University of São Paulo
"Luiz de Queiroz" College of Agriculture

Decomposition dynamics of sugarcane straw in the central-southern Brazil

Laisa Gouveia Pimentel

Thesis presented to obtain the degree of Doctor in Science. Area: Soil and Plant Nutrition

Piracicaba
2017

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Agronomist

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
Prof. Dr. **CARLOS EDUARDO PELLEGRINO CERRI**

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Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP

Pimentel, Laisa Gouveia

Decomposition dynamics of sugarcane straw in the central-southern Brazil / Laisa Gouveia Pimentel - - versão revisada de acordo com a resolução CoPGr 6018 de 2011. - - Piracicaba, 2017.

77 p.

Tese (Doutorado) - - USP / Escola Superior de Agricultura “Luiz de Queiroz”.

1. Bioenergia 2. Manejo de resíduos culturais 3. Qualidade da palha 4. Espectroscopia 5. Biologia molecular I. Título

ACKNOWLEDGMENTS

I would like to express my greatest appreciation to:

- The University of São Paulo, specifically the Graduate Program in Soils and Plant Nutrition of the College of Agriculture "Luiz de Queiroz" (Esalq);

- The São Paulo Research Foundation (FAPESP) (grant # 2015/00308-0);

- The Brazilian Federal Agency for the Support and Evaluation of Graduate Education (CAPES);

- The National Counsel of Technological and Scientific Development (CNPq);

- The Brazilian Development Bank, Raízen Company, and Delta CO_2;

- My adviseres Carlos Clemente Cerri (*in memoriam*) and Carlos Eduardo Pellegrino Cerri;

- All the professors and staff of the Department of Soil Science and Cena, especially Fernando Dini Andreote, José Alexandre Melo Demattê, and Brigitte Josefine Feigl;

- The staffs of the Laboratory of Biogeochemistry, and the technician of the Laboratory of Soil Microbiology Sônia, and also all the trainees;

- The Federal Universty of Viçosa, and the Federal University of Rio Grande do Sul;

- My lovely family, above all, my parents, José Maurício e Maria Aparecida, and my dear brother, Bruno;

- My great love, Dener, for always being by my side boosting me in every time, for your uncondiscional love, time and patience;

- All my posgraduate colleagues, especially the "Grupo Palha", Maurício, Baiano, Marcos, and Caio;

- My best friends Amanda, Marilene, Carla, Estefânia, and Jéssica;

- Especially to God;

Many thanks to all!

CONTENTS

RESUMO

Dinâmica da decomposição da palha de cana-de-açúcar na região centro-sul do Brasil

A adoção da colheita mecanizada sem queima prévia da cana-de-açúcar aumenta a quantidade de palha sobre o solo. Essa palha tem um alto potencial para produção de etanol 2G e bioeletricidade. No entanto, a manutenção da palha tem papel essencial nas propriedades do solo e no desempenho das culturas. A decomposição da palha é um processo chave para investigar e informar sobre corretas decisões acerca do manejo da remoção. Diversos fatores afetam a taxa de decomposição, tais como: qualidade e quantidade da palha; condições edafoclimáticas; e práticas de manejo. Portanto, realizamos um estudo de campo em dois locais no centro-sul do Brasil, maior região produtora de cana-de-açúcar no mundo, abrangendo duas épocas de colheita (chuvosa e seca) ao longo de dois anos para avaliar a dinâmica de decomposição da palha da cana-de-açúcar sob diferentes taxas de remoção. A principal hipótese é de que a alta remoção de palha desequilibre o ambiente edáfico e reduza o fornecimento de C para os microrganismos, diminuindo a atividade microbiana e consequentemente a taxa de decomposição da palha. O manejo de remoção afetou a taxa de decomposição da palha, a menor taxa de decomposição foi associada a maiores remoções. A perda de C e N foi duas e três vezes maior no segundo ano do que no primeiro ano de condução do experimento, respectivamente. Em geral, a celulose da palha diminuiu em 13%, a hemicelulose em 7% e a lignina proporcionalmente enriqueceu em 92% após dois anos. Mundancas na composição química da palha ao longo do processo de decomposição foram detectadas tanto utilizando o método tradicional, via extrações químicas sequenciais, quanto atravéz de técnica espectroscópica, como o Diffuse Reflectance Infrared Fourier Transform DRIFT. Assim, para verificar alterações da celulose e da hemicelulose da palha suger-se o uso das picos espectrais de 896, 987, 1173 e 1447 cm^{-1}, enquanto que para verificar mudanças na lignina os valores de 1510 cm^{-1} mostrou-se um eficiente preditor. A comunidade bacteriana do solo foi afetada pelo tempo de decomposição. A qualidade da palha explicou 23,2% da variação bacteriana total, onde a hemicelulose representou 17,2% dessa variação. Além disso, a estrutura bacteriana foi sutilmente afetada pelo manejo de remoção da palha da cana-de-açúcar. No geral, nosso estudo mostrou que a remoção de palha para a produção de etanol 2G e bioeletricidade afetará a dinâmica da decomposição da palha nas áreas comerciais de cana-de-açúcar do Brasil. O tempo foi o principal regulador das mudanças nos conteúdos bioquímicos da palha e na estrutura bacteriana do solo. O uso de resíduos de culturas para fins energéticos é uma das principais alternativas para aumentar a produção de bioenergia nos próximos anos. No entanto, a remoção da palha de cana-de-açúcar deve ser feita com prudência, uma vez que a taxa de remoção afetou a dinâmica da decomposição e conseqüentemente deverá afetar a ciclagem de nutrientes e o ciclo do C.

