



Three-Year Soil Carbon and Nitrogen Responses to Sugarcane Straw Management

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Abstract

Green harvest sugarcane management has increased soil organic C and N stocks over time. However, emerging sugarcane straw removal to meet increasing bioenergy demands has raised concerns about soil C and N depletions. Thus, we conducted a field study in southeast Brazil over nearly three years (1100 days) for assessing soil C and N responses to increasing sugarcane straw removal rates. In order to detect the C input as a function of the different amounts of straw over three years, a field simulation was performed, where the original soil layer (0–0.30 m) was replaced by another from an adjacent area with low total C and $\delta^{13}\text{C}$. The treatments tested were as follows: (i) 0 Mg ha⁻¹ year⁻¹ (i.e., 100% removal), (ii) 3.5 Mg ha⁻¹ year⁻¹ (i.e., 75% removal), (iii) 7.0 Mg ha⁻¹ year⁻¹ (i.e., 50% removal), (iv) 14.0 Mg ha⁻¹ year⁻¹ (i.e., no removal), and (v) 21.0 Mg ha⁻¹ year⁻¹ (i.e., no removal + extra 50% of the straw left on the field). The results showed that sugarcane straw removal affected the soil C and total N pools. In the first 45 days of straw decomposition, a small but important straw-derived C portion enters into the soil as dissolved organic carbon (DOC). The lower the straw removal rate, the higher was straw-derived DOC content found into the soil, down to 0.50 m depth. After 3 years of management, keeping sugarcane straw on soil surface significantly increased C and N stocks within surface soil layer (0–0.025 m). Our findings suggest that under no straw removal management (i.e., 14 Mg ha⁻¹), approximately 364 kg ha⁻¹ of C and 23 kg ha⁻¹ of N are annually stored into this low-C soil. The contribution of the straw-derived C (C-C4) to the total soil C increases over time, which accounted for about 60% under no straw removal rate. The greatest contribution of the C storage preferentially occurs into the fraction of organic matter (< 0.53 μm) associated with soil clay minerals. We concluded that indiscriminate sugarcane straw removal to produce cellulosic ethanol or bioelectricity depletes soil C stocks and reduces N cycling in sugarcane fields, impairing environmental gains associated with bioenergy production. Therefore, this information, linked with other agronomic and environmental issues, should be taken into account towards a more sustainable straw removal management for bioenergy production in Brazil.

Keywords Crop residues · Dissolved organic C · Soil C and N stocks · Bioenergy production

Carlos C. Cerri is deceased. This paper is dedicated to his memory.

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Introduction

Brazil is the largest sugarcane producer in the world, representing 40% of global production [1]. In the 2016/2017 season, 9.1 Mha were cultivated with sugarcane in Brazil, resulting in a stalk production of 657 million of tons. About half of this production (47%) was destined to produce 39 million of tons of sugar and the rest (53%) to produce 28 billion liters of ethanol [2]. Historically, sugarcane management included burning of straw before harvesting. However, due to socio-economic and environmental issues, since beginning of 2000s, burnt harvesting has been gradually replaced

by the unburnt harvesting (i.e., green harvesting), especially in São Paulo state, where more than 90% of fields are harvested without burning [3].

Green sugarcane harvesting results in large amounts of straw (i.e., 10–20 Mg ha⁻¹ of dry mass) left on the soil surface every year [4]. Several studies have shown that C inputs annually added to soil through sugarcane straw resulted in soil C and N stocks accretion over time [5–10]. Cherubin et al. [9] have compiled in-field available data from studies carried out in central-southern Brazil and found an average rate of soil C accretion in sugarcane areas without burning of 0.81 and 1.37 Mg ha⁻¹ year⁻¹ for the 0–0.3 and 0–1.0 m layers, respectively. Two recent studies predicted 30-year soil C changes induced by sugarcane straw management using DayCent model [8, 10]. Carvalho et al. [8] verified that the maintenance of sugarcane straw on the soil surface would increase soil C stocks into 0.09 and 0.19 Mg ha⁻¹ year⁻¹ in two study sites in central-southern Brazil. Oliveira et al. [10] found similar average accretion rate of soil C stocks (0.11 Mg ha⁻¹ year⁻¹) when sugarcane straw is left on the soil surface in this same region.

In order to meet increasing global demands for bioenergy and accomplish international commitments (e.g., iNDC assumed in COP 21, Paris), Brazil intends a significant increase of the bioenergy participation into the energy matrix by next few years [11]. Consequently, a new management of sugarcane straw has emerged in Brazil, in which straw has been collected and used to produce cellulosic ethanol and to co-generate bioelectricity. Both products are strategic towards meet these increasing demands for renewable bioenergies [12] as well as increase the productivity and profits of the sugarcane industry [13].

In light of this promising scenario, the total or partial sugarcane straw removal from the field has raised concerns about potential soil organic matter (SOM) depletion and its impacts on the ecosystem functioning [9, 14]. It also has driven the demand for additional studies to better comprehend the implications of straw management to avoid negative impacts, especially in terms of agronomic and environmental issues [14]. Aboveground, crop residues are the main C-residue source to the soil in the current bioethanol sector and that indiscriminate removal of crop residues to produce cellulosic biofuels can reduce the soil C stocks and attenuate GHG mitigation in comparison with fossil fuels [8]. The magnitude of the C losses resulting from residue removal varies by soil texture, tillage operations, weather conditions, and organic residue use [8, 10].

Although recent studies modeled soil organic carbon (SOC) changes induced by straw removal in Brazilian fields [8, 10], to our knowledge, there are no studies assessing in-field soil C changes induced by rates of sugarcane straw removal throughout more than one crop season in Brazil. We hypothesized that successive sugarcane straw removal

depletes soil C and N over time. In order to test this hypothesis, we conducted an in-field study to assess soil C and N responses to sugarcane straw management throughout three crop years. Specifically, we aimed to (i) assess the short-term dynamics of dissolved organic C (DOC) into the soil after sugarcane straw deposition, (ii) quantify soil C and N stock changes induced by straw removal rates, (iii) determine how much soil C is derived from the sugarcane straw, and (iv) evaluate C distribution within each physical fraction of the SOM.

