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Effects of burned and unburned sugarcane harvesting systems on soil CO_2 emission and soil physical, chemical, and microbiological attributes

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ABSTRACT

The mechanized sugarcane harvesting system has been replacing the old manual harvesting system with the burning of the sugarcane field. The purpose of the conversion of these systems is to obtain benefits related to the improvement of soil and environmental quality, minimizing the effects of greenhouse gas (GHG) emissions, especially carbon dioxide (CO_2). In this context, this study aimed to investigate the effect of burned and unburned sugarcane harvesting systems on soil CO_2 emission and soil chemical, physical, and microbiological attributes. Two adjacent areas were used in this study: an unburned sugarcane area, with an eight-year history without burning the sugarcane field and high amounts of crop residues (mean of 13 t ha^{-1}), and a burned sugarcane area, with manual harvesting after burning the sugarcane field and without crop residues on the soil surface. The soil of both areas is classified as an Oxisol. Soil CO_2 emission, soil temperature, and soil moisture were assessed from 20 randomly sampling points placed in each area. Soil samples were collected at the end of the soil CO_2 emission, soil temperature, and soil moisture assessments from each point at a depth of 0–0.20 m to determine soil physical, chemical, and microbiological attributes. Soil CO_2 emission was, on average, 37% higher in the burned sugarcane area (2.63 μmol m^{-2} s^{-1}) compared to the unburned sugarcane area (1.92 μmol m^{-2} s^{-1}). Soil moisture was higher in the unburned sugarcane area (25.30%) than in the burned sugarcane area (16.02%). An opposite effect was observed for soil temperature, which presented values 2.5 °C higher in the burned sugarcane area (21.5 °C) compared to the unburned sugarcane area (19.1 °C). Soil carbon decay constant *k* indicated that carbon was decomposed faster in the burned sugarcane area (0.00070 $days^{-1}$) than in the unburned sugarcane area (0.00046 $days^{-1}$). Thus, soil carbon half-life was longer in the unburned sugarcane area (1,572.82 days) compared to the burned sugarcane area (1,033.95 days), i.e., carbon permanence time in the unburned sugarcane area was 52% higher than in the burned sugarcane area. Soil temperature, soil moisture, air-filled pore space, P, the sum of bases (Ca^{2+} + K^{+} + Mg^{2+}), soil bulk density, soil carbon stock, soil C/N ratio, and the abundance of functional gene *nif*H are the most representative soil attributes that allows characterizing the CO_2 emission process in soils managed with sugarcane under unburned and burned harvesting systems. Therefore, the study of these attributes should be taken into account when assessing the variability of CO_2 emissions in agricultural soils. In conservationist terms, the unburned sugarcane system presents a higher potential for stabilizing soil carbon and reducing the contribution of agriculture to greenhouse gas emissions, especially CO_2, when compared to the burned sugarcane system.

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1. Introduction

The increase in the world population has been followed by the ever-increasing demand for food and, at the same pace, the alarming need for new agricultural areas and natural resources. In this sense, concerns about the sustainability of agricultural systems have emerged (FAO, 2015). Changes in soil use and management cause significant alterations in its attributes, influencing the magnitude of greenhouse gas (GHG) emissions, mainly carbon dioxide (CO_2) (De Figueiredo and La Scala Jr, 2011; Panosso et al., 2011). In addition to the increased CO_2 concentration in the atmosphere and the already known consequences related to climate change, carbon loss to the atmosphere via CO_2 ($C–CO_2$) emissions is also a major threat to the soil (IPCC, 2014).

Brazil is the world's largest producer of sugarcane (*Saccharum* spp.), with a cultivated crop area in the 2019/2020 growing season of approximately 8.5 million hectares. In this scenario, the state of São Paulo is the largest sugarcane producer, accounting for 51.7% or approximately 4.7 million hectares of this planted area (Conab, 2020). Therefore, changes in the sugarcane management system may have a significant impact on CO_2 emissions from agricultural areas.

Sugarcane field burning before manual harvesting has historically been a common management practice in Brazil and worldwide, aiming at reducing the amount of straw and hence facilitating cutting operations and mechanical loading. However, this practice causes high GHG (i.e., CO_2, N_2O, and CH_4) emissions (De Figueiredo and La Scala Jr, 2011; Almeida et al., 2018; Bordonal et al., 2018) and harmful particles into the atmosphere. On the other hand, the mechanical harvesting of sugarcane fields provides the deposition of up to 15 t ha^{-1} year^{-1} of the dry mass of crop residues on the soil surface, increasing the amount of carbon in the surface layers (Cerri et al., 2013; Carvalho et al., 2017). Thus, the carbon that would be emitted immediately into the atmosphere during the burning of the sugarcane field under the burned sugarcane harvesting system remains incorporated in the soil in the unburned sugarcane harvesting system, contributing to mitigating atmospheric CO_2 concentrations and reducing the contribution of agriculture to the additional greenhouse effect (Galdos et al., 2009).

Although changes in agricultural systems may contribute to GEE mitigation, soil attributes and climate conditions coordinate CO_2 emission potential from agricultural soils (De Figueiredo and La Scala Jr, 2011). Among the soil attributes related to the production and transport of CO_2 from soil to the atmosphere, soil temperature and soil moisture are controlling factors related to the intensity and frequency of these processes (Lal, 2009). The CO_2 transport process, i.e., the gas exchange process between soil and the atmosphere, are governed by two mechanisms (diffusion and mass flow) that respond to a pressure gradient, which varies as a function of texture, structure, and moisture content of soils (Ball, 2013). Thus, soil CO_2 emissions in agricultural areas are the result of the interaction between climate (Wang et al., 2015) and soil physical (Chen et al., 2010; Panosso et al., 2011), chemical (Lal, 2009; Moitinho et al., 2015), and biological attributes (Rosenzweig et al., 2016).

Moreover, most of these attributes are related to CO_2 emissions since they affect the soil microbiological community (Rosenzweig et al., 2016) and the formation, stabilization, and decomposition of soil organic matter (Panosso et al., 2011). Particular parameters known as indicators are usually used to assess the effectiveness of a management system or compare different effects of soil use. Thus, investigating the structure and functionality of specific microorganisms has been very useful to elucidate the dynamics of different soil management systems (Hayden et al., 2010; Lauber et al., 2013).

Several studies conducted in Brazil in the last decades have indicated that the conversion of sugarcane harvesting systems from burned to unburned directly influenced an increase in soil organic matter and, consequently, the long-term soil C stocks or at least the maintenance of their levels (Galdos et al., 2009; Sant'Anna et al., 2009; Panosso et al., 2011; Signor et al., 2014), soil fertility (Resende et al., 2006; Souza et al., 2012), and aggregate stability, as well as an improvement in soil structure (Oliveira et al., 2010; Vasconcelos et al., 2014), soil microbiological properties (Galdos et al., 2009; Paredes Júnior, 2012), and GHG mitigation (De Figueiredo and La Scala Jr, 2011; Panosso et al., 2011). However, there is a lack of studies that simultaneously assess physical, chemical, biological, and climatic attributes and their interactions with the soil CO_2 emission dynamics and compare these harvesting systems.

In this context, this study hypothesized that the conversion from burned into unburned sugarcane harvesting system contributes to reducing soil CO_2 emissions since it also affects soil physical, chemical, and biological characteristics, altering the microbiota dynamics in the processes of decomposition and mineralization of organic matter and incorporation of carbon into the soil. Thus, this study aimed to investigate the effect of burned and unburned sugarcane harvesting systems on soil CO_2 emission and soil chemical, physical, and microbiological attributes.

2. Material and methods

2.1. Characterization of the study areas

The study was conducted in two adjacent agricultural areas destined for sugarcane production for more than 35 years. These areas are located in Barrinha, state of São Paulo, Brazil, with geographical coordinates 21°14′ S and 48°05′ W. The soil of both areas is classified as an Oxisol (Haplorthox, USDA Soil Taxonomy) (Santos et al., 2013), with 677.5, 208.1, and 134.0 g kg^{-1} of clay, silt, and sand, respectively, in the unburned sugarcane area, and 669.9, 206.7, and 132.8 g kg^{-1} of clay, silt, and sand, respectively, in the burned sugarcane area, both at a depth of 0–0.20 m. According to the Thornthwaite classification, the regional climate is defined as $B_1rB'_4a'$, i.e., a humid mesothermal climate with a small water deficit and summer evapotranspiration lower than 70% of the annual evapotranspiration. The mean annual precipitation registered during the last 30 years was 1,560 mm, concentrated from October to March, and a mean annual temperature of 22.2 °C.

These areas present different management systems. The first area is an unburned sugarcane (US) area with an eight-year history without burning the sugarcane field and high amounts of crop residues (mean of 13 t ha^{-1}) left on the soil surface after mechanical harvesting. The second area is a burned sugarcane (BS) area with a history of sugarcane cultivation since 1992 and manual harvesting after burning the sugarcane field. The controlled burning of sugarcane straw was carried out at night in mid-August 2013 when the relative air humidity is around 62%, mean air temperature reaches 22 °C, and wind speed reaches 8 km h^{-1} (São Paulo, 2002). During the burning of sugarcane straw, the temperature reached over 100 °C up to 15 cm deep in the soil and reached 700 °C above the soil surface. It usually takes 10 min to burn a dry mass of approximately 20 t ha^{-1}.