Palavras-chave: Bioenergia; Manejo de resíduos culturais; Qualidade da palha; Espectroscopia; Biologia molecular.

ABSTRACT

Decomposition dynamics of sugarcane straw in the central-southern Brazil

The adoption of mechanical unburned sugarcane harvesting increases the quantity of straw left on the soil. This material has a high potential for 2G ethanol and bioelectricity production. Although the straw maintenance has an essential role in the soil properties and crop performance. The straw decomposition is a key process to investigate and to inform the correct removal management decisions. Diverse factors affect the decomposition rate, such as: quality and quantity of straw; edaphoclimatic conditions; and management practices. Therefore, we conducted a field study at two sites within central-southern Brazil, the largest sugarcane-producing region in the world, encompassing two harvesting seasons (rainy and dry) over two years to evaluate the sugarcane straw decomposition dynamics under different removal rates. The main hypothesis is that the high removal unbalances the soil environment and reduce de C supply for the microorganisms, decreasing the microbial activity and consequently the straw decomposition rate. The straw removal management affected the decomposition rate, which the lowest decomposition was associated with high removal. The C and N loss was two- and threefold greater in the second year than in the first year of experimentation, respectively. Overall, the straw cellulose decreased by 13%, the hemicellulose 7%, and the lignin proportionally enriched by 92% after two years. Throughout the decomposition process, the straw chemical changes were detected using the traditional method, wet chemical extractions, and using the spectroscopy technique, Diffuse Reflectance Infrared Fourier Transform DRIFT. In this sense, in order to trace straw cellulose and hemicellulose changes we suggested the use of 896, 987, 1173, and 1447 cm^{-1} peaks, whereas to trace lignin changes, the absorbance at 1510 cm^{-1} seems to be an efficient predictor. The soil bacterial community was most affected by the time of decomposition. The straw quality explained 23.2% of the total bacterial variation, in which hemicellulose accounting for 17.2% of this variation. Moreover, the bacterial structure was subtle affected by the sugarcane straw removal. Overall, our study showed that the straw removal for 2G ethanol and bioelectricity production affect the straw decomposition dynamics in commercial sugarcane areas in Brazil. The time was the main regulator of changes in straw chemical contents and in the soil bacterial structure. The use of crop residues for energy purposes is one of the principal alternatives to increase bioenergy production in the next few years. However, the sugarcane straw removal should be done with prudence, since the straw removal rate affected the straw decomposition dynamics and consequently it should affect the nutrient recycling and C cycle.

Keywords: Bioenergy; Crop residues management; Straw quality; Spectroscopy; Molecular biology.

1. GENERAL INTRODUCTION

Brazil is the world largest producer of sugarcane and the second biggest producer of bioethanol in the world (Kutas, 2016). In 2017/18 crop year, the Brazilian production is estimated in approximately 647 million tons in 9 million hectares cultivated with sugarcane. In this same year, the total sugarcane ethanol production was estimated at 26 billion liters (CONAB, 2017). Projections from the Ministry of Agriculture, Livestock, and Supply (MAPA, 2014) point out that in the coming year's ethanol production is expected to reach production of 59 billion liters. In order to double the ethanol production, as projected by MAPA, the main strategies are: i) exploration of new areas (Lapola *et al.*, 2014); ii) sugarcane breeding (Arruda, 2012); and iii) cellulosic ethanol (*i.e.*, second generation ethanol - 2G) (Karlen & Johnson, 2014).

This last strategy has been highlighted, mainly due to the recent process of mechanical harvesting of sugarcane. In Brazil, the adoption of mechanical unburned sugarcane harvesting (green management) potentially increases the quantity of residue left on the soil. On average, around 10-20 Mg ha^{-1} of sugarcane straw is left on the soil surface after each sugarcane harvest event (Galdos *et al.*, 2010). This raw material has a high potential to be used for 2G-ethanol and bioelectricity production (Menandro *et al.*, 2017). The use of crop residues for energy purposes is one of the principal alternatives to increase bioenergy production in the next few years. In addition, there is no cultivated area expansion (Lapola *et al.*, 2014), without threats to natural ecosystems and food production (Cherubin *et al.*, 2017), as well as being faster than sugarcane breeding programs (Arruda, 2012).

Although there are several benefits with the use of cellulosic ethanol, some aspects are still uncertain, mainly regarding the impacts of sugarcane straw removal on key processes in the soil (Blanco-Canqui *et al.*, 2006, Petersen *et al.*, 2013, Wiloso *et al.*, 2014, Pourhashem *et al.*, 2016). Certainly, the straw maintenance has an essential role in soil physical, chemical and biological properties and processes and in crop performance (Lal, 2009, Carvalho *et al.*, 2016, Cherubin *et al.*, 2017), contributing to the proper soil functioning and environmental sustainability (Lal, 2009, Carvalho *et al.*, 2016) (Figure 1).

