (> 53 μm), was dried at 50 °C. After drying, the sample was ground in a porcelain mortar and completely passed through a 0.149 mm sieve to determine PC by dry combustion in the elemental analyzer. The carbon associated with minerals (MC) was calculated as the difference between TC and PC.

The TC and PC stocks were estimated by the equivalent-layer technique (Ellert and Bettany, 1995). In each layer, C stocks were calculated by multiplying the C contents by the soil density (Table S1) and the layer thickness. For this purpose, a total of three undisturbed samples per experimental plot were collected from seven layers (0–20, 20–40, 40–60, 60–80, 80–100, 100–150 and 150–200 cm), in 5 cm high rings in the central position of the first five layers, and in the final position of each of the last two layers (145–150 and 195–200 cm), as shown in the sampling diagram for soil density (Fig. 3).

2.6. Statistical analyses

Two factors were considered in the statistical analysis: gypsum rate and soil depth. The factor "gypsum rate" was represented by gypsum and application and non-application, corresponding to the plots. The factor "soil depth" was represented by layers allocated to the sub-subplots. The following statistical model was used for analysis of variance (ANOVA) of the stalk and straw biomasses, Ca^{2+}, S-SO_4^2, Al^{3+} saturation, pH, RW, FFAC, PC and TC stocks and soil density in the layers:

$$Y_{ijm} = \mu + B_i + T_j + error1_{(ij)} + C_m + TC_{jm} + error2_{(ijm)}$$

where Y_{ijm} = observed value of the response variable in treatment j, block i, layer m; μ = overall data mean; B = block effect (i = 1,2,3,4); T = effect of gypsum rates (j = 1,2); C = soil depth effect (m = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10); $error1_{(ij)}$ = experimental error associated with treatment j, in block i; $error2_{(ijm)}$ = experimental error associated with treatment j, in block i, in layer m.

An analysis of variance (ANOVA) was performed with the PROC MIXED tool in the software SAS 9.1 (Statistical Analysis System) and in case of significance, the Tukey's test ($P < 0.05$) was used to distinguish the means (Little et al., 1996).

A principal component analysis (PCA) was carried out using the packages "FactoMineR" and "factoextra" contained in the statistical software R (version 3.4.0) (R Core Team, 2017) to identify which of the study variables (Ca^{2+}, S-SO_4^{2-} and Al^{3+} saturation, RW, FFAC and stocks of PC and TC) contributed with greatest weight to the linear combination of the first two principal components. This analysis addressed the variables with commonality values ≥0.6.

3. Results and discussion

3.1. Sugarcane stalk biomass, total straw production, root dry weight and soil chemical properties

Gypsum application increased the stalk biomass of the planted sugarcane and the six ratoon crops. Stalk biomass production with and without gypsum application was 237.3 and 200.0 t ha^{-1}, respectively, representing a total increase of 18.6% (Fig. 2a). Similar results were obtained for straw production, whereby gypsum application resulted in a 22.3% gain. Compared to our previous study conducted after four years of gypsum application (Araújo et al., 2016), the present study showed that stalk and straw productions continued to increase until the

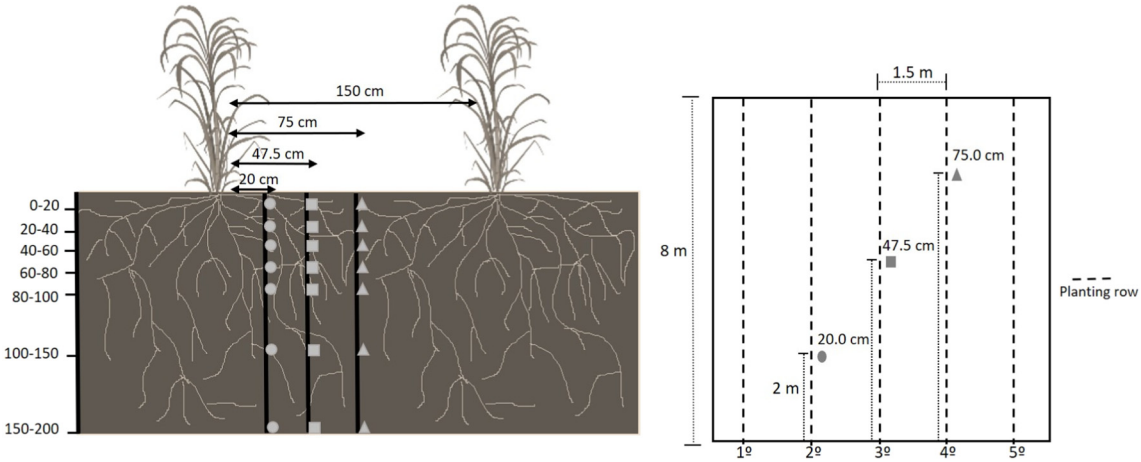

Fig. 3. Diagram of the sampling to determine soil bulk density, consisting of three sub-samples in the second, third and fourth plant row, at a distance of 20, 47.5 and 75 cm from the row, respectively.