Sugarcane variety planted in both areas in previous years was CTC-6 (CTC, 2012). The areas have produced, on average, from 88 to 90 t ha^{-1}, with no significant differences regarding production. The two studied areas are very close, neighbors, as their separation distances are smaller than 100 m. Differences in the landscape shape of the studied areas are minimum. The elevation of the unburned area is of 561–556 m, while the burned area has an elevation of 549–541 m. Sugarcane harvesting was performed simultaneously in both areas, one month before the beginning of this study (August 2013), being performed mechanically and without burning in US and manually after burning in BS.

2.2. Assessments of soil CO_2 emission, soil temperature, and soil moisture

Soil CO_2 emissions (FCO_2), soil temperature (Ts), and soil moisture (Ms) were assessed in both areas (US and BS) at 20 sampling points

separated at minimum distances of 10 m. PVC collars (0.10 m in diameter) were installed at each point at a depth of 3 cm. A total of nine assessments were performed during the initial stages of sugarcane growth for 28 days (September 25 and 27 and October 1, 4, 9, 11, 15, 17, and 22, 2013, which corresponded to the Julian days 268, 270, 274, 277, 282, 284, 288, 290, and 295) from 8:00 to 10:00 h.

A portable LI–8100 automated soil CO_2 flux system (LI-COR, Lincoln, NE, USA) was used to measure the emission (μmol m^{-2} s^{-1}) in the experimental areas. This system monitors changes in CO_2 concentration inside a closed chamber using optical absorption spectroscopy in the infrared spectrum. In the measurement mode, FCO_2 is determined inside the chamber every 2.5 s, and approximately 1.5 min were required to record it at each point. A portable sensor from the LI–8100 system was used to measure Ts. This sensor was inserted into the soil at a distance of 10 cm from the FCO_2 assessment points. Ms was measured by a Time Domain Reflectometry (TDR) system (Hydrosense TM, Campbell Scientific Inc., Logan, UT, USA), which consists of two 12-cm probes that are inserted into the soil, also at 10 cm from the PVC collars.

2.3. Determination of soil physical and chemical attributes

A soil sampling was performed on October 22 and 23, 2013, after FCO_2, Ts, and Ms measurements were completed. Disturbed and undisturbed soil samples were collected from a depth of 0–20 cm near each sampling point. For soil chemical analysis, samples were collected using a Dutch auger, being subsequently dried, decloded, and sieved through a 2-mm mesh sieve. Analyses included soil organic matter content (SOM), phosphorus availability (P), K, Ca, Mg, and H + Al contents (Raij et al., 2001), the sum of bases (Ca^{2+} + K^{+} + Mg^{2+}), aluminum saturation (m), and cation exchange capacity (CEC). The total organic carbon (TOC) was estimated by dividing SOM by 1.724. The analysis of the total soil nitrogen (N) was performed by the Kjeldahl method (Embrapa, 1997).

Particle size distribution (sand, silt, and clay) analysis was performed using the method proposed by Embrapa (1997). Soil bulk density (Ds) was determined using undisturbed soil samples collected with a sampler adapted to cylinders with an internal diameter of 5 cm and a height of 4 cm. The total pore volume (TPV) was calculated from Ds, with pore size distribution determined by a porous plate funnel under a 60-cm water column tension in previously saturated samples. The pore volume retained in the sample corresponded to the micropores, and the difference calculated between TPV and the micropores corresponded to the macropores (Embrapa, 1997). Air-filled pore space (AFPS) was calculated as the difference between the porosity fraction filled with water (Ms) and TPV (Panosso et al., 2011; Bicalho et al., 2014).

The corrected soil carbon stock (Cstock) was calculated using Eq. (1), based on the equivalent soil mass (Veldkamp, 1994; Carvalho et al., 2009), taking into account the differences in soil masses. In this method, the thickness of the studied soil layer is corrected in relation to a reference area. The reference soil density used in this study was that found in BS.

$$\text{Cstock} = \frac{\text{OC}\times\text{Ds}\times\frac{\text{Dref}}{\text{Ds}\times\text{L}}}{10} \quad (1)$$

where Cstock is the soil carbon stock (Mg ha^{-1}), OC is the organic carbon content (g kg^{-1}), Ds is the soil bulk density (Mg m^{-3}), Dref is the soil bulk density at the sampled depth in the reference area, and L is the soil layer depth (20 cm). The potential of soil carbon stability in BS and US was obtained by Eqs. (2) and (3):

$$k = \frac{\text{C}-\text{CO}_2}{\text{Cstock}} \quad (2)$$

where k is the soil carbon decay constant and C–CO_2 is the labile carbon emitted into the atmosphere as CO_2. Soil labile carbon half-life ($t_{1/2}$), i.e., the time required for 50% of the C–CO_2 be decomposed, was estimated from the constant k by Eq. (3).

$$t_{1/2} = \frac{1}{k} \times \ln \ (2) \quad (3)$$

2.4. Quantitative real-time PCR

2.4.1. Extraction of DNA from environmental samples

Soil samples were collected on October 24, 2013, from nine demarcated points at each experimental area using pre-sterilized PVC tubes with dimensions of 50 cm in length and 5 cm in diameter. These PVC tubes were inserted vertically into the soil to collect soil samples. Subsequently, all PVC tubes were sealed to prevent losses, stored in iceboxes, and sent to the Laboratory of Biochemistry of Microorganisms and Plants of the School of Agricultural and Veterinary Sciences of the São Paulo State University (FCAV–UNESP), Jaboticabal, state of São Paulo, Brazil, being stored in an ultra-freezer at − 80 °C to be used for subsequent genomic DNA extraction.

The genomic DNA from soil samples collected from each management system was extracted using the FastDNA® SPIN Kit for Soil (MP Biomedicals) and followed the manufacturer's instructions, being stored at − 20 °C. A 5-μL aliquot was submitted to electrophoresis in 1% (w/v) agarose gel stained in GelRed™ (Uniscience) in SB buffer to confirm the DNA extraction quality. An amount of 2 μL Low DNA Mass Ladder (Invitrogen) was used as a molecular standard. This gel was submitted to an electric field of 85 V for approximately 30 min. Subsequently, the DNA was quantified in a Nanodrop 2000c spectrophotometer (Thermo Scientific), being adopted a ratio of the optical density of 1.0 at 260 nm (OD_{260}) equal to 50 ng of DNA μ^{-1} (Sambrook et al., 1989).

2.4.2. Detection and quantification of bacterial 16S rRNA, pmoA, and nifH genes by quantitative real-time PCR

Quantitative real-time PCR (qPCR) reactions were conducted at the Laboratory of Cellular and Molecular Biology of the Center of Nuclear Energy in Agriculture of the University of São Paulo (CENA–USP), Piracicaba, state of São Paulo, Brazil. These reactions were performed on the StepOnePlus™ real-time PCR system (Applied Biosystems) for bacterial 16S rRNA, *pmoA*, and *nif*H genes using the SYBER Green I system. The established conditions for each gene are described in Table 1. The qPCR cycling parameters consisted of 10 min at 95 °C, 40 cycles of 95 °C for 15 s, 60 °C for 1 min, followed by a dissociation stage of 95 °C for 15 s, 60 °C for 1 min, and temperature increments of 0.7 °C each 10 s until 95 °C.

Standard curves were obtained by performing the amplification with the copy number of template DNA of *Pseudomonas fluorescens* for the bacterial 16S rRNA gene, environmental sample DNA for the *pmo*A gene, and *Bradyrhizobium liaoningensi* for the *nif*H gene. The data obtained by DNA amplification extracted from soil were interpolated to determine the number of copies of the genes under study. The real-time PCR reaction for each gene was prepared in a final volume of 10 μL, containing 5 μL SYBR Green ROX qPCR Master Mix (Thermo Scientific), 5 pmol of each forward and reverse primers, 1 μL DNA of the test sample, and sterile ultrapure water. The data of qPCR were obtained using the StepOne Software 2.2.2 (Applied Biosystems), being subsequently exported to Excel (Microsoft) to calculate the number of gene copies per gram of dry soil.

2.5. Microbiological analyses: Microbial biomass carbon and enzymatic activity

Microbial biomass carbon (Cmic) was determined using the irradiation-extraction method proposed by Islam and Weil (1998) and adapted by Barbosa (2015). The enzymatic activities of urease and dehydrogenase were determined according to McGarity and Myers

Table 1
Primers used for the amplification of bacterial 16S rRNA, *pmoA*, and *nifH* genes.

Target gene	Primer	Sequence (5′–3′)	Fragment size (bp)	Reference	Amplification conditions
Bacterial 16S rRNA	Eub 338f	ACTCCTACGGGAGGCAGCAG	180	Bakke et al. (2011)	95 °C for 10 min; 40 cycles of 94 °C for 15 s, 56 °C for 30 s, and 72 °C for 45 s
	Eub 518r	ATTACCGCGGCTGCTGG			
pmoA	A189 f	GGN GAC TGG GAC TTC TGG	273	Degelmann et al. (2010)	95 °C for 5 min; 40 cycles of 94 °C for 20 s, 55 °C for 1 min, and 72 °C for 45 s
	A682 r	GAA SGC NGA GAA GAA SGC			
nifH	*nifH*	AAA GGY GGW ATC GGY AAR TCC ACC AC TTG TTS GCS GCR TAC ATS GCC ATC AT	457	Wallenstein and Vilgalys (2005)	95 °C for 5 min; 40 cycles of 95 °C for 30 s, 59 °C for 30 s, 72 °C for 1 min, and 72 °C for 1 min

(1967) and Casida et al. (1964), respectively. Also, the enzymatic activity of amylase was performed according to the Barbosa (2015) method, in which the substrate extraction was conducted as the Cole (1977) method, followed by the determination of reducing sugar by the Somogyi (1952) method. The enzymatic activity of cellulase was analyzed following the methodology proposed by Kanazawa and Miyashita (1986).