Material and Methods

Site Description

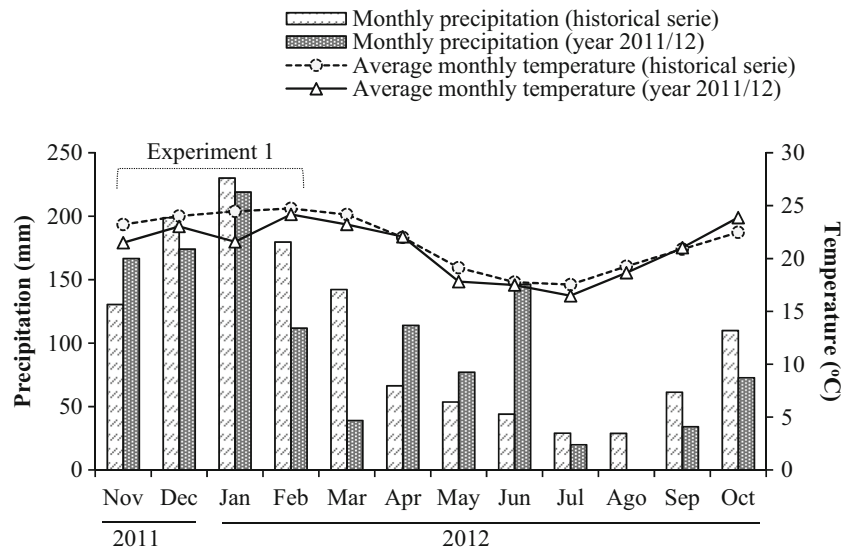
Two field experiments were carried out in the experimental area of Sugarcane Technology Center—“Centro de Tecnologia Canavieira—CTC” (Lat. 22° 41' 26" S, Long. 47° 33' 28"), located in Piracicaba, São Paulo state, southeastern Brazil. The regional climate is humid tropical, Cwa type (Köppen classification), with dry winter and wet summer. The historical monthly precipitation (mm) and average monthly temperature (°C), as well as these meteorological information from November 2011 to October 2012 (first experimental year), are shown in Fig. 1. The soil of the experimental area was classified as an Oxisol (i.e., *Latossolo Vermelho-Amarelo distrófico*—LVAd by Brazilian Soil Classification System [15]), with a sandy-clay-loam texture (300 g kg⁻¹ of clay content).

Experimental Design

The experimental area was cultivated with CTC 15 sugarcane variety, planted in May 2009 and mechanically harvested after 18 months in November 2010, resulting in 14 Mg ha⁻¹ dry mass of straw left on soil surface. After plant cane harvesting, two field experiments were established aiming to detect the soil C changes induced by different rates of straw removal. The amounts of straw left on soil surface in both field experiments were as follows: (i) 0 Mg ha⁻¹ year⁻¹ (i.e., 100% removal), (ii) 3.5 Mg ha⁻¹ year⁻¹ (i.e., 75% removal), (iii) 7.0 Mg ha⁻¹ year⁻¹ (i.e., 50% removal), and (iv) 14.0 Mg ha⁻¹ year⁻¹ (i.e., no removal). In order to evaluate an extra amount of straw, a treatment with 21.0 Mg ha⁻¹ year⁻¹ (i.e., no removal + extra 50% of the straw left on the field) was included. The experimental design was randomized block with split-plot in time and five replications.

In order to give a better understanding of the straw decomposition, at the time of the experiment implementation, samples of the straw were collected and analyzed in terms of total C, N and biochemical compounds (e.g., lignin, cellulose, and hemicellulose). The total soil C (SOC) and total nitrogen (TN)

Fig. 1 Monthly precipitation (mm) and average monthly temperature (°C) for a historical data series (1917–2014) and for the 2011/2012 season. The period of conduction of the experiment 1 was indicated in the graphic. Source: Departamento de Engenharia de Biosistemas—ESALQ-USP (<http://www.leb.esalq.usp.br/posto/>)



determination were performed by dry combustion according to Nelson and Sommers [16] using an elemental analyzer (Leco CN-2000®, St. Joseph, Michigan). The results indicated that the straw used in the experiments presented C content = 461 g kg⁻¹, TN = 4.3 g kg⁻¹, and C/N ratio = 108.

The concentrations of cellulose and hemicellulose were determined by neutral detergent fiber content (NDF) and acid detergent fiber (ADF) method, while lignin concentration was determined in acid detergent (ADL) with 72% sulfuric acid [17]. All determinations were completed using an Ankom²⁰⁰ fiber analyzer (Ankom Tech., Fairport, NY, 2000). The straw biochemical composition was 115, 403, and 333 g kg⁻¹ of hemicellulose, cellulose, and lignin, respectively.

To evaluate in details the influence of straw removal on the different SOM pools, two different experiments were conducted, as described below.

Experiment 1: Dissolved Organic Carbon - DOC

The first experiment was conducted for 120 days (November 2011 to February 2012) to evaluate the DOC from the process of sugarcane straw decomposition. The relative amount of straw to each treatment was placed inside of a metallic frame with dimensions of 0.64 × 0.64 allocated within inter-row position of the sugarcane area. In each inter-row the metallic frame were spaced five meters from each other. A total of 25 metallic frames were evaluated in this experiment (i.e., 5 treatments × 5 replications). In order to prevent contamination with external material (straw or exogenous material), frames of 0.05 × 0.05 m mesh were used to cover the metallic frames.

Soil solution to determine DOC was sampled at the 0.2 and 0.5 m depths using two tension lysimeters installed inside of each metallic frame. These devices consist of a porous capsule of 0.05 m in diameter coupled to PVC pipes. To collect the solution, vacuum was produced with a manual pump

connected to a plastic tube on the top of the lysimeter. More detail about this methodology can be found in Kolka et al. [18].

Dissolved Organic Carbon and ¹³C/¹²C Ratio

The soil solution sampling was carried out after each rainfall event within the first 120 days, which occurred at 17, 32, 40, 45, 77, 81, 87, 94, 106, and 116 days after the experiment installation. The samples were stored in a cold room with the addition of mercury chloride (HgCl₂ 30 mmol L⁻¹) to preserve and avoid variation in the C concentrations by microbial decomposition. Total soil C was analyzed using a Shimadzu TOC-V-cpn elemental analyzer.

The relative C-straw contribution (%) in the soil solution was verified in two moments, from the 32 to 40 days and from 116 to 120 days after installation. These composite samples were frozen, lyophilized, and then forwarded for δ ¹³C determination. The ¹³C/¹²C isotopic ratio was determined by the release of CO₂ from combustion at 550 °C in a sealed Pyrex tube in the presence of CuO in an elemental analyzer (Carbo Erba EA-110). Gases generated from this combustion were separated through gas chromatography and carried through continuous flux to the mass spectrometer (Finnigam Delta Plus).

Experiment 2: Changes on Soil C and N Stocks

The second experiment was conducted in the same sugarcane field of the previous experiment. This experiment aimed to quantify the contribution of sugarcane straw to supply C and N to the soil.

As changes of soil C and N stocks are difficult to detect in short-term evaluations, we conducted a 3-year experiment based on the following methodology: (i) selected an area

adjacent of the experiment with similar soil type and different $\delta^{13}\text{C}$ values, (ii) collected soil of 0.3-m soil layer at the adjacent area, and (iii) replaced the soil inside of the metallic frames (with dimensions of 1.10 m long, 0.90 m width, and 0.30 m depth) by the one collected in the adjacent area with low contents of total C, N, and $\delta^{13}\text{C}$ (i.e., baseline values: SOC = 6.16 g kg⁻¹; Total N = 0.42 g kg⁻¹ and $\delta^{13}\text{C} = -16.35\text{‰}$). This procedure was performed aiming to detect $\delta^{13}\text{C}$ enrichment over time and consequently to quantify how much C was added by straw decomposition in this period in the sugarcane area. (iv) After these steps, the specific amounts of straw (i.e., 0, 3.5, 7.0, 14, and 21 Mg ha⁻¹ year⁻¹) were added within each metallic frame. The deposition of the same amount of straw was repeated on the next two years after sugarcane harvesting. The experiment was conducted for three crop seasons (2011–2013), totaling 1100 days of evaluation in order to detect C and N changes induced by the different amounts of straw or different rates of straw removal.