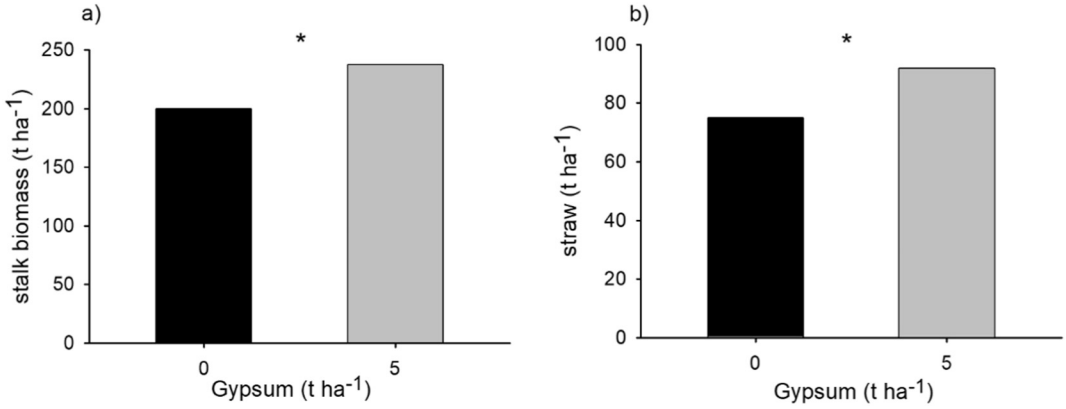

Fig. 4. Cumulative stalk (a) and straw (b) biomass productions in seven sugarcane cuts (planted sugarcane and six ratoon crops) in response to gypsum application at planting. * Significant by the Tukey's test at $P < 0.05$.

seventh sugarcane cut at 87 months after gypsum application. This long-term residual effect of gypsum on the increased stalk biomass and total straw production represents an important contribution to soil C storage, as highlighted by Galdos et al. (2009) (Fig. 4).

Our study demonstrates that the residual effect of gypsum promotes the enrichment of Ca^{2+} in the 40–200 cm layer, even after seven sugarcane cuts. Our previous study (Araújo et al., 2016) had already shown that gypsum increases the Ca^{2+} content at up to 100 cm deep. However, the residual effect of gypsum in deeper soil layers (100–200 cm) had remained unknown. As demonstrated below, this increase of Ca^{2+} in deep soil layers is important for the development of roots, and consequently C storage. The greatest increase in Ca^{2+} content was observed in the 40–100 cm layer, followed by the 100–200 cm layer (Fig. 5a). An increase in sulfate content ($S\text{-}SO_4^{2-}$) in the soil profile was, however, only observed in the 0–120 cm layer (Fig. 5b). In response to the application of 5 t ha^{-1} gypsum, an increase in Ca^{2+} and $S\text{-}SO_4^{2-}$ in the 60–120 cm layer was observed, along with a reduction in Al^{3+} saturation (Fig. 5c). Despite the increased Ca^{2+} levels in the soil