2.6. Data processing and analysis

Initially, an analysis of variance was performed in a completely randomized design, and the observations were repeated over time. Comparisons of daily means of FCO_2, Ts, and Ms were performed using the Tukey's test at a 5% significance level. The analyses of variance and regression between these variables were carried out using the software SAS (SAS version 9, SAS Institute, Cary, NC, USA). The descriptive statistics (mean, standard error of the mean, minimum, maximum, and coefficient of variation) was used to classify the variability of these attributes. The differences between the means of soil attributes for the different management systems were tested by the Student *t*-test ($p < 0.01$) using the software R (R Development Core Team, 2019).

The multivariate normality hypothesis was assessed, followed by the application of the multivariate statistical methods k-means non-hierarchical clustering analysis, principal components analysis (PCA), and heatmaps. These analyses aimed to reduce the number of variables and visualize the attributes that best interact with FCO_2. A clustering method was used to discriminate the soil management that presented higher similarity, and the generalized Mahalanobis distance matrix was applied to group them. The k-means clustering method minimizes the difference between points within the same group and maximizes the difference between k groups defined by the user.

The principal components whose eigenvalues were higher than the unity were considered in this analysis, according to the criterion established by Kaiser (1958). The coefficients of linear functions, which define the principal components, were used to interpret their meaning using the signal and relative size of the coefficients as an indication of the weight to be assigned to each variable. Only coefficients with high values, usually those higher than or equal to 0.70 in absolute value, were considered in the interpretation. The multivariate analyses (k-means and PCA) were processed in the software Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA). Heatmaps were generated using the Euclidean distance as the distance method, and this analysis was processed using the package gplots of the software R (R Core Team, 2019).

3. Results

3.1. Temporal variation of FCO_2, soil temperature, and soil moisture

The analysis of variance of repeated measures for soil CO_2 emission (FCO_2) did not indicate significance (F-test = 0.74, p = 0.6642) for the interaction between management systems and time (assessment days). Therefore, FCO_2 presented a similar pattern of temporal variability for both unburned (US) and burned sugarcane (BS) areas. However, a significant difference was observed when FCO_2 were analyzed separately relative to management (F-test = 45.58; $p < 0.0001$) and time (F-test = 9.94; $p < 0.0001$) (Table 2). BS presented the highest FCO_2 mean (2.63 $\mu mol\, m^{-2}\, s^{-1}$), which was 37% higher than in US (1.92 $\mu mol\, m^{-2}\, s^{-1}$). Also, higher values of FCO_2 were observed in both areas between Julian days 277 and 284, followed by a decrease up to the end of the experiment (Table 2). This effect is evident when observing the behavior of FCO_2 over the days in Fig. 1a.

On the Julian day 274, after the measurements were conducted, precipitation of 68 mm was observed after 28 days without precipitation, and its effect on FCO_2 was observed in the next measurement, carried out on the Julian day 277 in US (2.88 $\mu mol\, m^{-2}\, s^{-1}$) and BS (3.98 $\mu mol\, m^{-2}\, s^{-1}$). An increase of 81 and 107% in FCO_2 was

Table 2
Slicing of interaction between harvesting systems and time (days) for soil CO_2 emission, soil temperature, and soil moisture.

Julian day	Soil CO_2 emission (μmol m^{-2} s^{-1})			Soil temperature (°C)			Soil moisture (%)		
	US	BS	Mean	US	BS	Mean	US	BS	Mean
268	1.79	2.73	2.26 bc	19.31 Bbc	22.40 Aa	20.86	19.95	10.85	15.4 e
270	1.64	1.83	1.74c	18.50 Bc	22.00 Aab	20.25	18.80	10.70	14.7 e
274	1.59	1.92	1.75c	20.34 Ba	22.80 Aa	21.57	20.65	11.50	16.0 de
277	2.88	3.98	3.43 a	18.80 Bc	20.64 Acd	19.72	32.20	23.20	27.7 a
282	2.63	3.24	2.93 ab	18.95 Bbc	20.40 Ad	19.68	31.80	21.35	26.5 a
284	1.97	3.08	2.53 bc	18.68 Bc	21.40 Abc	20.04	30.15	20.35	25.2 a
288	1.61	2.27	1.94c	18.84 Bc	21.50 Ab	20.17	27.35	16.80	22.0b
290	1.78	2.46	2.11 bc	19.71 Bab	22.40 Aa	21.06	22.70	13.90	18.30 cd
295	1.46	2.20	1.83c	18.68 Bc	20.10 Ad	19.39	24.10	15.50	19.8 bc
Mean	1.92B	2.63 A		19.08	21.54		25.3 A	16.0B	

N = 20. US, unburned sugarcane harvesting system; BS, burned sugarcane harvesting system. Means followed by the same uppercase letters in the rows and lowercase letters in the columns do not differ from each other by the Tukey's test at 5% probability.

observed for US and BS, respectively, relative to the values observed in the assessment carried out on the Julian day 274 (1.59 and 1.92 μmol m^{-2} s^{-1}, respectively). Thus, the soil under BS presented a high sensitivity of FCO_2 to the intense precipitation. The assessment of the Julian day 277 occurred three days after precipitation. Moreover, precipitation of 75 mm was observed in the area in the afternoon of the Julian day 277, which, together with the previous precipitation, maintained high the FCO_2 values, as observed in the subsequent assessments conducted between the Julian days 282 and 284.

Soil temperature was the only variable measured on the temporal scale that showed a significant effect on the interaction between management systems and time (F-test = 7.14; $p < 0.0001$). Therefore, Ts did not present the same temporal variability pattern when both management systems were compared. Similar to FCO_2, the lowest Ts values were observed in US for all assessed days (Table 2 and Fig. 1b), with values ranging from 18.5 (Julian day 270) to 19.7 °C (Julian day 290), thus presenting a mean variation of 1.2 °C throughout the experimental period. In BS, on the other hand, Ts varied from 20.1 (Julian day 295) to 22.8 °C (Julian day 274), with a mean variation of 2.7 °C, indicating the effect of soil vegetation cover on this variable.

The straw on the soil surface preserved soil moisture (Fig. 1c), as shown by the highest values of Ms in US compared to BS, mainly after the precipitations that occurred on the Julian days 277 to 284, remaining high up to the end of the experiment. Although an increase in Ms has been observed in BS after precipitation (Fig. 1c), US had already been presenting higher Ms values on all assessed days even before these precipitations. For Ms, the repeated measures analysis also did not indicate an interaction between management systems and time (F-test = 0.52; p = 0.8461). However, significant values were observed when the management systems (F-test = 587.40; $p < 0.0001$) and time (F-test = 73.13; $p < 0.0001$) were analyzed separately. The mean values of Ms were 58% higher in US (25.30%), differing significantly ($p < 0.05$) from BS (16.02%) (Table 2). Over the experimental period, Ms varied from 18.80 to 32.20% in US on the Julian days 270 and 277, respectively. In BS, Ms variation was lower and presented higher values, also occurring on the Julian days 270 (10.70%) and 277 (23.20%).

Fig. 1 shows similarities in the temporal variability patterns of FCO_2 (Fig. 1a), Ts (Fig. 1b), and Ms (Fig. 1c), indicating a possible relationship between these variables. A regression analysis was performed to test this hypothesis (Table 3). We observed that FCO_2 was associated with Ms both in US ($R^2_{adjusted} = 0.64$; p = 0.01) and in BS ($R^2_{adjusted} = 0.66$; $p < 0.01$). The adjusted linear model explained 64% of the temporal variability of FCO_2 in US and 66% in BS, indicating that, over time, FCO_2 was controlled by the variation of soil water content and suggesting that microbial activity was not limited by the low soil aeration during the experimental period in both harvesting systems.

3.2. Effect of management on soil attributes

The attributes soil potential acidity (H + Al), calcium (Ca), potassium (K), the sum of bases, cation exchange capacity (CEC), carbon stock (Cstock), soil carbon decay constant (k), soil labile carbon half-life ($t_{1/2}$), soil bulk density (Ds), macropores (Macro), micropores (Micro), total pore volume (TPV), and air-filled pore space (AFPS) differed significantly ($p < 0.01$) as a function of the management systems (Table 4). BS presented the highest values of H + Al, Ca, and the sum of bases. The variables CEC (11.82 $cmol_c$ dm^{-3}) and K (0.90 $cmol_c$ dm^{-3}) were significantly higher in US (Table 4). K was 52% higher in US compared to BS (0.59 $cmol_c$ dm^{-3}). Soil organic matter (SOM) did not differ as a function of the management systems (Table 4). Moreover, Cstock was 12% higher in the unburned sugarcane (US) area (44.54 Mg ha^{-1}) than in the burned sugarcane (BS) area (39.85 Mg ha^{-1}), differing between the management systems (Table 4).