Soil Organic Carbon, Total Nitrogen, and ^{13}C

Soil samples were taken from the 0–0.025, 0.025–0.05, and 0.05–0.10 m layers to quantify the baseline of C, N, and $\delta^{13}\text{C}$ at the installation of the experiment and annually after sugarcane harvesting (i.e., 334, 685 and 1100 days). Five disturbed subsamples were collected to compose a sample within each plot (field replications) in the same depths mentioned above, totaling 75 samples (i.e., 5 straw amounts \times 3 soil depth \times 5 replications) in each sampling campaign. An additional soil sampling was carried out after 469 days, in order to verify the soil C changes within a shorter period, intermediate between the first to the second year after straw deposition. Undisturbed samples were also collected using volumetric cylinder method (785 cm³), and soil bulk density was determined by the relationship between soil dry mass and the cylinder volume.

Soil samples were dried and sieved at 2 mm. From each sample, 20 g was ground and sieved at 100 mesh (150 μm) to determinate total C and N content by dry combustion, according to Nelson and Sommers [16], through a Carbon Analyzer - LECO CN-2000 as well as $\delta^{13}\text{C}$ content by an elemental analyzer (Carbo Erba EA-110) coupled to the mass spectrometer (Finnigan Delta Plus).

Soil C and N Stock Calculations and Estimates of Carbon Origin

For each soil layer, C and N stocks were calculated through the Eq. 1:

$$C \text{ or } N \text{ stock} = C \text{ or } N \times LT \times BD \quad (1)$$

Given that, C or N stock is in Mg ha⁻¹, C or N is element content in percent, LT is soil layer thickness in centimeters, and BD is bulk density in Mg m⁻³.

In order to determine the proportion of soil C derived from sugarcane straw (C4 plant), the total C content derived from sugarcane was quantified at the beginning of the experiment and annually after harvesting, during three crop years.

The $^{13}\text{C}/^{12}\text{C}$ isotopic ratio for each sample is expressed in delta (δ) unit per million (‰) in relation to the international standard Vienna Pee Dee Belemnite (PDB), according to the Eq. 2 [19]:

$$\delta^{13}\text{C} = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1000 \quad (2)$$

Given that, R_{sample} is the $^{13}\text{C}/^{12}\text{C}$ isotopic ratio of sample and R_{standard} is the $^{13}\text{C}/^{12}\text{C}$ isotopic ratio of PDB standard.

To calculate the relative contribution of soil C derived from sugarcane straw, the Eq. 3 was used [20]:

$$X (\%) = [(\delta_{\text{sample}} - \delta_{\text{origin}})/(\delta_{\text{straw}} - \delta_{\text{origin}})] \times 100 \quad (3)$$

Given that, X is the proportion of C derived from sugarcane straw, δ_{sample} is the $\delta^{13}\text{C}$ of total organic C of local samples; δ_{origin} is the $\delta^{13}\text{C}$ of total organic C of origin soil evaluated (taken at the experiment installation), and the δ_{straw} is the $\delta^{13}\text{C}$ of sugarcane straw (C4 plants).

Physical Fractionation of SOM

Based on the assumption that the SOM changes due to sugarcane straw decomposition, soil was sampled from the 0–0.025-m layer at 685th day to perform physical fractionation of SOM. Subsamples of 20 g of air-dried soil were sieved at 2 mm and completed with 70 mL of distilled water. The suspensions were put in a freezer for 4 h in order to reduce heating during the sonication. An ultrasonic device was used (Sonic Vibra Cell Ultrasonic Processor—VC505) for 15 min at 70% of power (500 W). The suspension was then passed through a set of sieves (53 μm). The organomineral fraction < 53 μm was oven dried at 40 °C, weighed, and ground to 100 mesh. The C content and $\delta^{13}\text{C}$ in each sample were determined by the same methods described above.

Statistical Analysis

The raw data were subjected to a one-way analysis of variance (ANOVA) to test the influence of the sugarcane straw removal on soil C and N within each sampling time and over time. When ANOVA revealed a significant effect (F test $p < 0.05$), the means were compared by Tukey's test ($p < 0.05$). In addition, linear regressions were performed to estimate soil C and N accumulation as function of C-straw inputs. All statistical procedures were computed using the PROC GLM procedure

in the Statistical Analysis System (SAS) v.9.3 software (SAS Inc., Cary, USA).

Results and Discussion

DOC from Sugarcane Straw Decomposition

Sugarcane straw removal did not significantly change DOC dynamics over time in the upper soil layer (0–0.20 m) (Fig. 2a). During the first rainfall after straw deposition, soil DOC content averaged approximately 15 kg ha⁻¹. This content significantly decreased to approximately 3 kg ha⁻¹ and stabilized after 45 days. In the underlying soil layer (0.3–0.5 m), a significant decrease in DOC content with the increase of straw removal rate was observed from the beginning of the experiment up to 32 days (Fig. 2b). Dissolved organic C contents reached 26 and 10 kg ha⁻¹ in the first two evaluations under 14 Mg ha⁻¹ year⁻¹ of straw, whereas under 0 and 3.5 Mg ha⁻¹ year⁻¹ (100 and 75% removal), DOC contents were significantly lower, averaging 11.3 and 13.5 kg ha⁻¹ to the first evaluation and 7.0 and 8.1 kg ha⁻¹ to the second evaluation, respectively. Afterwards, DOC contents did not change due to straw removal, reducing and stabilizing at approximately 4 kg ha⁻¹ (Fig. 2b). The peak of DOC until 45 days after straw deposition is consistent with the period of highest straw decomposition as reported by Sousa Jr. et al. [21]. These authors verified that in the early stage of decomposition (first 45 days), dry mass loss ranged from 11 to 36% according to the initial straw amounts left on the soil.

In addition, DOC presents a fast turnover in the soil, reducing quickly after rainfall events [22]. Once the DOC is an organic matter fraction readily available for microbial decomposition [23, 24], its concentrations in the soil tend to reduce rapidly [23, 25–27]. According to Kalbitz et al. [25], from 10 to 40% of DOC can be easily decomposed by microorganisms over a period of time, which may range from days to a few months. Although DOC dynamics is often investigated in natural ecosystems, it is still poorly understood in agricultural soils [24]. Dissolved organic C is highly reactive in soil [27]; therefore, it is considered an important fraction of SOM, representing between 5 and 25% of soil total C [23]. De Troyer et al. [27] observed that the initial DOC flush originating from the residue mainly consists of hydrophilic compounds (98%) with low aromaticity, and consequently, most of the DOC released by the plant material is easily degradable. Thus, studies have shown that DOC inputs in the soil can promote a primer effect, accelerating soil C turnover [24, 27–29], consequently increasing CO₂ emissions to atmosphere [24].