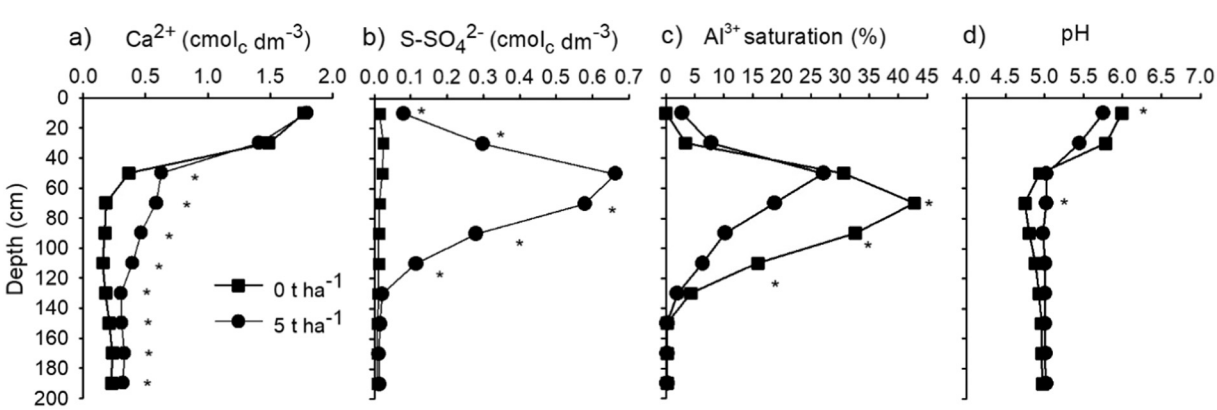

Fig. 5. Contents of exchangeable Ca^{2+} (a), $S\text{-}SO_4^{2-}$ (b), aluminum saturation (Al^{3+}) (c) and pH (d) in soil samples 87 months after gypsum application to a sugarcane plantation. * Significant according to the Tukey's test at $P < 0.05$.

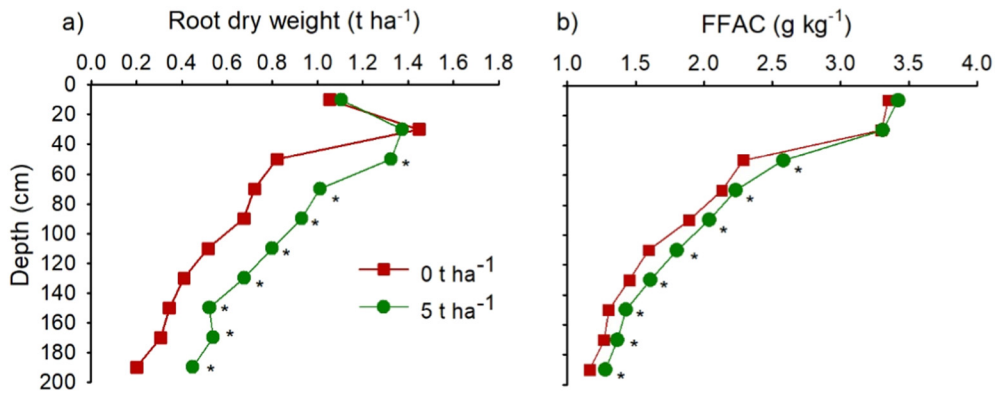

Fig. 6. Root dry weight (a) and free fulvic acid carbon (FFAC) (b) in soil samples 87 months after gypsum application to the sugarcane plantation. * Significant according to the Tukey's test ($P < 0.05$).

layers below 120 cm with the use of gypsum, no decrease in Al^{3+} saturation was observed, possibly due to the low Mg^{2+} contents in these layers (Table S1).

Gypsum contains approximately 19% calcium and 15% sulfur (de Sousa and Lobato, 2004), promoting an increase of these nutrients after gypsum application to the soil (Fig. 5a, b). Due to the high solubility of gypsum and low reactivity (adsorption) of sulfate in the upper layers, especially in agricultural soils with corrected acidity, gypsum application promotes leaching and increases sulfate contents and exchangeable bases in the soil profile (Pavan et al., 1984; Zoca and Penn, 2017). The long-term residual effect of gypsum observed is due to the generally high adsorption capacity of sulfate in the subsurface layers of the acidic soils of variable charge, associated with the concomitant development of a negative charge, allowing retention of bases in these layers, confirmed by the decrease in pH values (Fig. 5d) (Marcano-Martinez and McBride, 1989; Bolan et al., 1991).

As observed for Ca^{2+}, the present study shows that gypsum increased the sugarcane root growth in the deeper soil layers (40–200 cm) (Fig. 6a). The means that RW increases in the 40–100 and 100–200 cm soil layers were 46 and 67%, respectively. Considering the entire evaluated soil profile, RW reached 6.2 and 8.3 t ha^{-1} without and with gypsum application, respectively. Araújo et al. (2016) showed that gypsum increases the Ca^{2+} and C contents at depths up to 100 cm. The current study now demonstrates that in addition to the enriching of Ca^{2+}, gypsum increases RW in the deeper soil layers (100–200 cm).

An appropriate availability of Ca^{2+} in the subsurface layers positively influences sugarcane yields (Landell et al., 2003), which is related to its important role in cell growth (Hawkesford et al., 2012). An adequate Ca^{2+} content in the soil leads to intensified root growth (Ritchey et al., 1982).