In this way, considering the scenario in which the use of crop residues for energy production is a promising strategy on a global scale, the residue management cannot be overlooked. Therefore, one of the main challenges is the definition of the correct straw management strategy, without jeopardizing soil functioning and other ecosystem services (Carvalho *et al.*, 2016, Cherubin *et al.*, 2017).

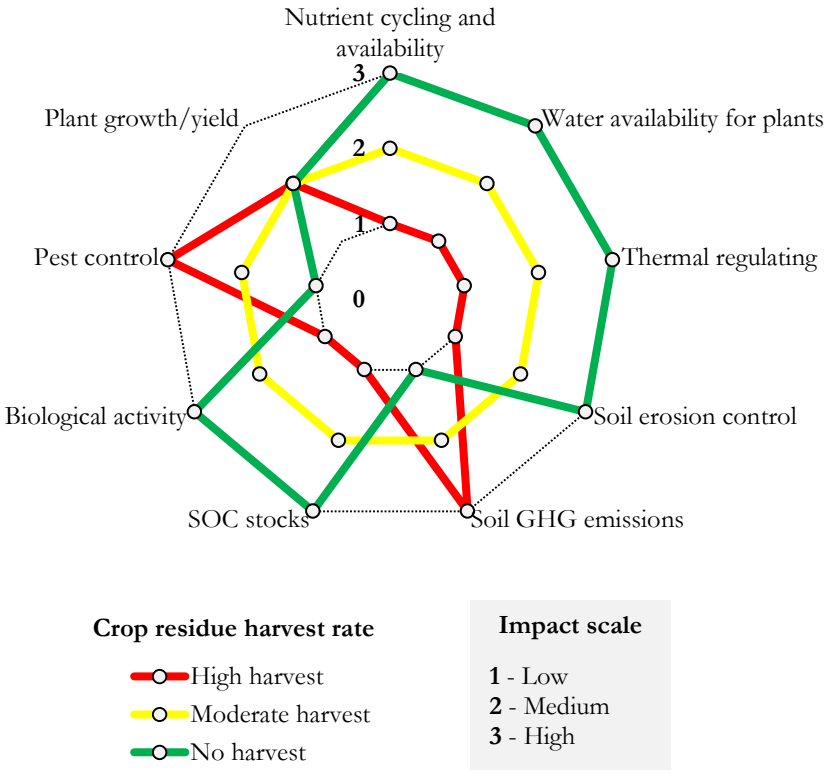

Figure 1. Impacts of crop residue management on soil functions and plant growth. The soil functions and plant growth were graded for each crop residue harvest rate (high, moderate and low) according to the impact scale (*i.e.*, 1 - low, 2 - medium and 3 - high). Source: Cherubin *et al.* (2017).

The straw decomposition is a key process to investigate the straw removal management decisions. The decomposition dynamics controls the nutrient recycling, contributes to mitigation of greenhouse gases emissions by increasing the soil C input, and affects the soil organic matter formation (Mitchell *et al.*, 2016). The process of straw decomposition is very complex (Figure 2), driven by diverse abiotic and biotic factors, such as: quality (Gao *et al.*, 2016, van Huysen *et al.*, 2016) and quantity of residue (Galdos *et al.*, 2010; Sousa Jr *et al.*, 2017); climate conditions (Zhang *et al.*, 2008); soil properties (Sun *et al.*, 2013, van Huysen *et al.*, 2016); soil biota (Paredes *et al.*, 2015; Wall *et al.*, 2008) and management practices (Thorburn *et al.*, 2012; Sousa Jr. *et al.*, 2017).

The main hypothesis tested was that the high straw removal rates unbalance the soil environmental by increasing temperature fluctuations, and decreasing soil moisture and the C supply for soil microorganisms. Thereby, the microbial activity is reduced, decreasing the straw decomposition. In this sense, high straw removal is associated with low decomposition rate. Therefore, we conducted a field study at two sites within central-southern Brazil, the largest sugarcane-producing region in the world, encompassing two harvesting seasons (rainy and dry) over two years to evaluate the sugarcane straw decomposition dynamics under different removal rates. Specifically, we aimed to (i) characterize straw composition changes over time, by different

techniques; and (ii) study the effect of the straw removal rates on the soil bacterial community structure.