However, in addition to assessing soil carbon stock, measuring the stability of this carbon in the studied ecosystems is essential. In our study, this stability was estimated by calculating the soil carbon decay constant (k) and the soil labile carbon half-life ($t_{1/2}$). Unburned and burned sugarcane management systems showed a different behavior regarding k and $t_{1/2}$ (Table 4). The constant k values indicated that the carbon was decomposed faster in the burned sugarcane area (0.00070 $days^{-1}$) than in the unburned sugarcane area (0.00046 $days^{-1}$) (Table 4). Thus, soil carbon half-life was longer in the unburned sugarcane area (1,572.82 days) than in the burned sugarcane area (1,033.95 days), i.e., the carbon permanence time in the unburned sugarcane was 52% higher than in the burned sugarcane (Table 4).

The soil chemical attributes pH, Mg, P, Al, the sum of bases, and aluminum saturation (m) did not present significant differences, considering the sugarcane management systems (Table 4). However, soil structure was influenced by the management since all soil physical attributes differed significantly according to the management system (Table 4). In this sense, the unburned sugarcane area presented the highest mean values of Ds (1.28 g cm^{-3}) and Micro (39.79%) compared to the burned sugarcane area. High values of Ds and Micro and low values of Macro (11.43%) in the unburned sugarcane area were mainly due to the natural arrangement of the non-disturbed soil and the mechanical harvesting system without burning. Also, Macro (19.88%) TPV (56.31%), and air-filled pore space (AFPS) (40.29%) presented high values in the burned sugarcane area (Table 4), indicating the characteristics of a non-compacted area.

In addition, no difference was observed between the unburned sugarcane and the burned sugarcane areas for the enzymatic activity of urease, amylase, and cellulase (Table 4). On the other hand, the enzymatic activity of dehydrogenase was significantly higher ($p < 0.05$) in the unburned sugarcane area (29.75 μg TPF g^{-1} dry soil 24 h^{-1}) than in the burned sugarcane area (23.65 μg TPF g^{-1} dry soil 24 h^{-1})

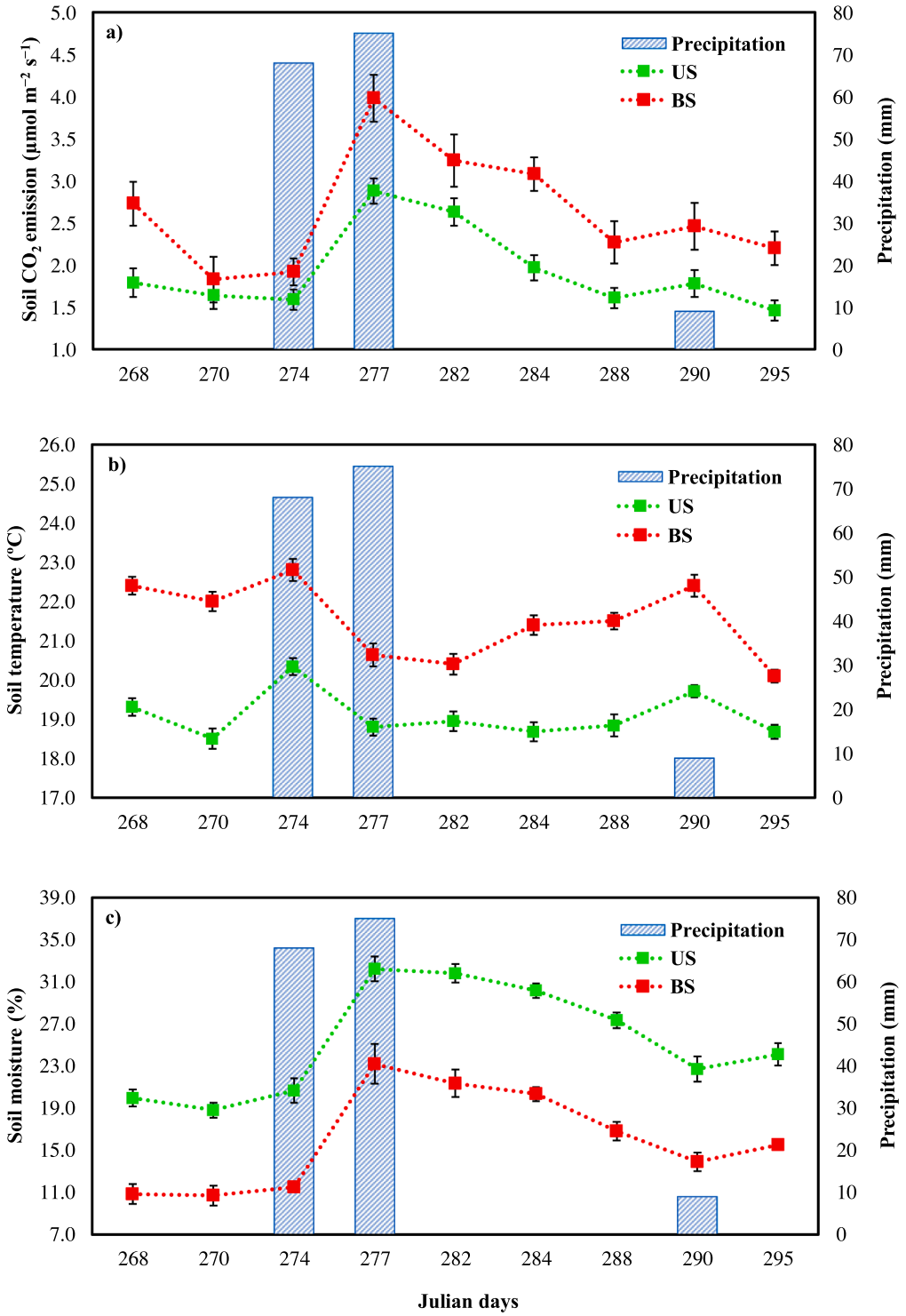

Fig. 1. Mean daily values of soil CO_2 emission (a), soil temperature (b), and soil moisture (c), with bars of the standard error of the mean, as well as the precipitation that occurred during the study period in the unburned (US) and burned (BS) sugarcane harvesting systems.

(Table 4). Moreover, the variable microbial biomass carbon (Cmic) was 92% higher in the unburned sugarcane area (25.43 µg g^{-1} soil C) compared to the burned sugarcane area (13.25 µg g^{-1} soil C) (Table 4). The soil C/N ratio was also higher in the unburned sugarcane area (14.8), differing from the burned sugarcane area (11.75) (Table 4).

Considering the microbiota characteristics due to the management and the choice of the three genes (bacterial 16S, *pmo*A, and *nif*H) for this study, it is important to highlight that the quantification of the bacterial 16S gene allows estimating the total number of the soil microbial community, while *pmo*A and *nif*H genes allow estimating the microorganisms that act directly in the biogeochemical cycles of carbon and nitrogen, respectively, as biodegradable agents. However, in our study, a difference was observed only for the number of copies of the *nif*H gene when comparing the unburned sugarcane area (434 copies g^{-1} soil) with the burned sugarcane area (4,436.67 copies g^{-1} soil) (Table 4), indicating that the FCO_2 variation in the management systems may not be directly related to the total amount of microorganisms active in the soil but to the specific role they play in this agroecosystem.

Table 3
Parameters of the linear regression between soil CO_2 emission (FCO_2) and soil moisture (Ms) for both harvesting systems.

Management	Linear regression $FCO_2 = a + b \times Ms$			
	a	b	*R^2	*p*
Unburned sugarcane	0.03 ± 0.54	0.08 ± 0.02	0.64	0.010
Burned sugarcane	0.66 ± 0.51	0.12 ± 0.03	0.66	0.005

* Adjusted R^2.

3.3. Interdependence relationship between management and soil attributes

Considering that FCO_2 has a complex relationship with edaphoclimatic conditions, a multivariate analysis was conducted to verify which attributes under study were the most expressive to explain the characteristics of unburned and burned sugarcane management systems.

3.3.1. Non-hierarchical clustering analysis (k-means)

The results presented in the analysis of the temporal variation (Table 2 and Fig. 1) were corroborated by the non-hierarchical clustering analysis (k-means), which presents the standardized means of soil physical, chemical, and biological attributes, highlighting the individual behavior of each variable for the identified groups (US and BS). In this context, Group 1 (BS) presented the highest values of FCO_2 and soil temperature (Ts), and the lowest values of soil moisture (Ms) compared to Group 2 (US) (Fig. 2). The attributes sum of bases, P, Cstock, *k*, and $t_{1/2}$ were also selected by the multivariate analysis and showed distinct behavior as a function of the management system (Fig. 2).

Regarding soil enzymatic activity, the multivariate analysis selected the enzymes dehydrogenase, amylase, and cellulase, in addition to the microbial biomass carbon (Cmic). Among them, dehydrogenase and Cmic stood out in US and amylase in BS (Fig. 2). On the other hand, the functional *nif*H and *pmo*A genes were more expressive in BS. Also, US showed high Ds and a low AFPS, corroborating the results shown in Table 4, related to soil aeration in the areas under study.

3.3.2. Correlation of characteristics (management and attributes) according to similarities and dissimilarities

Fig. 3 shows the principal components, while Table 5 shows the variance assigned to them. Two components were extracted from this analysis, indicating that two independent processes were observed in the soil, which together explained 81.8% of the total variance observed in the original data. The first process (PC1) represented 63.5% of the total variance, while the second process (PC2) represented 18.3% of the total variance (Fig. 3 and Table 5).