The DOC content found in the soil under 100% straw removal (Fig. 2) indicated that crop residue is not the only C source of the DOC present in the soil. Although the origin of

soil DOC is not totally understood [25, 27], the main sources are plant residues, C washed from plants, humified organic matter, microbial biomass, and the C-rich products released by multiple mechanisms [30] from the root system of the crop itself [25].

The δ¹³C values of the DOC allowed determining the C contribution from sugarcane straw to soil DOC (Table 1). In general, lower proportions of C from straw (C4) were found in soil DOC content under smaller straw amounts (higher removal rates). For example, in the beginning of the experiment (32–40 days), only 4% of DOC was derived from C4 plants under 100% removal, whereas under 14 Mg ha⁻¹ year⁻¹ (no removal), about 37% of C was originated from C4. When extra amount of straw was added (21 Mg ha⁻¹ year⁻¹), the contribution of C-C4 plants increased to 53%. At the end of the rainy season (116–120 days), 31% of the DOC originated from C4 at the 21 Mg ha⁻¹ year⁻¹ plots, whereas no longer C-C4 was found in plots under 100% of straw removal (Table 1). Decreases of C from crop residue within soil DOC pool were also traced by De Troyer et al. [27]. They verified that soil solution DOC appeared mainly (65%) residue derived in short term, while in the long term, the DOC is mainly derived from soil organic matter (i.e., 5.1 and 1.3% of residue-C was found in the DOC after 57 and 240 days of incubation).

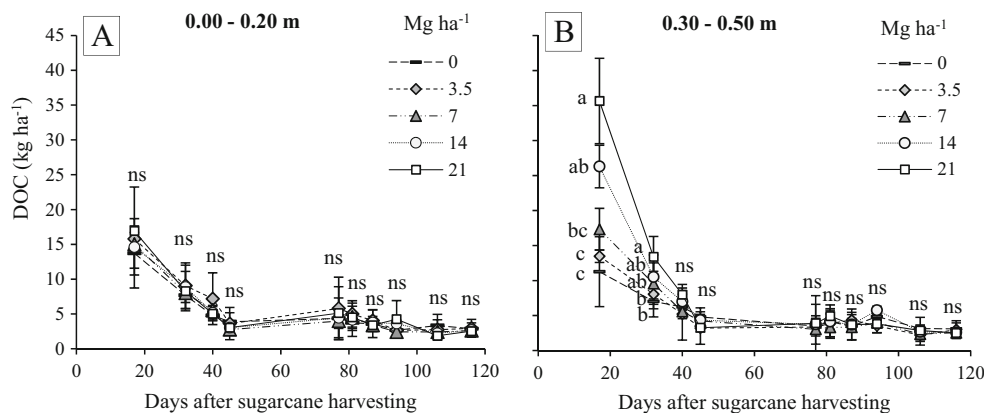
These results showed that straw removal for bioenergy production might decrease the DOC pool in deeper layers, altering the turnover of SOM and soil C sequestration potential in these areas. It was the first study that assessed DOC changes due to straw removal management. Therefore, additional studies are necessary to understand better the DOC dynamics in the soil and its contribution to soil C balance in sugarcane fields.

Soil C and N Contents and Stocks

The straw maintenance on soil surface increased significant soil C after three years (i.e., 1100 days), but this effect was restricted to the top soil (0–0.025 m) (Fig. 3a; Table 2). In the same way, results from long-term studies conducted in Australia showed that straw maintenance on soil led to significant C increases in the 0–0.02 m soil layers relative to burnt sugarcane plots, but few differences were observed in deeper layers [31]. In Brazil, Razafimbelo et al. [32] observed a significant soil C increases of 20% in the 0–0.05 m layer after 6 years of green harvesting compared to burnt areas, whereas these effects were not significant in the deeper layer (0.05–0.10 m). Galdos et al. [33] verified that soil C stocks increase of 30% in the 0–10 cm layer in after 8 years of green harvesting and did not find significant effects for the 10–20 cm soil layer.

Temporal soil C changes for the 0–0.025 m layer are shown in Fig. 3b and Table 2. The results showed that the

Fig. 2 Dissolved organic carbon (DOC) in the soil for the 0.00–0.20 (a) and 0.30–0.50 m (b) layers evaluated in the experiment 1, under increasing amounts of sugarcane straw (0, 3.5, 7, 14, 21 Mg ha⁻¹) throughout 120 days after sugarcane harvesting. Means followed by the same letter do not differ among themselves according to Tukey’s test ($p < 0.05$), ns: non significant



maintenance of 3.5 and 7 Mg ha⁻¹ year⁻¹ (i.e., 50 and 75% straw removal rates) had no impact on soil C compared to the 100% straw removal rate. Otherwise, keeping larger amounts of straw (14 and 21 Mg ha⁻¹ year⁻¹) led to significant C content increases only after 469 days, reaching increments of 3.32 and 4.85 g kg⁻¹ after 1100 days. Consequently, the soil C stock change rates were 0.36 and 0.53 Mg ha⁻¹ year⁻¹

respectively. These results also are consistent with literature [6, 34], indicating that regardless of the amount of straw retained on the soil, no or little changes of C can be observed in the short time, i.e., one year after deposition.

In longer-term studies, Thorburn et al. [6] observed C increases of 4.0 and 6.5 g kg⁻¹ in the 0–0.02 m layer at sites that had 5 and 6 years of straw retention on the soil surface. Other