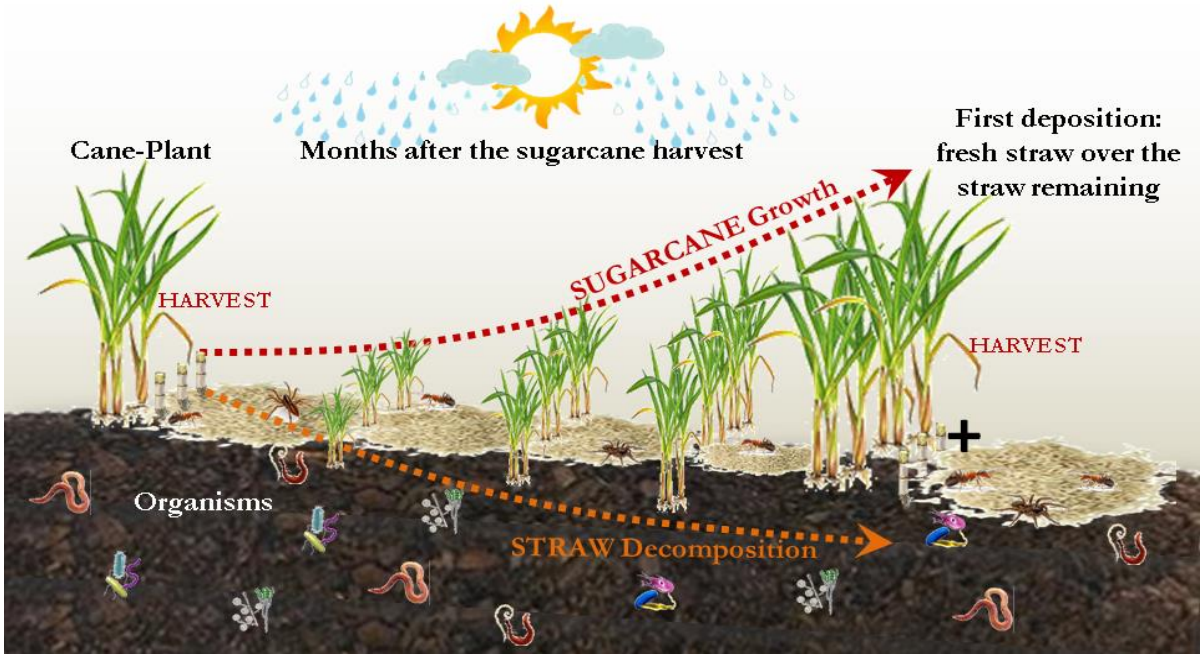

Figure 2. Schematic representation of the sugarcane straw decomposition dynamics affected by abiotic and biotic factors.

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2. DECOMPOSITION OF SUGARCANE STRAW IN THE CENTRAL-SOUTHERN BRAZIL: BASIS FOR MANAGEMENT DECISIONS FOR BIOENERGY PRODUCTION

ABSTRACT

The straw decomposition is one of the main factors that should drive the straw removal management decisions for bioenergy production. Therefore, we conducted a field study at two sites in Brazil for two harvesting seasons and over two years, to evaluate the sugarcane straw decomposition under different straw removal rates and characterize straw composition changes over time. The straw rates tested were: no removal (~14 Mg ha^{-1} of dry mass left on the soil surface), 25% straw removal (~11 Mg ha^{-1}), 50% straw removal (~7 Mg ha^{-1}), and 75% straw removal (~3.5 Mg ha^{-1}). Our results show that straw removal management had no effect on the decomposition in the first year, but the reverse occurred in the second year, which the lowest decomposition was associated with high straw removal rates. The dry mass loss was significantly higher (25%) in the second year than the first year since there was the greater amount of straw left on the soil and by the "legacy effect". The negative exponential model was efficient to predict the C loss over time. The C and N loss was two- and threefold greater in the first years than in the second year of straw deposition, respectively. Overall, the cellulose decreased by 13%, the hemicellulose 7%, and the lignin proportionally enriched by 92% in two years of experiment. In this sense, our findings can be useful to assist sugarcane producers and government to decision-making about better sugarcane straw removal management practices to sustain 2G ethanol e bioelectricity production in Brazil.

Keywords: Bioenergy; Carbon dynamics; Nutrient recycling; Lignin

2.1. Introduction

Crop residues are the portion of the plant that remains in the field after harvest. It is estimated that approximately 5 Pg of crop residue are produced annually in the world (Cherubin *et al.*, 2017). However, globally, there is an estimated potential to increase the residue production by 1.3 Pg yr^{-1} (Bentsen *et al.*, 2014). The residue maintenance on the soil surface has an essential role in soil physical, chemical and biological properties and processes and in crop performance (Carvalho *et al.*, 2016, Cherubin *et al.*, 2017, Lal, 2009), contributing to the proper soil functioning and environmental sustainability (Carvalho *et al.*, 2016, Lal, 2009).

However, there has been a growing interest and investment to use part of these crop residues as the raw material for the bioenergy production, especially cellulosic ethanol (*i.e.*, second generation ethanol - 2G), and bioelectricity (Karlen & Johnson, 2014). In this sense, it is expected a gradual change in the energy mix, substituting the use of fossil fuels for this renewable energy sources (Holma *et al.*, 2013) and consequently, mitigating greenhouse gases (GHG) emissions and its negative impacts on global warming (IPCC, 2007).

Brazil is the world's largest sugarcane (*Saccharum* spp.) producer and the second biggest bioethanol producer, having an outstanding role in the global biofuel market (Filoso *et al.*, 2015). In 2017/18 crop season, the Brazilian production of the sugarcane is supposed to be approximately 647 million Mg at 9 million hectares cultivated, resulting in 39 million Mg of sugar and 28 billion liters of ethanol production (CONAB, 2017). Since the early 2000s, the gradual banishment of burning on sugarcane harvesting has increased the quantity of straw left on the soil. After each sugarcane harvest event around 10-20 Mg ha^{-1} of sugarcane straw are left on the soil surface (Carvalho *et al.*, 2016), and this material has a high potential to produce 2G-ethanol or bioelectricity. Therefore, one of the main challenges is to quantify the potential amount of straw that can be removed from sugarcane fields for energy production without jeopardizing soil functioning and other ecosystem services (Carvalho *et al.*, 2016, Cherubin *et al.*, 2017).