The process contained in PC1 was the most important for our study, as it is derived from the highest eigenvalue and has the highest percentage of explanation (63.5%). The variables that contributed the most with this process were Ms (0.95), P (−0.93), Cstock (0.92), Ts (−0.90), dehydrogenase (0.87), FCO_2 (−0.82), soil C/N ratio (0.82), AFPS

Table 4
Descriptive statistics of soil CO_2 emission, soil temperature, soil moisture, soil carbon decay constant, soil labile carbon half-life, and soil chemical, physical, and microbiological attributes in the assessed harvesting systems.

Attribute	Unburned sugarcane					Burned sugarcane					
	Mean	SE	Minimum	Maximum	CV (%)	Mean	SE	Minimum	Maximum	CV (%)	F-test
FCO_2 (μmol m^{-2} s^{-1})#	1.92b	0.06	1.29	2.65	39.06	2.63 a	0.11	1.83	3.40	59.79	45.58**
Ts (°C) #	19.08b	0.07	18.56	19.66	5.26	21.54 a	0.09	21.04	22.35	5.91	87.40**
Ms (%)#	25.30 a	0.49	21.89	28.00	26.29	16.02b	0.37	15.00	17.56	31.30	635.70**
pH ($CaCl_2$)	5.18 a	0.03	4.90	5.40	2.39	5.14 a	0.03	5.00	5.40	2.71	0.92 ns
H + Al ($cmol_c$ dm^{-3})	4.21b	0.09	3.40	5.20	10.10	4.75 a	0.15	3.40	5.8	13.71	9.81**
Mg^{2+} ($cmol_c$ dm^{-3})	1.18 a	0.03	0.89	1.52	13.49	1.27 a	0.06	0.97	1.99	19.81	1.63 ns
Ca^{2+} ($cmol_c$ dm^{-3})	4.45b	0.06	3.91	4.96	6.65	5.20 a	0.07	4.56	5.66	5.75	62.89**
K^+ ($cmol_c$ dm^{-3})	0.90 a	0.03	0.80	1.27	16.51	0.59b	0.03	0.35	0.89	24.56	44.75**
P (mg dm^{-3})	23.15 a	0.74	19.00	32.00	14.31	25.15 a	1.00	21.00	39.00	17.79	2.58 ns
Sum of bases ($cmol_c$ dm^{-3})	6.50b	0.10	5.23	7.16	6.63	7.05 a	0.08	6.42	7.82	5.06	19.94**
CEC ($cmol_c$ dm^{-3})	11.82 a	0.10	9.46	11.65	4.10	10.72b	0.14	10.49	12.76	5.39	40.18**
Al ($cmol_c$ dm^{-3})	0.02 a	0.02	0.01	0.19	47.21	0.07 a	0.06	0.01	0.13	45.49	2.14 ns
V (%)	60.59 a	0.77	53.08	67.03	5.70	59.81 a	0.85	53.07	67.33	6.39	0.46 ns
SOM (g dm^{-3})	33.90 a	0.50	29.00	39.00	7.02	32.15 a	0.42	30.00	37.00	5.70	1.69 ns
Cstock (Mg ha^{-1})	44.54 a	1.71	37.34	65.63	17.13	39.85b	1.02	30.11	45.07	11.41	9.32**
Soil C/N ratio	14.80 a	0.50	14.00	16.00	6.09	11.75b	0.85	9.00	13.00	15.46	7.02*
k (day^{-1})	0.00046b	0.00	0.00031	0.00060	18.32	0.00070 a	0.00	0.00046	0.0010	21.73	106.85**
$t_{1/2}$ (day)	1,572.82 a	72.26	1,174.53	2,263.86	20.54	1,033.95b	44.99	676.98	1,503.55	19.46	77.96**
Ds (g cm^{-3})	1.28 a	0.02	1.14	1.48	6.13	1.11b	0.02	0.98	1.25	6.93	47.79**
Macro (%)	11.43b	1.17	11.70	20.91	45.80	19.88 a	0.83	11.53	26.13	18.62	34.77**
Micro (%)	39.79 a	0.45	36.03	43.58	5.08	36.42b	0.51	32.89	40.96	6.22	24.57**
TPV (%)	51.22b	0.94	40.11	56.95	8.20	56.31 a	0.41	51.93	59.02	3.26	24.67**
AFPS (%)	26.14b	1.03	14.45	33.06	17.64	40.29 a	0.45	36.38	43.91	5.01	158.00**
Dehydrogenase (μg TPF g^{-1} dry soil 24 h^{-1})	29.75 a	1.99	26.43	34,53	13.34	23.65b	1.45	20.34	26.92	12.23	6.17*
Urease (μg TPF g^{-1} dry soil 24 h^{-1})	15.97 a	3.02	10.33	24.41	37.83	15.61 a	1.75	12.23	19.37	22.44	0.51 ns
Amylase (μg TPF g^{-1} dry soil 24 h^{-1})	47.86 a	3.56	41.17	54.83	14.87	55.96 a	2.01	50.43	59.60	7.17	3.94 ns
Cellulase (μg TPF g^{-1} dry soil 24 h^{-1})	89.51 a	0.88	86.93	90.82	1.96	89.65 a	0.41	89.01	90.84	1.02	0.22 ns
Microbial biomass carbon (μg g^{-1} soil C)	25.43 a	1.32	17.32	32.53	26.10	13.25b	2.86	14.75	30.38	32.01	22.52**
Bacterial 16S rRNA (copies g^{-1} soil)	3.77^{E+09} a	1.52^{E+08}	2.63^{E+09}	4.23^{E+09}	20.04	3.45^{E+09} a	1.31^{E+08}	3.00^{E+09}	3.66^{E+09}	8.85	0.60 ns
*pmo*A (copies g^{-1} soil)	5.13^{E+04} a	3.23^{E+03}	5.94^{E+03}	1.81^{E+05}	38.06	1.21^{E+05} a	1.18^{E+04}	9.46^{E+04}	1.42^{E+05}	19.01	2.45 ns
*nif*H (copies g^{-1} soil)	434.00b	46.00	244.70	568.05	26.63	4,436.67 a	945.90	3,897.90	5,654.34	18.11	23.94**

N = 20 except for #, in which N = 180; ns = non significant; * significant at 5% ($p < 0.05$); ** significant at 1% ($p < 0.01$). Means followed by the same letter in the rows did not differ from each other by the Student *t*-test. SE, standard error of the mean; CV, coefficient of variation; FCO_2, soil CO_2 emission; Ts, soil temperature; Ms, soil moisture; H + Al, potential acidity; Mg, exchangeable magnesium content; Ca, exchangeable calcium content; K, exchangeable potassium content; P, phosphorus content; CEC, cation exchange capacity; Al, aluminum content; V, base saturation; SOM, soil organic matter; Cstock, soil carbon stock; *k*, soil carbon decay constant; $t_{1/2}$, soil labile carbon half-life; Ds, soil bulk density; Macro, macroporosity; Micro, microporosity; TPV, total pore volume; AFPS, air-filled pore space; Bacterial 16S rRNA, number of copies of the bacterial 16S rRNA; pmoA, number of copies functional gene pmoA; nifH, number of copies functional gene nifH.

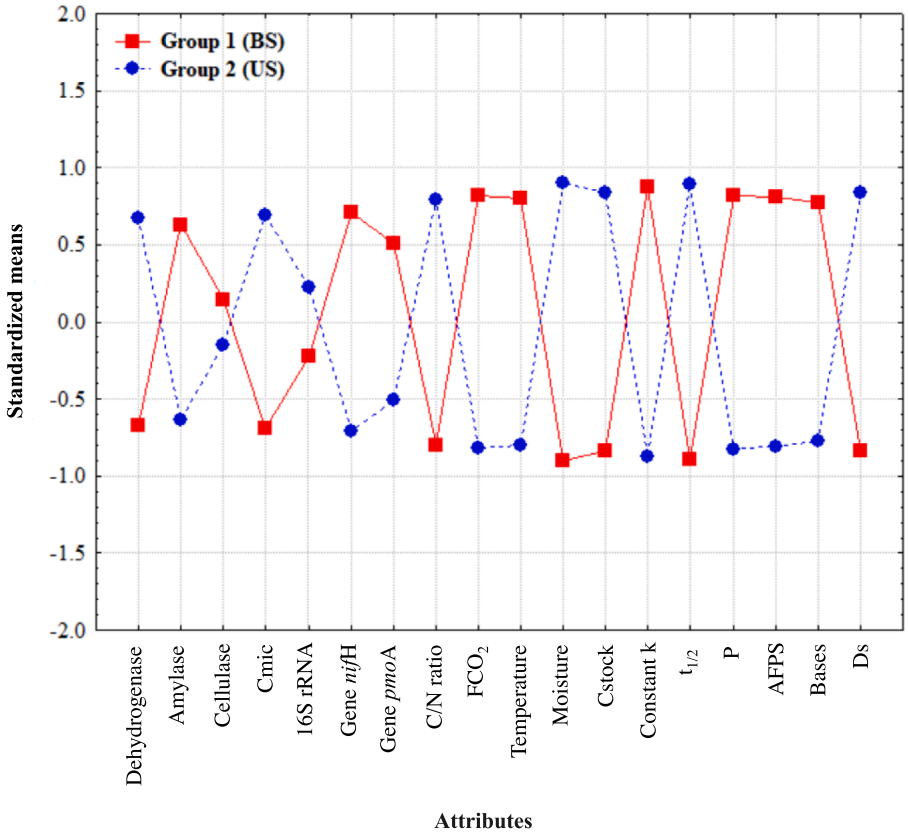

Fig. 2. Standardized means of the attributes studied under different harvesting systems (US and BS), according to the k-means non-hierarchical clustering analysis. US, unburned sugarcane harvesting system; BS, burned sugarcane harvesting system; FCO_2, soil CO_2 emission; Temperature, soil temperature; Moisture, soil moisture; AFPS, air-filled pore space; Bases, sum of bases; 16S rRNA, number of copies of the bacterial 16S rRNA gene; Gene *pmo*A, *pmo*A functional gene; Gene *nif*H, *nif*H functional gene; Cmic, microbial biomass carbon; C/N ratio, soil C/N ratio; Ds, soil bulk density; Dehydrogenase, enzymatic activity of dehydrogenase; Amylase, enzymatic activity of amylase; Cellulase, enzymatic activity of cellulase; P, phosphorus content; Cstock, soil carbon stock; Constant *k*, soil carbon decay constant; $t_{1/2}$, soil labile carbon half-life.