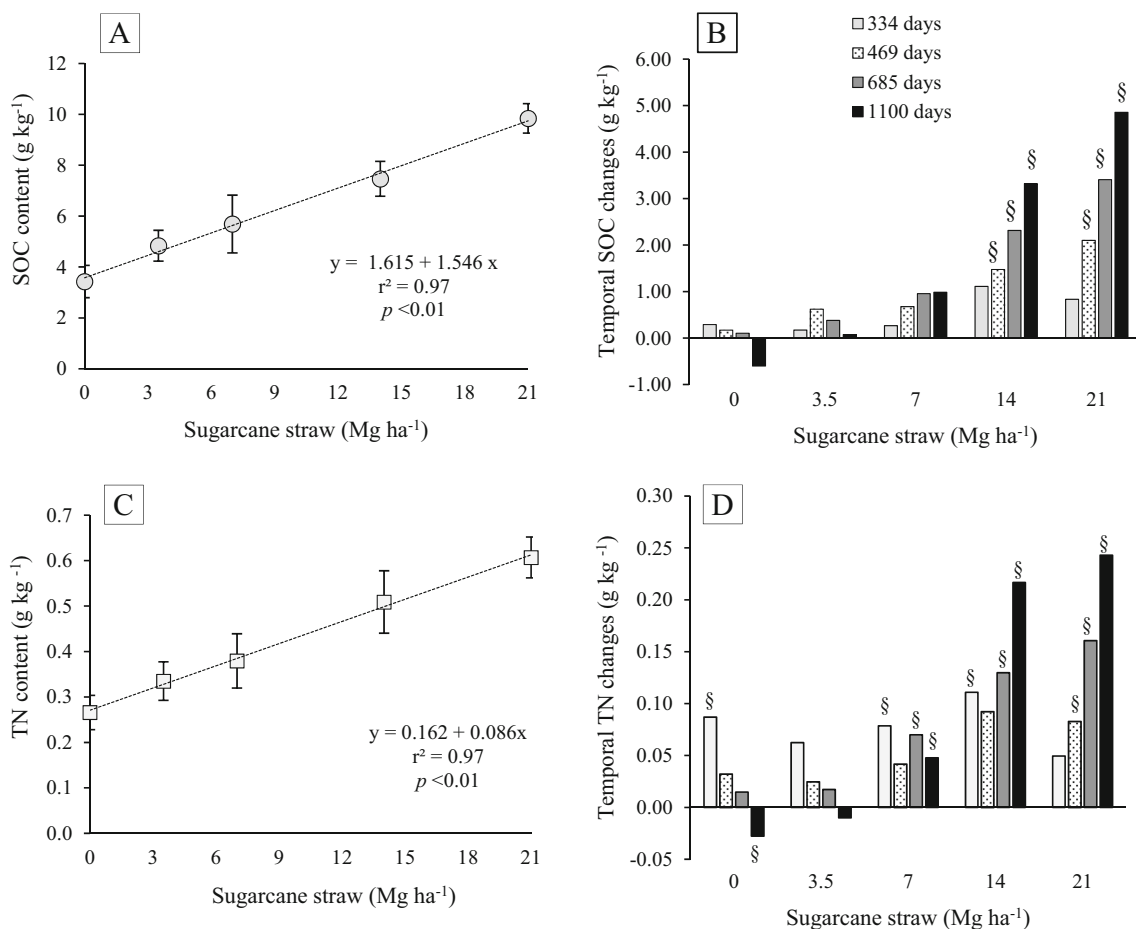


Fig. 3 Changes in soil organic carbon (SOC) and total nitrogen (TN) due to increasing amounts of sugarcane straw (0, 3.5, 7, 14 and 21 Mg ha⁻¹) kept on the field, where a, c SOC and NT contents for the 0–0.025 m layer after 1100 days and b, d SOC and NT content changes between sampling

campaigns for the 0–0.025 m layer. Error bars represent standard deviation of means; § denotes significant differences in the SOC or NT contents over time compared to the initial contents, according to Tukey’s test ($p < 0.05$)

Table 1 $\delta^{13}\text{C}$ and relative contribution of C3 and C4 plants in the soil dissolved organic carbon (DOC) measured in the experiment 1 under increasing amount of sugarcane straw keep on the soil

	Sugarcane straw (Mg ha^{-1})	Soil depth			
		0.20 m		0.50 m	
		$\delta^{13}\text{C}$	$\delta^{13}\text{C}$	%C4	%C4
Days of sugarcane straw decomposition: 32–40					
			– 27.40		4.17
3.5		– 22.10	– 23.39	37.12	29.14
7.0			– 23.65		27.50
14.0		– 21.39	– 22.18	41.54	36.64
21.0			– 19.52		53.17
Days of sugarcane straw decomposition: 116–120					
0.0		– 26.72	– 28.07	8.38	0.00
21.0		– 25.60	– 23.10	15.32	30.90

studies also indicated that retention of straw in the field increased soil C, for example, 2 g kg^{-1} (0–0.2 m) after 6 years [35] and 5 g kg^{-1} (0–0.2 m) after 8 years [33]. Variations of annual soil C increases reported among studies may be related to factors such as: initial soil C stock (baseline), soil type, annual straw inputs, sugarcane variety, and local climatic conditions [6–8].

Soil N content decreased linearly according to the straw removal rate after 1100 days (Fig. 3c). Straw removal rates of 75 and 100% (0 and $3.5 \text{ Mg ha}^{-1} \text{ year}^{-1}$) led to a gradual depletion of soil N over time (Fig. 3d). Thus, soil N stocks were significantly lower in 75 and 100% straw removal rates than no-removal straw plots after 1100 days (Table 2). Under no-removal and no-removal + extra 50% straw rates (14 and $21 \text{ Mg ha}^{-1} \text{ year}^{-1}$), soil N increments averaged 0.22 and 0.24 g kg^{-1} (Fig. 3d), representing an N stock accretion of 23 and $26 \text{ kg ha}^{-1} \text{ year}^{-1}$ (Table 2). These results agree to those obtained by Thorburn et al. [6], which verified significant increase of N contents (0.05 to 0.5 g kg^{-1}) in the 0–0.02 m layer in four of the five regions where there was retention of different amounts of straw. As observed in the present study, more pronounced N increases were found associated with greater soil C increases. Higher soil C storage implies a greater availability of N, since more than 95% of the total soil N is in the organic form [36].

Sugarcane straw removal led to a significant decrease in soil surface C/N ratio from 16 to 13 after 1100 days (Fig. 4). Decomposition of higher amounts of straw on the soil with high C/N ratio (i.e., 108) induced increases in soil C/N ratio.

Conversion Rate from Straw C and N to Soil C and N Stocks

Based on C and N inputs by straw and soil C and N stock changes after 1100 days (Fig. 5), we verified that 3.8 Mg ha^{-1} of C or 8.3 Mg ha^{-1} of straw (i.e., 46% C in straw) and

35 kg ha^{-1} of N or 8.1 Mg ha^{-1} of straw (i.e., 0.43% N in straw) were necessary to sustain soil C and N stocks over time. As this amount of straw was divided by 3 (i.e., three sugarcane season—1100 days), the straw amount that needs to be left annually on the field to sustain C and N stocks is about 2.8 and 2.7 Mg ha^{-1} . This small amount of straw ($< 3 \text{ Mg ha}^{-1}$) is directly associated with the low-C soil used in this experiment and the soil layer (0–0.025 cm) used in this calculation. In addition, sugarcane-replanting time was not included, in which soil C and N losses are much more intensive due to soil tillage [37]. Therefore, although these pioneer field data are important to understand the sugarcane straw removal effects on soil C and N, new experiments conducted in different soils under sugarcane cultivation are encouraged. It is worth mentioning that a sustainable straw removal rate should be defined considering not only soil C and N stocks, but also other agronomic and environmental aspects (e.g., such as nutrient and water fluxes in the soil, soil structure and resistance to erosion, weed, pest and pathogen control, greenhouse gas emissions [9, 14]), as well as the best management practices associated with straw removal management [9].