The straw decomposition, by controlling nutrient mineralization and contributing to GHG emissions to the atmosphere and SOM formation (Mitchell *et al.*, 2016), is a key process to inform about straw removal management decisions. Diverse factors affect directly or indirectly the decomposition rate, such as: climate, associated with precipitation, radiation and atmospheric temperature (Zhang *et al.*, 2008, Zhou *et al.*, 2015); soil, especially aeration, temperature (Sousa Jr *et al.*, 2017, van Huysen *et al.*, 2016) and microbial community (Paredes *et al.*, 2015, Sun *et al.*, 2013); quality of plant material, with emphasis on lignin, cellulose, hemicellulose content, and C/N ratio of straw (Gao *et al.*, 2016, van Huysen *et al.*, 2016); quantity of this material, by varying the straw mass to be removed from the production fields (Galdos *et al.*, 2010, Zheng & Marschner, 2017); management practices, including straw incorporation by soil tillage, irrigation, organic amendments, N fertilization and type and time of harvesting (Carmo *et al.*, 2013, Fortes *et al.*, 2012, Thorburn *et al.*, 2012).

There are few studies that integrated all these complex factors to evaluate sugarcane straw decomposition dynamics (Robertson & Thorburn, 2007) towards a more sustainable straw removal management for bioenergy production (Sousa Jr. *et al.*, 2017). However, to our knowledge, there are no studies carried out in the field that firstly evaluate the effects of fresh sugarcane straw left on the soil surface (plant-cane harvesting) and after the effects of the new

fresh straw on sugarcane straw remaining (ratoon harvesting) on decomposition rate. Some studies tracked sugarcane straw decomposition for more than a year (Fortes *et al.*, 2012, Meier & Thorburn, 2016), but they did not consider annual straw deposition (straw over remaining straw), making these results little applicable to direct the straw removal management.

The straw maintenance should be improving the soil environmental, and it is a source of energy for the soil microorganisms (Carvalho *et al.*, 2016, Cherubin *et al.*, 2017). In this sense, our hypothesis is that high straw removal rates should decrease microbial activity, reducing the straw decomposition rates. In addition, straw deposition in the rainy season promotes faster decomposition compared to deposition during the dry season, reducing the amount of straw that can be sustainably harvested to bioenergy production. Therefore, we conducted a field study at two sites within central-southern Brazil encompassing two harvesting seasons (rainy and dry) over two years, to: i) evaluate the decomposition process of sugarcane straw under different removal rates; and ii) characterize straw composition changes over time.

2.2. Material and Methods

2.2.1. Study sites and experimental design

The field study was conducted at two experimental sites within central-southern, main sugarcane-producing region, which represents 93% of Brazilian sugarcane production. The first site is located near Capivari city, southeast region of São Paulo state, at the Bom Retiro mill (Lat. 22°59'42"S; Long. 47°30'34" W) and the second site is near Valparaiso city, northwest region of São Paulo state, at the Univalem mill (Lat. 21°14'48" S; Long. 50°47'04" W) (Fig. 1).

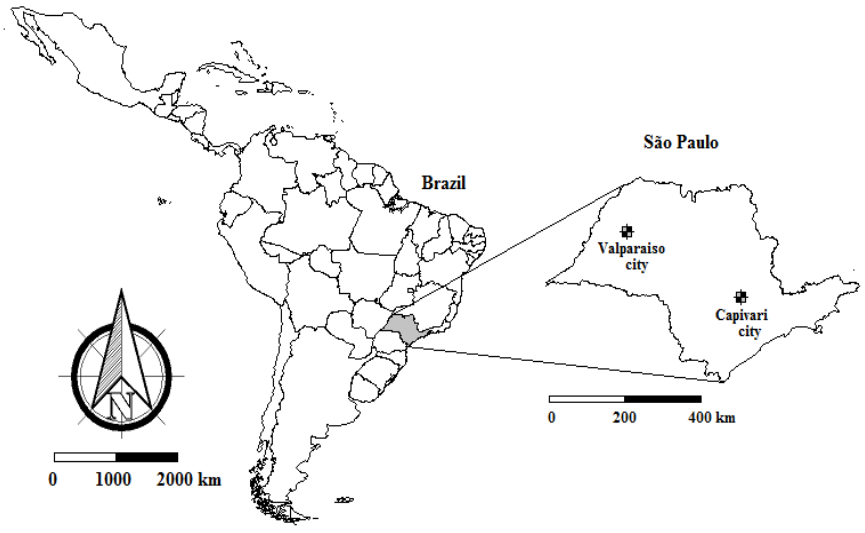

Figure 1. Geographic location of the study sites in central-southern Brazil.

Soil was classified as Rhodic Kandiudox at the Bom Retiro site and as Kanhaplic Haplustults at the Univalem site (USDA, 2014). These soils are typical of the tropical region, covering approximately 60% of the Brazilian territory (Santos *et al.*, 2013). Some selected soil physical and chemical parameters are presented in Table 1.

Table 1. Soil physical and chemical attributes on 0-0.3 m of soil depth of studied sites under sugarcane straw removal in central-southern Brazil.