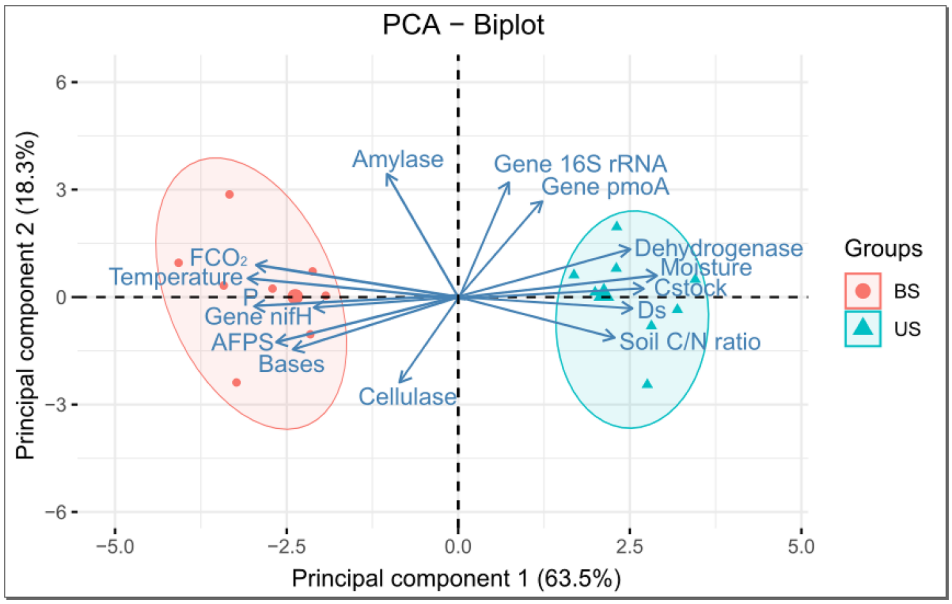

Fig. 3. Biplot graph with soil attributes in the unburned (US) and burned (BS) management systems and confidence ellipses (95% confidence). FCO_2, soil CO_2 emission; Temperature, soil temperature; Moisture, soil moisture; AFPS, air-filled pore space; Bases, sum of bases; 16S rRNA, number of copies of the bacterial 16S rRNA gene; *pmo*A, *pmo*A functional gene; *nif*H, *nif*H functional gene; Ds, soil bulk density; Dehydrogenase, enzymatic activity of dehydrogenase; Amylase, enzymatic activity of amylase; Cellulase, enzymatic activity of cellulase.

(−0.80), Ds (0.80), the sum of bases (−0.77), and *nif*H (−0.76). According to the signals of loadings, Ms, Cstock, dehydrogenase, soil C/N ratio, and Ds, grouped to the right of the biplot graph (Fig. 3) in PC1, are direct and strongly related to each other, but inversely related to Ts, P, FCO_2, AFPS, the sum of bases, and *nif*H, grouped to the left of the biplot graph, also in PC1 (Fig. 3). Similarly, Ts, P, FCO_2, AFPS, the sum of bases, and *nif*H are directly associated with each other but inversely associated with attributes located on the opposite side of the graph (Ms, Cstock, dehydrogenase, soil C/N ratio, and Ds) (Fig. 3).

Another important factor that Fig. 3 shows is the dispersion of US and BS, whose groupings are arranged on opposite sides of the graph. The dispersion of sampling points that represent US faces the same projection site of Ms, Cstock, dehydrogenase, soil C/N ratio, and Ds, indicating that these attributes are more expressive in US than in BS. Similarly, Ts, P, FCO_2, AFPS, the sum of bases, and *nif*H were more expressive in BS and less representative in US. Therefore, all attributes retained in PC1 are responsible for the characteristics that distinguished US from BS in the present study. For instance, a characteristic of soil under unburned management is to have high Ms, Cstock, dehydrogenase, soil C/N ratio, and Ds, as well as low Ts, P, FCO_2, AFPS, the sum of bases, and *nif*H. On the other hand, a soil under burned sugarcane management presents as main characteristic high Ts, P, FCO_2,

Table 5
Correlation between soil attributes and the first two principal components (PC1 and PC2).

Attribute	PC1 63.5%*	PC2 18.3%*
FCO_2	**−0.82**	0.32
Temperature	**−0.90**	0.19
Moisture	**0.95**	0.15
Soil C/N ratio	**0.82**	−0.13
Cstock	**0.92**	0.04
AFPS	**−0.80**	−0.12
Sum of bases	**−0.77**	−0.30
16S rRNA	0.31	**0.81**
*pmo*A	0.47	**0.71**
*nif*H	**−0.76**	−0.20
Ds	**0.80**	−0.12
Dehydrogenase	**0.87**	0.12
Amylase	−0.22	**0.70**
Cellulase	−0.25	**−0.76**
P	**−0.93**	−0.05
Interpretation	Production and transport of CO_2	Enzymatic activity of amylase, cellulase, and *pmo*A and 16S rRNA genes

* Value refers to the percentage of variation of the original dataset retained by the respective factors. Loading values in bold (higher than 0.70 in absolute value) were considered in the factor interpretation. FCO_2, soil CO_2 emission; Temperature, soil temperature; Moisture, soil moisture; AFPS, air-filled pore space; 16S rRNA, number of copies of the bacterial 16S rRNA; *pmo*A, *pmo*A functional gene; *nif*H, *nif*H functional gene; P, phosphorus content; Ds, soil bulk density; Dehydrogenase, enzymatic activity of dehydrogenase; Amylase, enzymatic activity of amylase; Cellulase, enzymatic activity of cellulase.

AFPS, the sum of bases, and *nif*H and hence low values of Ms, Cstock, dehydrogenase, and soil C/N ratio. From these associations, PC1 can be interpreted as the process that occurs in the soil related to the production and transport of CO_2, resulting in high or low FCO_2 values in the assessed management systems.

The behavior observed in PC1 (Fig. 3) was confirmed by the construction of the heatmap graph using the multivariate cluster analysis and Euclidean distance as a method of the metric distance between groups (Fig. 4). Two significant clusters were found with the distinction between US and BS from the attributes retained in PC1 (Fig. 3 and Table 5). In addition, the scale of colors in the heatmap showed in which management the soil attributes are more or less expressive (Fig. 4), corroborating the results obtained with PCA (Fig. 3).

4. Discussion

4.1. Effects of straw and seasonal changes on FCO_2, soil temperature, and soil moisture

Several studies conducted in short periods in sugarcane areas have reported low FCO_2 values under unburned areas (Panosso et al., 2011; Silva-Olaya et al., 2013; Acreche et al., 2014; Bicalho et al., 2014). According to these authors, this result is mainly due to the remaining crop residues left on the soil surface after harvesting the sugarcane areas, and the low emissions are related to the effects of these residues on soil temperature and soil moisture. Climate conditions are determinant on FCO_2 intensity (Table 2 and Fig. 1a), leading to daily variations in the emissions (Panosso et al., 2011; Moitinho et al., 2015), as they alter soil temperature (Table 2 and Fig. 1b) and soil moisture (Table 2 and Fig. 1c), which are controlling factors of the CO_2 emission process (Wang et al., 2015).

In a similar study also conducted in an Oxisol, Panosso et al. (2011) observed that FCO_2 was 32% higher in the burned sugarcane area (2.74 $\mu mol\ m^{-2}\ s^{-1}$) than in the unburned sugarcane area (2.07 $\mu mol\ m^{-2}\ s^{-1}$) at seven years after the conversion from the burned to the unburned sugarcane harvesting system. De Figueiredo and La Scala Jr (2011) elaborated a GHG balance due to the conversion of these systems and estimated a replacement from the burned to unburned sugarcane areas from 310.7 (not considering soil carbon sequestration) to 1484.0 kg CO_2eq $ha^{-1}\ year^{-1}$ (considering soil carbon sequestration). In contrast, Vargas et al. (2014) observed that sugarcane crop residues increased CO_2 and N_2O emissions under high soil moisture conditions, as the presence of crop residues promoted a higher microbial activity, in addition to the effect on soil moisture conservation. In this scenario, there is not only the emission of CO_2 from the soil but also straw decomposition and consequent release of soluble C, which is also one of the main propellants for N_2O losses.