Because SO_4^{2-} did not increase in soil layers below 120 cm (Fig. 5b), other inorganic or organic anions may have favored higher Ca^{2+} contents in these layers (Fig. 5a). Nitrates and chlorides from fertilizers (urea, ammonium nitrate and potassium chloride) and low and high molecular weight organic acids that constitute the soluble organic matter may promote the movement of cations to deeper soil layers (Ritchey et al., 1980; Franchini et al., 2001; Pavinato and Rosolem, 2008). The high sulfate contents in the layers < 100 cm in the gypsum treatment inhibit nitrate and chloride adsorption (Donn et al., 2004), favoring their movement and that of the accompanying cations (Ca^{2+}) to deeper layers, which could partly explain the higher Ca^{2+} accumulation in these layers (Fig. 5a).

Gypsum application also favored the increase of FFAC in the layers at depths > 40 cm, as similarly observed for Ca^{2+} and RW (Fig. 6b). Distribution of the percent increases of FFAC in the 0–40, 40–100 and 100–200 layers in response to gypsum was 6, 41 and 53%, respectively, indicating that the highest FFAC gains occurred in the soil depth layers of > 40 cm. Increasing this SOM fraction in deeper soil layers may result in higher C storage and soil quality (SQ) since this fraction is a good indicator of SQ (Souza et al., 2016a). Additionally, the increase in the FFAC after gypsum application (Fig. 6b) may also have favored a more intensive Ca^{2+} movement, especially in the 100–200 cm layer where the effect of S-SO_4^{2-} was weak, since FFAC is moveable in the soil and therefore subject to displacement to deeper layers (Souza et al., 2016b).

The contribution of sugarcane roots to increase C in the soil, especially in the deeper soil layers (Silva-Olaya et al., 2017) is an important effect which increases over the long term. Around 55 to 65% of the sugarcane root system dies after harvesting (Otto et al., 2014), and new roots grow from the new shoots. Thus, C from this dead root tissue contributes to soil C. Aside from the contribution of C from root tissue, living roots are also an important source of soluble C (Ota et al., 2013) as well as C input to the soil by rhizodeposition (Pausch and Kuzyakov, 2018).

3.2. Particulate, mineral-associated and total soil carbon stocks

The particulate C (PC) stock in the 0–200 cm layer was 17.1 t ha^{-1} without gypsum and 19.3 t ha^{-1} with gypsum application. Accordingly, gypsum promoted a gain of 2.2 t ha^{-1} PC (Fig. 7a), of which 7, 29 and 64% were observed in the 0–40, 40–100 and 100–200 cm layers, respectively. Our previous study (Araújo et al., 2016) showed that after four sugarcane cultivations, gypsum application increased the PC stock in the 40–100 cm layer by 18.4%. In the current study, it is evident that the residual effect of gypsum may be longer, and that over time the PC gain is higher in the deeper soil layers (100–200 cm), thus representing an important strategy for the storage and stability of soil C. Moreover, increases in the PC stock were similar to those observed for RW (Fig. 6a). Thus, the increase in PC stock is mainly related to the root growth induced by gypsum. As pointed out by Ontl et al. (2015), the root volume and soil PC are strongly correlated. Carmeis Filho et al. (2017) also described a positive effect of gypsum-limestone combination on PC contents, since they observed an increase in the PC/TC ratio in the 0–10 and 20–40 cm layers after an application of 2.1 t ha^{-1} gypsum to annual crops.

The same trend as that of PC stock was observed for the stock of mineral-associated C (MC) (Fig. 7b). Gypsum application promoted an increase of 10.1 t ha^{-1} MC in the evaluated soil profile (0–200 cm), with the highest increases in the layers below 40 cm (81.2%). These results demonstrate that gypsum increases C with different degrees of stability, reinforcing the strategy of gypsum application with regards to C accumulation. Thus, it is clear that the effect of gypsum on the soil C accumulation occurs through the increase of different SOM fractions, both labile (PC) and those considered stable (MC).