Soil attributes[§]	Study Site	
	Bom Retiro	Univalem
Clay content (g kg^{-1})	331	116
Silt content (g kg^{-1})	67	23
Sand content (g kg^{-1})	602	861
Bulk density (g cm^{-3})	1.32	1.51
pH $CaCl_2$	5.2	5.2
SOC (%)	1,2	0.6
TN (%)	0.09	0.05
CEC_{pH7} ($cmol_c$ kg^{-1})	6.5	3.0

[§]SOC = Soil Organic Carbon, TN = Total Nitrogen, CEC_{pH7} = Potential Cation Exchange Capacity.

The climate in Bom Retiro site is subtropical (Cwa type - Köppen-Geiger classification), with rains concentrated in the summer (October to April) and a dry season in winter (May to September), with a mean annual temperature of 21.8 °C and an annual precipitation of 1260 mm. At the Univalem site the climate is tropical (Aw type), with the dry season in the winter (April to September), and mean annual temperature of 23.4 °C and an annual precipitation of 1240 mm. Data of average daily temperature and daily rainfall collected throughout of experiment are shown in Figure 2.

In each site, the field study was installed in the dry (August 2014) and the rainy seasons (October 2014) as pointed out in Figure 2. The study replication in these two seasons allowed us to represent the main sugarcane harvesting time in Brazil and their influence in the straw decomposition over time.

The experimental design consisted of a randomized complete block design with split-plots and four replications, where straw removal rates served as the main plot factor and time of sampling as the split-plot factor. Four sugarcane straw removal rates were studied, as follows: no removal, 25, 50 and 75% of straw removal. The amount of straw (Mg ha^{-1} of dry mass) in the no removal treatment (reference) was verified flipping randomly a metallic frame with 1 m^2 just after sugarcane harvesting. Based on this average straw amount, the other treatments were defined by regulating the primary and secondary extractor of the harvester. For more details of the harvester

setup and the respective amount of straw harvested, see Lisboa *et al.* (2017). The amount of sugarcane straw left on the soil surface in each treatment and each site is presented in Table 2.

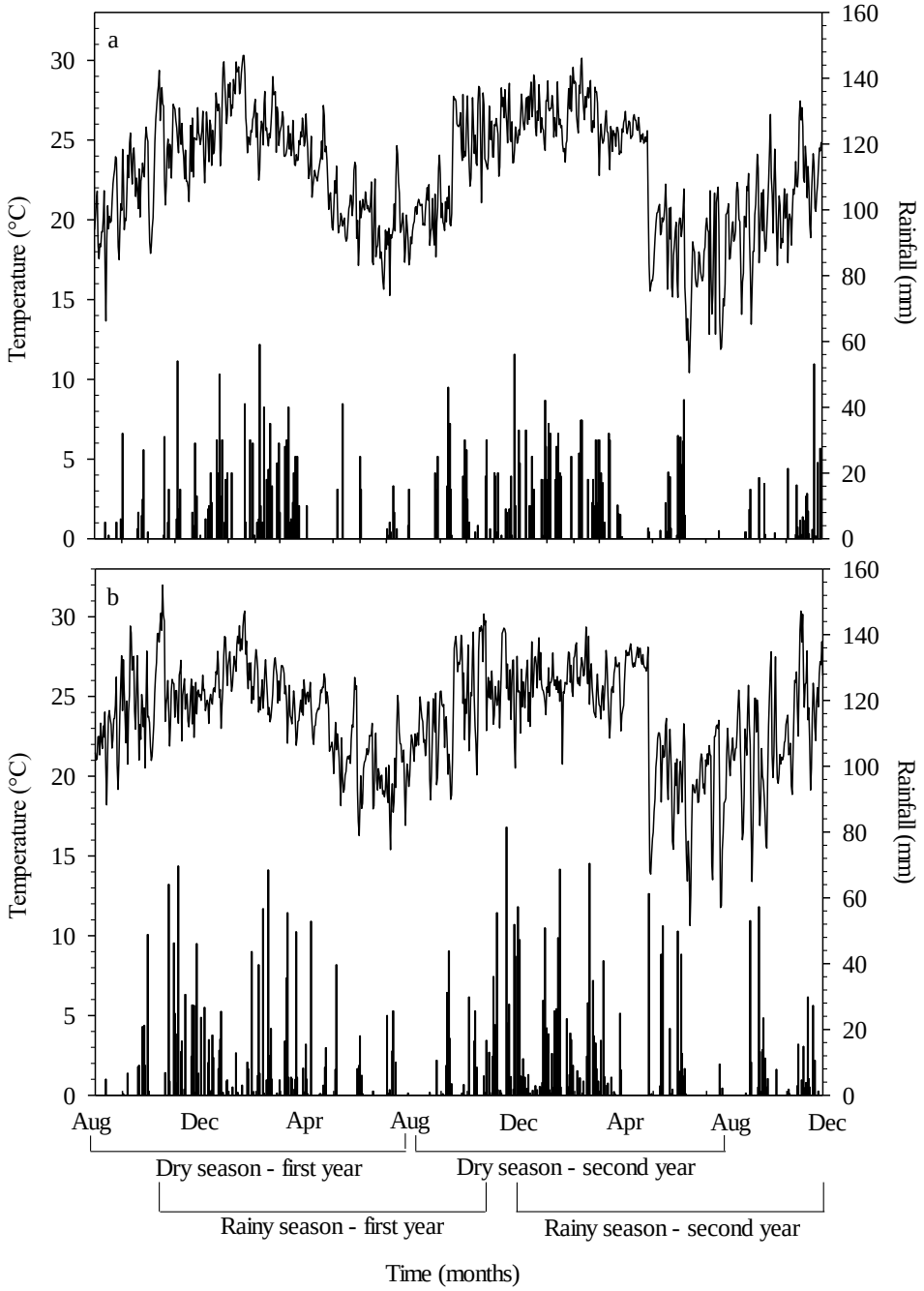

Figure 2. Daily average temperature (°C) and daily rainfall (mm) between August 2014 and December 2016 at the Bom Retiro (a) and Univalem (b), central-southern, Brazil.