The mean daily values of soil temperature were always lower in US than in BS, which was already expected because, depending on the amount and distribution of soil vegetation cover, the temperature of soil surface can be reduced by up to 4 °C. In addition, the soil evaporation rate is reduced to about 25%, with a vegetation cover of approximately 70%. Soil surface temperature below the straw decreases up to 5 °C compared to the ambient temperature (Tominaga et al., 2002). Soil temperature is one of the most important factors for the CO_2 emission process during and between the days because its increase accelerates organic matter decomposition and microbial and root activities (Silva-Olaya et al., 2013), thus influencing FCO_2, which responds exponentially to increases in soil temperature (Acreche et al.,

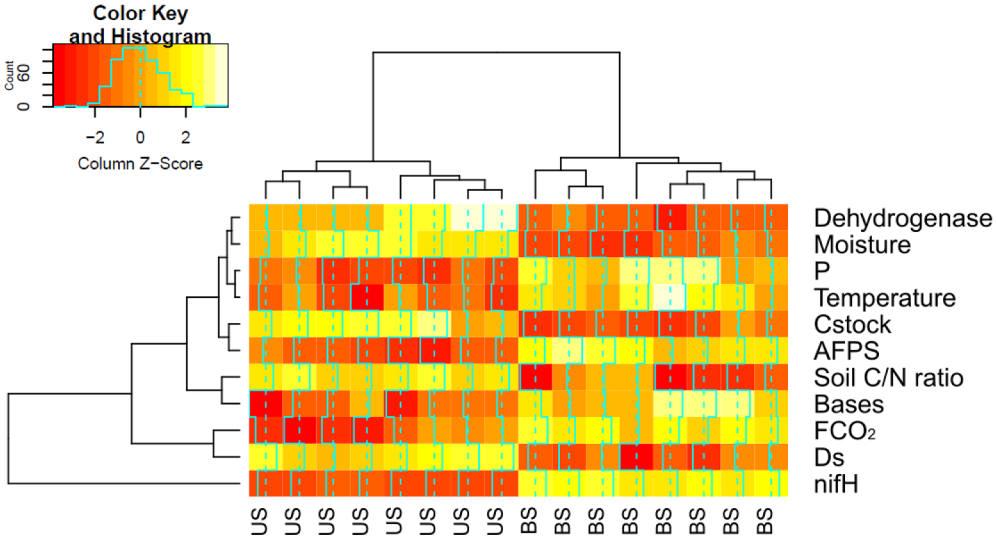

Fig. 4. Heatmap graph ($r = 0.98$; $p < 0.0001$) with soil attributes in the unburned (US) and burned sugarcane (BS) harvesting systems. Heatmap shows the variations of soil attributes as a function of management. The dendrogram above the heatmap represents the clustering of the management based on similar patterns of variation. FCO_2, soil CO_2 emission; Temperature, soil temperature; Moisture, soil moisture; AFPS, air-filled pore space; Bases, sum of bases; *pmo*A, *pmo*A functional gene; *nif*H, *nif*H functional gene; Ds, soil bulk density; Dehydrogenase, enzymatic activity of dehydrogenase.

2014; Karhu et al., 2014).

In addition to the thermal insulation effect promoted by straw on the reduction of soil temperature and maintenance of soil water content (Ussiri and Lal, 2009), another advantage of the unburned on burned sugarcane is that soil burning contributes to creating hydrophobic substances, whose effect is greater the lower soil particles are (Primavesi, 2006; Vogelmann et al., 2010). Soils that present these organic substances have a strong repellency to water, becoming drier than soils without burning (Primavesi, 2006).

On the other hand, no significant ($p > 0.05$) linear or quadratic models between FCO_2 and Ts were found for both management systems possibly due to the low Ts variation throughout the experimental period, as can be observed by the range of variation (minimum, maximum, and CV values) shown in Table 4. In addition, the effect of Ts on FCO_2 can be partially hidden by the Ms effect, as soil moisture and temperature are interdependent variables and commonly change simultaneously (Ding et al., 2010).

Soil moisture content is another factor of great importance when assessing FCO_2. Soil moisture is related to the processes of production (Lal, 2009), transport (Ball, 2013), and hence emission of CO_2 from soil to the atmosphere (Table 3). Depending on the soil moisture content, these processes may be favored or inhibited, as they affect microbial activity and gas diffusion. These effects are mainly due to the interaction between moisture content and soil porosity (Ordóñez-Fernández et al., 2008). The movement of air and water replacement inversely relates aeration and moisture (Chen et al., 2010). Changes in soil and air constitution are related to the growth and activity of the microbiota, as CO_2 and O_2 are required for its growth. From a microbiological point of view, a well-aerated soil is that in which the oxygenation activity is maximal (Tsai et al., 1992). Other authors also observed a positive relationship between soil CO_2 emission and soil moisture (Morell et al., 2010; Vargas et al., 2014; Wang et al., 2015).

However, the effect of soil moisture on FCO_2 is more accentuated when precipitation occurs (Fig. 1a and c) (Panosso et al., 2011; Wang et al., 2015), especially after a dry season period (Moitinho et al., 2015). During a precipitation event, or even in a short period after it, the physical expulsion of CO_2 from soil pores occurs due to the water entry into the pores (Chen et al., 2010). After precipitation and with soil still soaked, there is a decrease in FCO_2 due to a protection layer (sealing) caused by water in the soil. However, the emission increases as water evaporates because soil pores are no longer filled with water in this process and, consequently, soil reestablishes its aeration condition, favoring the aerobic activity of microorganisms (Zanchi et al., 2003).

4.2. Carbon stock and stability in soil

Positive results related to an increase in the carbon stock and its stability in the soil were observed with the conversion of BS into US (Table 4 and Fig. 2). Similar results have been widely reported in the literature and are directly related to the time of adoption of the unburned sugarcane management system with crop residues left on the soil surface, leading to increases in carbon stocks usually in the most superficial soil layers (Galdos et al., 2009; Panosso et al., 2011; Souza et al., 2012; Cerri et al., 2013; Carvalho et al., 2017).

According to some long-term studies, the maintenance of around 15 t ha^{-1} $year^{-1}$ of dry matter from sugarcane crop residues would result in an accumulation of carbon in the superficial soil layers (Cerri et al., 2013). Other studies also have suggested that, in addition to post-harvesting sugarcane residues deposited on the soil surface (sugarcane straw), the root system (rhizomes and roots) could also contribute to increasing organic carbon reserves in the soil (Carvalho et al., 2017). Carvalho et al. (2017) conducted a literature review associated with modeling results and observed that although the root system may contribute to soil carbon inputs, sugarcane crop residues on the soil surface were the main C inputs, exceeding, on average, more than three times the potential of C entry by the root system. According to Bordonal et al. (2018), estimations of the effects of land-use change and soil management on soil C balance should also take into account CO_2 savings from sugarcane cultivation.

According to Souza et al. (2012), the burning of sugarcane fields also leads to higher availability of mineralizable C, with higher proportions of recalcitrant C in sugarcane areas without burning. Therefore, the unburned sugarcane system favors the increased stability of soil aggregates and, therefore, high carbon stocks (Galdos et al., 2009) due to the high physical protection of organic matter inside the aggregates, hampering its decomposition by microorganisms (Souza et al., 2012). Also, this system reduces O_2 availability for the oxidative processes of decomposition (Tominaga et al., 2002).

Although the unburned sugarcane harvesting system contributes to high amounts of carbon deposited on the soil surface during several growing seasons, a great part of this carbon can be lost in the short term (La Scala et al., 2009) during sugarcane field reform activities, which include conventional tillage and soil correction (lime and gypsum application), reducing the potential for carbon sequestration in this agrosystem. In this context, the maintenance of sugarcane straw on the soil surface associated with the minimum tillage is, therefore, a more sustainable management option to be considered during the sugarcane replanting period (Bordonal et al., 2018).

In addition, the soil C/N ratio was also higher in US (14.8) than in BS (11.75) (Table 4 and Fig. 2). Canellas et al. (2003) conducted a study in a Cambisol aiming at assessing different sugarcane management systems and also observed high C/N values in the unburned sugarcane area (10.1), which differed from the burned management (8.7), indicating the presence of stable organic matter. The highest mean values of Cstock and soil carbon stability (k and $t_{1/2}$), enzymatic activity of dehydrogenase, Cmic, and soil C/N ratio, and the lowest mean value of FCO_2 (Table 4 and Fig. 2) in US compared to BS indicated that the unburned sugarcane area is a balanced ecosystem (Canellas et al., 2003).

4.3. Straw versus soil aeration

Soil aeration was influenced by management because soil physical attributes showed a distinct behavior when BS and US were compared (Table 4 and Figs. 2 to 4). High values of soil density (Ds) and soil microporosity (Micro) associated with low values of air-filled pore space (AFPS), soil macroporosity (Macro), and total pore volume (TPV) were observed in US compared to BS (Table 4). It is related to the mechanized harvesting system of the unburned sugarcane system, which causes the soil compaction up to a depth of 0–0.20 m, caused by the machinery traffic (Tominaga et al., 2002). High soil compaction levels in this mechanized harvesting system have been recognized as the main issue related to the negative points in the conversion of systems (Otto et al., 2011; Souza et al., 2014). Soil compaction occurs mainly in soils with a clayey texture and high moisture contents, as observed in the US area of our study.