The conversion rate from C-straw to soil C stock was calculated for the no-straw removal plots (14 Mg ha^{-1} of straw) that represent green harvest sugarcane fields without straw removal in central-southern Brazil. Our findings showed that conversion rate was approximately 5.6% for C (i.e., each 1000 kg of straw-C, soil C stock increased by 56 kg ha^{-1}) or 2.6% considering straw mass basis (i.e., each 1000 kg of straw, soil C stock increased by 26 kg ha^{-1}) (Fig. 5a). In terms of N, the conversion rate was 39% N (i.e., each 1 kg of straw-N, soil N stock increased by 0.39 kg ha^{-1}) or 0.16% considering straw mass basis (i.e., each 1000 kg of straw, soil N stock increased by 1.67 kg ha^{-1}) (Fig. 5b). Thus, the data suggest that keeping 100% of straw on the field enables the accumulation of approximately $364 \text{ kg ha}^{-1} \text{ year}^{-1}$ of C and $23 \text{ kg ha}^{-1} \text{ year}^{-1}$ of N in soil. These results confirm the

Table 2 Soil organic C (SOC) and total N (TN) stocks for the 0–0.025, 0.025–0.05, and 0.05–0.10 m layers measured in the experiment 2 under increasing amounts of sugarcane straw (0, 3.5, 7, 14, and 21 Mg ha⁻¹) left on the field 1100 days after sugarcane harvesting

Sugarcane straw (Mg ha ⁻¹)	Days of decomposition				
	0 (initial)	334	469	685	1100
----- SOC stock (Mg ha ⁻¹) -----					
0–0.025 m					
0	1.31 a A*	1.40 c A	1.37 c A	1.34 c A	1.11 d A
3.5	1.55 a A	1.60 bc A	1.75 bc A	1.67 c A	1.57 cd A
7	1.53 a A	1.62 bc A	1.75 bc A	1.84 bc A	1.85 c A
14	1.35 a D	1.80 ab CD	1.83 ab BC	2.10 ab AB	2.43 b A
21	1.62 a D	1.89 a CD	2.30 a BC	2.73 a AB	3.20 a A
0.025–0.05 m					
0	1.43 ns	1.41 ns		1.35 ns	
3.5	1.51	1.43		1.33	
7	1.59	1.57		1.50	
14	1.29	1.41		1.42	
21	1.61	1.55		1.62	
0.05–0.10 m					
0	2.80 ns	2.77 ns		2.44 ns	
3.5	3.20	3.20		2.54	
7	3.22	3.31		2.80	
14	2.62	3.20		2.56	
21	3.23	3.12		2.66	
----- TN stock (kg ha ⁻¹) -----					
0–0.025 m					
0	95.39 a AB*	123.63 a A	105.83 b AB	100.16 b AB	86.42 c B
3.5	112.12 a A	132.38 a A	120.15 a A	117.71 b A	108.82 bc A
7	107.78 a B	133.31 a A	121.34 a AB	130.54 b A	123.30 b AB
14	94.96 a C	130.97 a AB	124.95 a BC	137.10 ab AB	165.41 a A
21	118.28 a D	134.38 a CD	145.20 a C	170.50 a B	197.26 a A
0.025–0.050 m					
0	100.92 a A	113.37 a A		97.14 a A	
3.5	109.28 a AB	127.61 a A		97.87 a B	
7	107.50 a A	122.75 a A		109.86 a A	
14	90.91 a B	117.86 a A		102.06 a AB	
21	116.17 a A	122.28 a A		114.36 a A	
0.050–0.10 m					
0	203.09 a A	234.31 a A		182.17 a A	
3.5	231.11 a AB	248.40 a A		189.52 a B	
7	227.53 a AB	247.90 a A		209.71 a B	
14	191.32 a B	239.12 a A		189.42 a B	
21	240.25 a A	238.90 a A		202.30 a B	

*Mean values followed by the same small letter (column) and capital letter (line) do not differ among themselves according to Tukey's test ($p < 0.05$)

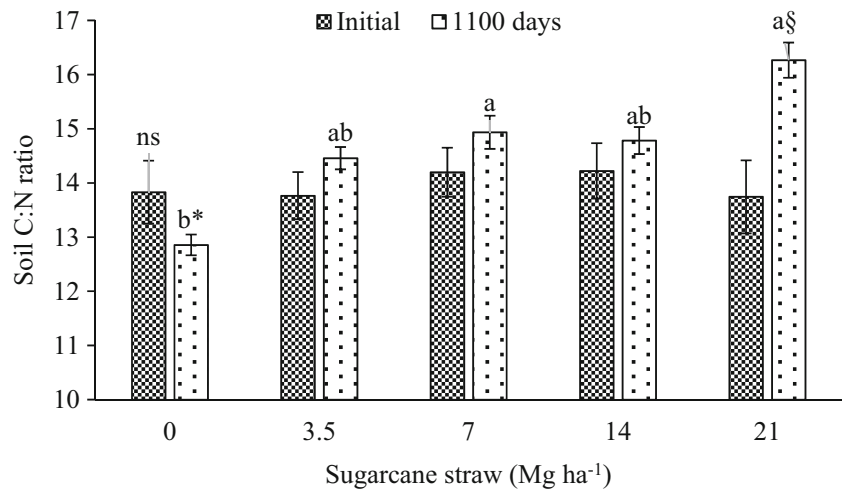
essential role of straw in sustain SOM and that indiscriminate straw removal can result in soil C and N losses, affecting multiple soil functions and ecosystem services [9, 14].

Low rate of straw-C accumulation into the soil is associated with microbial respiration during decomposition process where most of the C added by straw is lost to atmosphere as

C-CO₂ [21, 24, 35, 38, 39]. In addition, part of the C can be leached to deeper soil layers as DOC [23, 27, 35], as observed in this study (Fig. 2), and finally, part of C remains in non-decomposed straw [21].

In contrast, high rate of N accumulation into the soil per N-straw added is consistent with those rates found in Australian

Fig. 4 Soil C/N ratio for the 0–0.025 m layer at the installation of experiment (initial) and after 1100 days under increasing amounts of sugarcane straw (0, 3.5, 7, 14, 21 Mg ha⁻¹) kept on the soil surface. *Mean values followed by the same letter within each time (i.e., 0 and 1100 days) do not differ among themselves according to Tukey’s test ($p < 0.05$). ns: non significant. Section sign (§) denotes significant differences within each sugarcane straw rate between C/N ratio values from 0 (initial) and 1100 days; error bars represent standard deviation



sugarcane fields [35]. The fraction of non-accumulated N in the soil could have been lost by volatilization, denitrification, leaching, crop uptake [35, 40] or also remaining in non-decomposed straw [21]. Studies have confirmed that the main fate of N added via straw is remaining storage in the soil. For instance, Fortes et al. [41] observed after four years that sugarcane recovered 26% of the N-straw added, 51% remained in the soil, and 23% was no longer found in the soil-plant system in sugarcane fields in Brazil. Ferreira et al. [42] verified that the N amount recovered by the crop (16.2%) from straw after three years, representing only 2.1% of total crop needs. Nevertheless, although the straw removal has a small effect on crop nutrition in short term [42, 43], it can lead to the C and N stock depletion in long term, increasing N fertilizer requirements to supply crop needs [7, 35, 43].