Table 2. Sugarcane straw removal rates and respective dry mass amount (Mg ha^{-1}) of straw left on the soil surface in central-southern Brazil.

Sites	Harvesting season	Sugarcane straw removal rates (%)			
		0	25	50	75
Bom Retiro	Rainy	16.0	12.0	7.0	3.0
Bom Retiro	Dry	15.0	12.5	7.0	3.5
Univalem	Rainy	11.5	9.0	6.0	3.5
Univalem	Dry	14.5	11.0	9.5	3.5

The straw amount corresponding to each treatment was added into metallic frames with a 0.25 m^2 area and heights ranging from 0.07 to 0.14 m, according to the treatment. Each metallic frame corresponded to an experimental unit, *i.e.*, 192 metallic frames were installed in the field, at each site. Frames were placed in the inter-row within the sugarcane field. Before straw addition into the frames, the soil surface was cleaned to avoid the presence of other residues, and the straw was placed in direct contact with the soil. In order to prevent not only fresh litter inputs but also residue to blow out of the frame, a metal net (0.05 x 0.05 m mesh) covered frames.

After one year, the same amount of sugarcane straw was added inside the respective frames to simulate the effect of a new straw deposition over the remaining straw, exactly as happen in commercial sugarcane fields. Therefore, the studies were carried starting in two harvesting seasons for two years [*i.e.*, August 2014-2016 (dry season sites) and October 2014-2016 (rainy season sites)].

2.2.2. Field sampling and analytical procedures

The total straw remaining contained into the frames was collected by hand after 1, 2, 4, 6, and 12 months of decomposition, in the first year (straw left on the soil) and in the second year (straw over remaining straw). To quantify the dry matter (DM) losses, the straw was washed to remove any mineral impurities and then dried at 65°C for 72 hours and weighed.

Decomposition rates (k) were estimated by the quantification of DM loss data with the following the equation 1, proposed by Thomas & Asakawa (1993):

$$X = X_0 \times e^{-k \times t} \quad (1)$$

where: X is the straw mass recovered from frames at time t, X_0 is the initial straw mass and k is the decomposition rate.

Furthermore, the half-lives ($t_{1/2}$) (*i.e.*, the period of time (t) required to decompose 50% of the initial DM amount) were calculated for each site using the equation 2.

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

A sub-sample of 100 g of straw was finely ground and sieved (100 μm) for elementary (total C and N) and biochemical analyses. The C and N concentrations from sugarcane straw were determined using an elemental analyzer (Leco CN-2000®, St. Joseph, Michigan). The biochemical compounds (g kg^{-1}) were determined using the methodology proposed by Van Soest *et al.* (1991). The neutral detergent fiber content (NDF), acid detergent fiber or insoluble fraction (ADF), and acid unhydrolyzable residue (AUR), which is commonly associated with lignin content, were determined in an Ankom200 fiber analyzer (Ankom Tech., Fairport, NY, 2000).

Moreover, C and N loss rates (Mg ha^{-1}) were determined by multiplying these elements concentration of straw and the amount of remaining DM into the frames. Straw-C:N ratio was also calculated.

2.2.3. Statistical analyses

An analysis of variance (ANOVA) was performed to test the effects of straw removal rates and time of decomposition on straw DM loss and straw biochemical compound changes. If the ANOVA results were significant ($p < 0.05$), the means were compared according to Tukey's test ($p < 0.05$) using SAS/STAT® software.

The straw-C and N content were subjected to polynomial regression with SigmaPlot® software following the type of response obtained from SAS/STAT output, and the treatments with highest R^2 and probability levels (1 or 5%) could best explain the C and N loss over time.

2.3. Results

2.3.1. Dry mass loss of sugarcane straw by the decomposition

The effects of straw removal rates on decomposition were site-specific (Table 3). Straw DM losses were similar during the first year, regardless of the straw removal rates and site. At the end of the first year, DM loss averaged by 71 and 64% in Bom Retiro dry and rainy seasons, and 58 and 52% in Univalem dry and rainy seasons, respectively. In contrast, straw removal affected significantly the DM losses in the second year. In Bom Retiro site DM loss showed small significant variations over time, and the average DM loss was 11 and 20% in the dry and rainy seasons, respectively. On the other hand, in Univalem site the highest DM loss was found under no removal and 50% straw removal rate in the dry and rainy seasons, respectively.