Roots are rich in lignin, which is mineralized slowly, and therefore are important contributions to the formation of humic substances in the

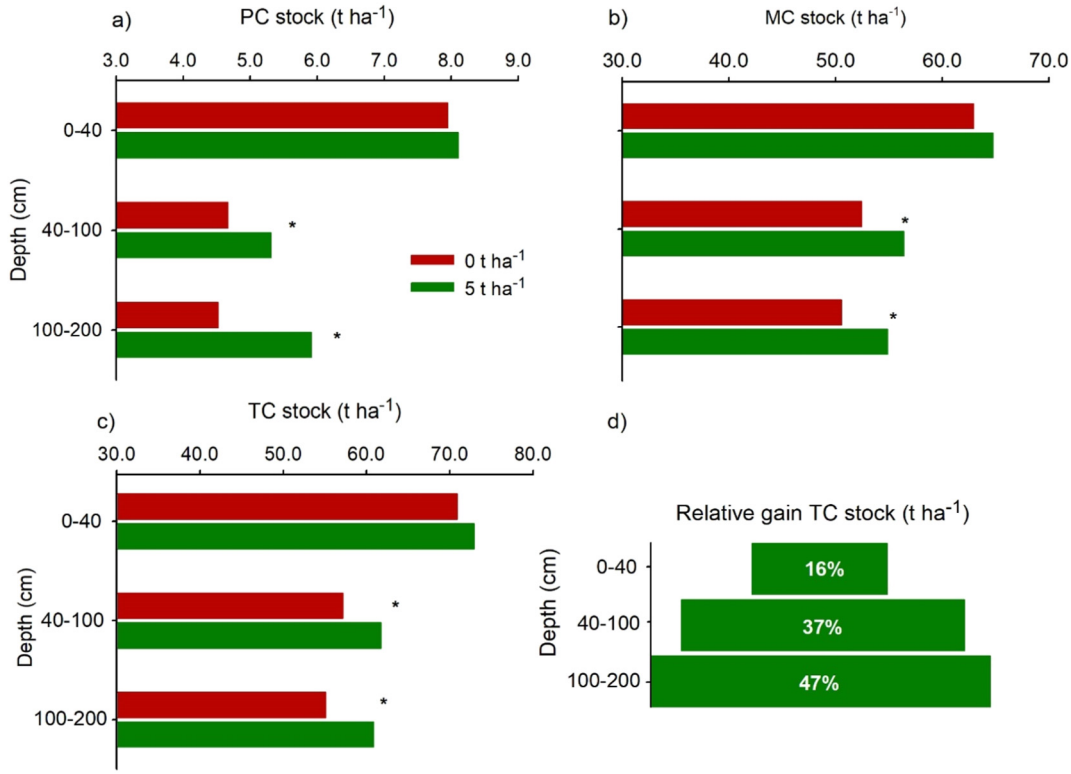

Fig. 7. Particulate carbon (PC) (a), mineral-associated carbon (MC) (b) and total carbon (TC) stocks (c) in soil samples 87 months after gypsum application to the sugarcane plantation; relative gain due to gypsum application in the TC stock in the 0–40; 40–100 and 100–200 cm layers (d). * Significant according to the Tukey's test ($P < 0.05$).

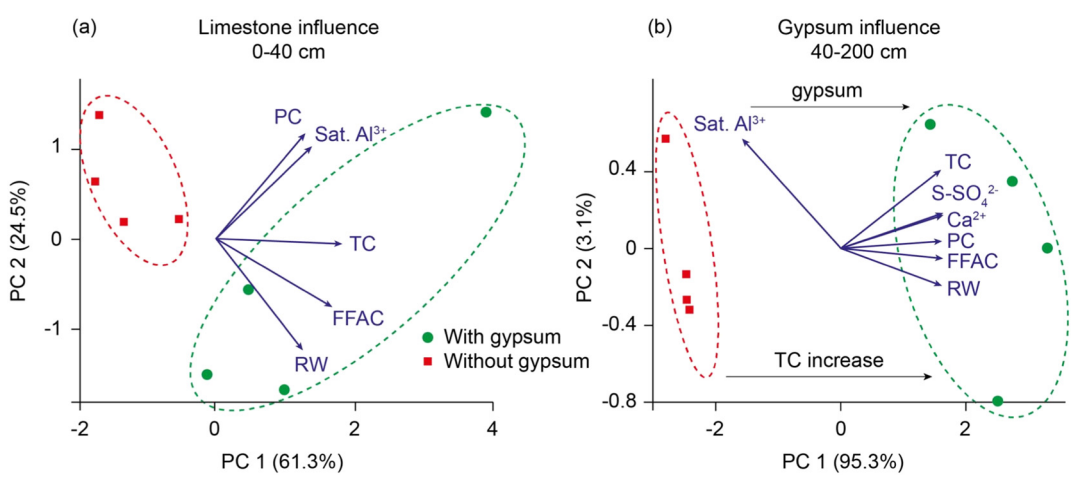

Fig. 8. Principal component analysis (PCA) ordering diagrams involving soil chemical properties, sugarcane roots and soil carbon stocks. Sat.Al: aluminum saturation of the soil; Ca^{2+}: calcium contents; $S\text{-}SO_4^{2-}$: sulfate contents; RW: root dry weight; PC: particulate carbon stock; FFAC: free fulvic acid carbon; TC: total carbon stock.