Soil δ¹³C natural Abundance and Proportion of C Derived from Straw

The maintenance of higher amounts of sugarcane straw on the soil promoted an enrichment of soil surface ¹³C (0–0.025 m

layer) over time (Table 3). The δ¹³C increases were observed from the 334 days after straw deposition, resulting in higher proportions of C-C4 into the total soil C. The most significant increases were obtained by maintaining 21 Mg ha⁻¹ of straw on the soil, where the C-C4 represented 25, 36, 52, and 60% of the total C after 334, 469, 685, and 1100 days, respectively.

In the deeper soil layers (0.025–0.05 m and 0.05–0.10 m), there was a significant increase of δ¹³C for most treatments over time compared to the initial values. On the other hand, no significant differences were found among the straw removal rates. The pattern of the measured δ¹³C—higher in upper layers—indicated an increase in the humification degree, since isotope ¹²C can be easier lost by microbial respiration relative to ¹³C [44]. Increases in δ¹³C values in depth can be resulting from a preferential decomposition and removal of ¹³C-impooverished components or molecules, increased humification, and even due to deposition of organic matter from a former ¹³C-rich vegetation [45]. Slight increases of δ¹³C in the soil under 100% straw removal, i.e., without straw addition over time (Table 3), were not expected; however, these results might be associated with C inputs from leaves/stalk washing

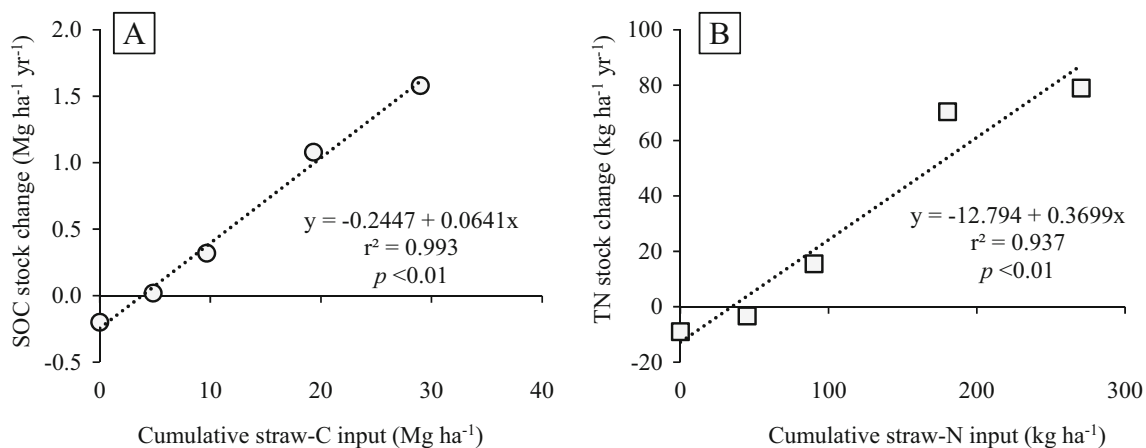


Fig. 5 Relationship between cumulative straw-C (a) and straw-N (b) inputs and soil organic C (SOC) and total nitrogen (TN) stock changes into the 0–0.025 m layer after 1100 days

Table 3 Soil $\delta^{13}\text{C}$ and relative contribution of C derived from C4-photosynthetic-cycle plants for the 0–0.025, 0.025–0.05, and 0.05–0.10 m layers measured in the experiment 2 under increasing amounts of sugarcane straw (0, 3.5, 7, 14, and 21 Mg ha^{-1}) left on the field over 1100 days after sugarcane harvesting

Sugarcane straw (Mg ha^{-1})	Days of decomposition									
	0		334		469		685		1100	
	(Initial) $\delta^{13}\text{C}$ (‰)	$\delta^{13}\text{C}$ (‰)	%C4	$\delta^{13}\text{C}$ (‰)	%C4	$\delta^{13}\text{C}$ (‰)	%C4	$\delta^{13}\text{C}$ (‰)	%C4	
0–0.025 m										
0	–19.32 a B*	–18.56 b AB	10.6	–18.77 b AB	7.8	–18.40 c A	12.9	–18.88 d A B	6.2	
3.5	–18.92 a D	–17.99 ab C	13.7	–17.41 a B	22.4	–16.95 b A	29.3	–16.50 c A	35.9	
7	–18.99 a C	–17.50 a B	21.9	–17.09 a B	27.9	–16.95 b B	29.9	–15.57 b A	50.3	
14	–19.04 a D	–17.51 a C	22.2	–16.72 a BC	33.8	–16.15 ab B	42.2	–14.92 a A	59.8	
21	–19.29 a C	–17.53 a B	24.8	–16.72 a B	36.1	–15.58 a A	52.2	–14.88 a A	60.1	
0.025–0.05 m										
0	–19.44 a A	–18.47 a A	13.3			–18.06 a A	18.9			
3.5	–19.21 a B	–18.66 a AB	7.7			–18.17 a A	14.8			
7	–19.07 a B	–18.26 a A	11.7			–17.97 a A	16.0			
14	–18.79 a B	–17.98 a A	12.2			–17.55 a A	18.8			
21	–19.18 a B	–18.04 a A	16.3			–17.52 a A	23.8			
0.05–0.10 m										
0	–19.21 a B	–18.44 a AB	31.8			–17.87 a A	38.0			
3.5	–18.97 a A	–18.72 a A	28.7			–18.40 a A	32.3			
7	–18.87 a B	–18.43 a AB	31.9			–18.14 a A	35.1			
14	–19.09 a B	–18.28 a A	33.5			–17.64 a A	40.6			
21	–19.12 a B	–18.09 a A	35.6			–18.19 a AB	34.5			

*Mean values followed by the same small letter (column) and capital letter (line) do not differ among themselves according to Tukey's test ($p < 0.05$)

and C released by sugarcane roots. Anyway, these values remained lower than those found in the soil with straw.

Physical Fractionation of Soil Organic Matter

In order to a better understanding straw removal effects in the SOM dynamics, physical fractionation of SOM was performed, resulting in an average recovery ratio of 98% (Table 4). The contribution of 2000–53 μm fraction (62 to 67%) was predominant compared to the <53 μm fraction (31 to 37%). The 2000–2053 μm fraction is sensitive to fluctuations in the organic residues contributions, showing spatial and seasonal variabilities [46]. Studying SOM changes in

conventional and organic sugarcane field in Brazil, Signor et al. [47] and Brandani et al. [48] verified an opposite distribution of SOM fractions, with predominance of <53 μm followed by the 2000–2075 μm fraction. The difference in the fractions distribution likely is associated with soil texture, in which SOM is preferentially stored in finer fractions in clay soils, as those studied by Signor et al. [47] and Brandani et al. [48].