High FCO_2 values in the burned sugarcane area may be related to these soil structural characteristics because higher aeration, with a consequent reduction in density, as observed in our study in BS (Table 4), optimizes the gas transport processes in the soil, i.e., the oxygen entry into the soil, required for the aerobic microbial activity, and the escape of CO_2 as a byproduct of this activity (Teixeira et al., 2012). Moreover, gas exchange between soil and the atmosphere can only occur adequately in well-drained soils since the path through which CO_2 and O_2 move inside the soil consists of large and continuous pores (Chen et al., 2010). Thus, soils with characteristics of burned sugarcane areas (more aeration) can provide conditions that favor the aerobic respiration of macro- and microorganisms, allowing more air to enter the soil when compared to unburned sugarcane areas, as observed in our study.

Among the several effects caused by the thick straw cover remaining from the harvest process, the high soil compaction due to the reduced

porous space and increased soil density in unburned sugarcane areas was undoubtedly the driver of several studies that have recently emerged addressing alternative practices associated with this management, such as the machinery traffic control in sugarcane areas, but mainly the partial straw removal (Castioni et al., 2019; Cherubin et al., 2019) to produce cellulosic ethanol or bioelectricity (Carvalho et al., 2019; Menandro et al., 2019).

4.4. Effect of management on soil dehydrogenase activity and microbial biomass

Soil enzymes are related to important microbial functions in the cycling of nutrients in ecosystems. Among the several soil enzymes, the enzymatic activity of dehydrogenase is associated with the intracellular metabolic activity, thus reflecting microbial growth and its association with the active fraction of soil microbial community (Tan et al., 2008). Soil moisture stands out among the edaphic factors that stimulate dehydrogenase activity (Uhlirova et al., 2005). In this sense, soil microbial activity, community composition, and hence soil enzymatic activity decrease as soils become drier.

On the contrary, soils with high moisture content may favor an increase in the number of bacterial populations (Subhani et al., 2001). Thus, any change in the number or activity of microorganisms is reflected in the soil enzyme biosynthesis. Soils with high moisture content or under soaked conditions present high dehydrogenase values (Zhao et al., 2010). In this context, the high enzymatic activity of dehydrogenase in US (Table 4 and Fig. 2) may be related to a mean soil moisture 58% higher in this management system (25.30%) than in BS (16.02%) (Table 4 and Fig. 2).

Microbial biomass carbon (Cmic) was also higher in US than in BS (Table 4 and Fig. 2). Souza et al. (2012) conducted a study in sugarcane areas under different harvesting management systems and observed that Cmic was 102% higher in the soil of the unburned sugarcane area compared to the burned sugarcane area, and 222% higher in the soil of a forest area compared to the unburned sugarcane area. In this case, the authors concluded that Cmic could be a reliable quality indicator for monitoring soils under different sugarcane harvesting systems. Tavares et al. (2015) assessed soil CO_2 emissions and soil microbial activity in one burned and two unburned sugarcane harvesting systems with a 5- and 10-year conversion history and observed that Cmic assessed in the winter showed no significant differences when comparing the three areas. However, in the summer, Cmic was 6.5 and 5.6% higher in the 10-year history unburned area than in the burned and 5-year history unburned areas, respectively. Galdos et al. (2009) observed that Cmic was significantly higher in an unburned area than in a burned sugarcane area, indicating that this fraction of organic matter is sensitive to changes in the surface residue management in the short term.

A detailed study on how microorganisms influence and are influenced by the environment is required for a better understanding of the environmental processes controlled by microbial communities (Rosenzweig et al., 2016). Much of the efforts to describe the relationship between emission/concentration of CO_2 in the atmosphere and soil microbial communities have been focused on their phylogenetic composition (Macedo, 2012). However, studies on the characterization of functional diversity of the microbial community mediating important biogeochemical processes, such as carbon and nitrogen cycling in the soil, are still scarce (Xu et al., 2013).

4.5. Main soil attributes related to variations in soil CO_2 emissions in the burned and unburned harvesting systems

The biplot (Fig. 3) and heatmap (Fig. 4) graphs represent the selection of the most expressive attributes in each management. Thus, they show which of the 30 attributes assessed in this study were associated with FCO_2 and were responsible for characterizing the burned (BS) and unburned sugarcane (US) areas. The production of CO_2 is a biochemical process related to the root respiration and microorganism dynamics, which are influenced by soil temperature, soil moisture, and soil nutritional status (Lal, 2009). That is the reason why FCO_2 is directly associated with Ts, the sum of bases (Ca + K + Mg), P, and *nif*H in BS (Table 5 and Fig. 3). Also, the available P content, besides being associated with soil nutritional status, is a chemical element related to soil microbial activity and can be considered a limiting factor to the dynamics of the intensity of this activity due to its importance in the metabolism of microorganisms (Duah-Yentumi et al., 1998).

In addition, the fact that FCO_2 is directly associated with AFPS and inversely related to Ds and Ms (Table 5 and Fig. 3) is in agreement with the responsive characteristics of CO_2 transport from soil to the atmosphere, as it occurs through diffusion and mass flow mechanisms, which vary as a function of soil texture, structure, and moisture content (Ball, 2013). An increased soil porosity (aeration) and low soil density make the gas exchange between soil and the atmosphere easier (Chen et al., 2010), favoring soil respiration and, consequently, increasing FCO_2. Also, the biodegradation processes related to carbon and nitrogen cycles that directly influence FCO_2 depend on the structure, composition, and functional potential of soil microbial communities (Xu et al., 2013).

In our study, *pmo*A and *nif*H genes represented the functional structure of the microbiota, while the bacterial 16S rRNA gene represented the total amount of microorganisms in the soil. Considering that the principal components are orthogonal to each other (not correlated), the processes retained in PC2, related to soil enzymatic activity (amylase and cellulase) and genes (bacterial 16S rRNA and *pmo*A), are independent of the process that occurs in PC1 (Table 5 and Fig. 3). Thus, the variation in the FCO_2 dynamics is not directly related to the total amount of soil microorganisms (bacterial 16S rRNA), but to the specific function these microorganisms have. Moreover, only the functional *nif*H gene is directly associated with the CO_2 production in the present study.

Although the functional *nif*H gene is related to the biogeochemical nitrogen cycle, it also has important interactions with the increase of CO_2 in the atmosphere (Xu et al., 2013) and the response of different ecosystems regarding the CO_2 emission potential. Soil N availability is probably an important factor for the metabolic activity of microorganisms. Therefore, the dynamics between C and N influences CO_2 release (Xu et al., 2013). Thus, as the *nif*H gene encodes a subunit of the nitrogenase enzyme, it acts in the ammonification phase, i.e., in the mineralization of incorporated N (Philippot et al., 2013) and, therefore, in the metabolic processes of microorganisms.

The soil C/N ratio, besides indicating the performance of the metabolic activity of microorganisms in the SOM decomposition, allows establishing relationships on its capacity to produce assimilable nitrogen forms (Silva, 2014). In this sense, the C/N ratio was lower in BS, which also showed a high expression of the *nif*H gene and high FCO_2 (Table 4 and Figs. 2–4). The lower the C/N ratio, the higher the decomposition process of organic material in which N is released. On the contrary, the higher the C/N ratio, the slower the decomposition rate in which N is immobilized by microorganisms (Dorodnikov et al., 2011), which possibly has been occurring in US, as the soil C/N ratio is high in this system (Table 4 and Figs. 2–4).

In short, the unburned sugarcane harvesting system showed the highest mean values of carbon stock, carbon stability (estimated by the half-life and the decay constant of soil carbon), and enzymatic activity of dehydrogenase and the lowest mean values of soil CO_2 emission (Figs. 3 and 4), allowing inferring that this system is a balanced ecosystem that presents a great potential for stabilizing soil carbon and reducing the contribution of agriculture to the emissions of greenhouse gases, mainly CO_2.

5. Conclusions

According to our results and main conclusions, the hypothesis raised in our study was accepted. The presence of straw on the soil surface

influences the cause-and-effect relationship between FCO_2 and Ms after precipitation since, under this condition, soil moisture is preserved for a longer period compared to the soil without straw on its surface. Also, crop residues on the soil surface lead to a decrease in the soil–water infiltration rate. Thus, CO_2 loss from soil to the atmosphere due to its physical expulsion by water occurs more slowly than in soils without this type of cover. Moreover, temporal variations of FCO_2 and Ms are correlated in both unburned and burned sugarcane harvesting systems.

The conversion of burned into unburned sugarcane harvesting systems influenced an increase in the carbon stock and its stability in the soil. Considering that soil carbon stock is one of the key indicators for characterizing and monitoring a given area regarding soil quality, we recommend that, besides quantifying the stock, carbon stability should also be estimated for a more effective study of the soil potential promoted by good agricultural practices.

Soil temperature, soil moisture, air-filled pore space, P, the sum of bases, soil bulk density, carbon soil stock, soil C/N ratio, and abundance of the functional *nif*H gene are the most representative soil attributes that allows characterizing the CO_2 emission process in soils managed with sugarcane under unburned and burned harvesting systems. Therefore, the study of these attributes should be taken into account in future studies that assess the variability of CO_2 emissions in agricultural soils.

Specific environmental legislation and protocols in Brazil have established a progressive reduction in the burning of sugarcane fields, aiming at the total elimination of this procedure until the next decade. However, the unburned sugarcane harvesting system provides higher soil compaction levels than the burned sugarcane harvesting system due to heavy and intense machinery traffic during mechanical harvesting and transport. Thus, the assessment of different forms of traffic, such as controlled and reduced traffic in the area of mechanical harvesting, can be important for future studies aiming at the high environmental sustainability of the sugarcane sector.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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