soil (Zech et al., 1997). Another importance aspect of gypsum with regards to C storage in soil is the supply of Ca^{2+}, since this nutrient can stabilize organic C by intensifying soil aggregation, promoting the physical protection of C (Rowley et al., 2018). In addition, Ca^{2+} induces the formation of a cationic bridge between organic matter and the clay fraction, reducing C mineralization by the soil microorganisms (Tisdall and Oades, 1982; Briedis et al., 2012).

The total C (TC) stocks in the treatments without and with gypsum were 183 and 195 t ha^{-1}, respectively, in the 0–200 cm layer. Therefore, the increase in TC stock in response to gypsum application was 12.3 t ha^{-1}. The highest gains were observed in the 40–100 and 100–200 cm layers, which accounted for 37 and 47% of the total observed increase (Fig. 7c, d). This increase in TC represented an average accumulation rate of 1.76 t ha^{-1} year^{-1} in the 0–200 cm layer due to gypsum application. Therefore, gypsum application to sugarcane fulfills the objective proposed by the ABC Plan, a Brazilian government effort to establish agriculture with low C emission, seeking to fixate CO_2 in the plants and soil of agricultural areas (MCTI, 2016).

The deepest soil layers have a high potential for C sequestration since they have low C contents and are therefore not saturated (Rumpel and Kögel-Knabner, 2011), in addition to the larger soil volume than the surface layer (Angst et al., 2018). Furthermore, higher C levels in deeper soil layers, where the oxygen flow is reduced, may represent an important strategy for long-term C accumulation (Lal, 2018). Considering that the gains in TC stock in this study were highest in the 40–200 cm layer, gypsum may be indicated as a successful technology

for implementing agriculture with low carbon emission.

3.3. Multivariate analysis involving gypsum application

The principal component analysis (PCA) was carried out to determine a pattern in the treatments with and without gypsum application in the layers treated with limestone (0–40 cm) and gypsum (40–200 cm), taking the following properties into consideration: Ca^{2+}, SO_4^{2}, Al^{3+} saturation, RW and FFAC, PC and TC stocks (Fig. 8).

In PC1 and PC2 in the 0–40 cm layer, contribution of the Ca^{2+} and SO_4^{2-} contents to the commonality value was low (< 0.6) (Fig. 8a). This can be explained by the fact that this is the layer influenced by limestone, as well as phosphorus accumulation added in the fertilizers. The SO_4^{2-} of gypsum is moved to deeper soil layers due to the pH increase with liming and competition with the phosphate anion, carrying Ca^{2+}.

In the 40–200 cm layer, PC1 corresponded to most of the total variation of the analyzed parameters, i.e., 95.3% (Fig. 8b). Grouping of the variables by PCA clearly showed that in the layer influenced by gypsum (40–200 cm), this input promotes C accumulation in the soil as a result of the improved chemical properties, such as higher Ca and S and reduction of Al^{3+} saturation, resulting in intensified development of the root system and greater accumulation of the C fractions, mainly PC and FFAC. As a result, the TC stock in the soil subsurface of this environment is increased. Therefore, due to the higher root growth favored by gypsum application, the main factor that contributed to soil TC storage was PC.

4. Conclusions

The results of this study help explain how gypsum increases soil C sequestration. The influence of gypsum is primarily due to the improvement of chemical properties in the subsurface soil layers (40–200 cm), with increased Ca^{2+} and S-SO_4^{2-} contents and reduction of Al^{3+} saturation. Improvement of the chemical environment due to gypsum application favors sugarcane root development. The good relationship between root dry weight and C stocks allows for concluding that roots are the main C supplier in acidic soils treated with gypsum. Multivariate analysis confirmed that the increment of TC stock in the subsurface layer was favored by gypsum application. It was therefore concluded that the characteristics of gypsum indicate it as an important strategy of soil conditioning when considering agriculture with low carbon emissions.

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