Straw removal management led to decreased C and N contents in both SOM fractions (Fig. 6). The <53 μm fraction presented the highest levels of C and N, reaching 14.2 and 1.2 g kg^{-1} , respectively, under 21 Mg ha^{-1} , i.e., 74 and 46% higher than C and N contents (8.2 and 0.8 g kg^{-1}) found in the

Table 4 Relative proportion of the each physical fraction of the soil organic matter (SOM) and its average recovery ratio (\pm standard deviation)

Sugarcane straw (Mg ha^{-1})	SOM fractions		Recovery ratio
	2000–53 μm	< 53 μm	
0	67.09 \pm 0.84 ns	31.43 \pm 0.66 ns	98.52 \pm 0.23
3.5	63.04 \pm 1.43	35.41 \pm 1.39	98.45 \pm 0.17
7	62.44 \pm 1.13	35.79 \pm 1.25	98.23 \pm 0.51
14	61.79 \pm 0.39	36.94 \pm 0.61	98.71 \pm 0.44
21	62.46 \pm 1.15	35.83 \pm 1.35	98.28 \pm 0.36

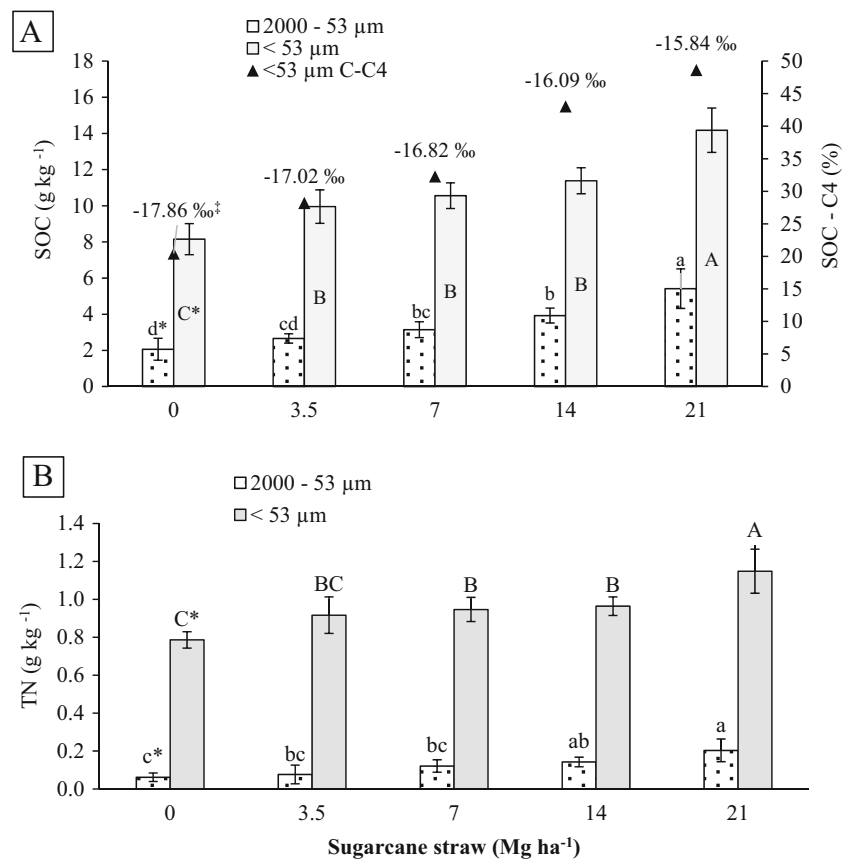
ns: non significant by ANOVA *F* test ($p < 0.05$)

same fractions under no straw (100% straw removal). The < 53 μm fraction corresponds to the more stabilized (recalcitrant) SOM linked to clay minerals and Fe and Al oxides. The protection effect of SOM caused by clay involves two mechanisms: the interaction of SOM with the clay particles surface (cationic bridges, H bonds, electrostatic interactions, and van der Waals' forces) and organic material occlusion in the soil aggregates matrix [49]. In addition, the high concentration of Fe and Al oxides in Oxisols can strongly affect the SOM availability for decomposition. On the other hand, the lower C and N contents found in 2000–53 μm fraction are associated with soil sand particle that presenting lower

specific surface, electric charges, and, consequently, reduced number of organomineral complexes [46].

Within the < 53 μm fraction, no straw removal rates (14 and 21 Mg ha^{-1}) resulted in higher soil C contents and C-C4 contribution into the total SOC (43 to 49%, respectively), whereas 100, 75, and 50% straw removal rates (0, 3.5 and 7 Mg ha^{-1}) showed a decreased contribution ranging from 20 to 33% (Fig. 6a). These results are in line with those found by Brandani et al. [48], who found C-C4 contributions within < 53 μm fraction around 80% in longer-time (4 to 12 years) green harvesting managed sugarcane soils. Therefore, our finding clearly shows the importance of straw inputs into the

Fig. 6 Soil organic carbon (SOC) (a) and total nitrogen (TN) (b) content in the physical fractions (i.e., 2000–53 and < 53 μm) of soil organic matter (SOM) for the 0–0.025 m layer under increasing amounts of sugarcane straw (0, 3.5, 7, 14, and 21 Mg ha^{-1}) left on the field after 685 days. Double dagger symbol denotes $\delta^{13}\text{C}$ (‰) and relative contribution of C derived of C4 plants within total SOC in < 53 μm fraction. *Mean values followed by the same small letter (2000–53 μm) and capital letter (< 53 μm) do not differ among themselves according to Tukey's test ($p < 0.05$). Error bars represent standard deviation



soil to sustain and increased SOM levels and consequently a sustainability of sugarcane production system.

Conclusions/Summary

Sugarcane straw removal affected significantly the C and total N pools in this low-C soil. In the first 45 days of straw decomposition, a small but important straw-derived C portion enters into the soil as dissolved organic carbon (DOC), down to 0.50 m depth. Future studies are encouraged to include DOC into soil C balance in sugarcane agroecosystems. After three years, sugarcane straw maintenance in the field increased C and N stocks at the soil surface layer (0–0.025 m). Our findings suggest that under no straw removal (i.e., 14 Mg ha⁻¹), approximately 364 kg ha⁻¹ of C and 23 kg ha⁻¹ of N are annually stored into this low-C soil. The straw-derived C contribution to total soil C increase over time accounted for ~60% under no straw removal rate. The greatest contribution of the C storage preferentially occurs by the fraction of organic matter (< 0.53 μm) associated with soil clay minerals.

Indiscriminate sugarcane straw removal induced negative impacts on soil C stocks and N cycling and should be accounted into the C footprint of cellulosic ethanol and/or bioelectricity. This information can also foster and/or validate local/regional soil C and N models used for evaluating agricultural management practices effects. Adoption of conservationist practices coupled with straw removal should be further investigated as a strategy to offset part of soil C losses. Integrated assessments including SOC data associated with other agronomic and environmental implications [9, 14] need to be performed towards more sustainable straw removal management for bioenergy production in Brazil.

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