The k and $t_{1/2}$ value were slightly affected by the straw removal rates (Table 3), in which lower values of k (0.073 $month^{-1}$) were found for 50% straw removal rate at Bom Retiro rainy season in the first year and higher values (0.210 $month^{-1}$) in Bom Retiro dry season in the second year under no straw removal. The $t_{1/2}$ value was only influenced in the second year at Univalem dry season, the higher value (5.9 months) was verified for 25% of straw removal rate.

The DM loss was higher in the early stages of decomposition, in each year of sampling (Table 3). On average, higher DM losses were observed after the first six months (39, 45, 34, and 38% of decomposition) compared to after the last six months of the first year (33, 20, 25, and 15% of decomposition) in the Bom Retiro dry and rainy seasons, and Univalem dry and rainy seasons, respectively. In the second year, this pattern was even more evident, presenting DM losses of 74, 68, 62, and 49% after the first six months, and only 15, 13, 6, and 14% after the last half year. The DM loss was significantly higher by nearly 25% in the second year than the first year (Table 3), ranging from seasons and sites from 48-71% in the first year to 63-89% in the second year.

In Bom Retiro site, in the first six months (first year) DM loss was lower in the dry season (38.5% of DM loss) than those verified in the rainy season (45% of DM loss); nevertheless, this pattern has reversed in subsequent six months (33% and 19% of DM loss in dry and rainy season, respectively), remaining 27% (dry season) and 32% (rainy season) at the end of the year. The same pattern was verified in the second year of sampling. However, the increase of straw DM loss on the dry season was earlier in the second year than the first year, from the fourth months. In Univalem site, the decomposition in the rainy season (21 and 38% of DM loss in the first and in the second year, respectively) was higher than dry season (10 and 30% of DM loss in the first and in the second year, respectively) only in the first two months. After this period, dry season induced highest DM loss, until the end of the year.

2.3.2. Straw-C and -N losses

The models for straw-C and -N losses in the field after 12 months of decomposition are presented in Table 4. The negative exponential model used in the study properly described larger losses at the beginning of the decomposition process, decreasing over time. The model was more efficient to estimate straw-C loss than straw-N loss, with R^2 values ranging from 0.81 to 0.98 for C and from 0.11 to 0.96 for N.

Table 3. Percentage of remaining sugarcane straw after two years of decomposition under increasing straw removal rates in central-southern Brazil.

Sugarcane straw removal (%)	First year						Second year					
	2 months	4 months	6 months	12 months	k(*) (month^{-1})	$t_{1/2}$(§) (months	2 months	4 months	6 months	12 months	k (month^{-1})	$t_{1/2}$ (months)
	Straw remaining (%)											
Bom Retiro dry season												
0	88 Aa¢	70 Bb	59 Ac	24 Bd	0.124 A	5.8 A	60 Ba	53 ABa	24 ABb	8 Ac	0.210 A	3.3 A
25	91 Aa	75 ABb	61 Ac	26 ABd	0.113 A	6.2 A	64 ABa	58 Aa	21 Bb	13 Ab	0.170 B	4.1 A
50	87 Aa	77 ABb	65 Ac	33 Ad	0.093 A	7.5 A	72 Aa	43 Bb	28 ABc	13 Ad	0.170 B	4.1 A
75	89 Aa	78 Ab	61 Ac	31 Ad	0.099 A	7.1 A	64 ABa	49 ABb	32 Ac	10 Ad	0.200 B	3.6 A
Bom Retiro rainy season												
0	78 Aa	66 Ab	56 Ab	32 ABc	0.096 A	7.3 A	54 Aa	33 Bb	30 BCb	16 Ac	0.153 A	4.6 A
25	77 Aa	63 Ab	51 Ac	38 ABd	0.081 AB	8.5 A	57 Aa	40 ABb	36 ABb	22 Ac	0.129 A	5.4 A
50	73 Aa	64 Aab	59 Ab	42 Ac	0.073 B	9.5 A	52 Aa	44 Ab	38 Ab	22 Ac	0.128 A	5.5 A
75	74 Aa	60 Ab	55 Ab	31Bc	0.099 A	7.1 A	54 Aa	35 Bb	26 Cc	18 Ad	0.145 A	4.9 A
Univalem dry season												
0	91 Aa	68 Ab	63 Ab	46 Ac	0.064 A	10.9 A	56 Ba	37 Bb	31 Cbc	27 Bc	0.118 A	5.9 B
25	88 Aa	71 Ab	65 Ab	41 ABc	0.073 A	9.7 A	76 Aa	51 Ab	41 ABc	36 Ac	0.092 A	7.5 A
50	90 Aa	75 Ab	68 Ab	44 ABc	0.068 A	10.7 A	71 Aa	47 Ab	34 BCc	32 ABc	0.104 A	6.7 AB
75	90 Aa	77 Ab	68 Ab	35 Bc	0.089 A	8.6 A	79 Aa	52 Ab	46 Ab	34 ABc	0.100 A	7.0 AB
Univalem rainy season												
0	77 Ba	73 Aa	63 Ab	45 Ac	0.067 A	10.4 A	60 Ba	57 Aba	50 ABb	40 Ac	0.084A	8.3A
25	76 Ba	72 Aa	59 Ab	49 Ac	0.061 A	11.5 A	70 Aa	61 Ab	54 Ac	35 Ad	0.097A	7.1A
50	79 Ba	71 Ab	63 Ac	48 Ad	0.062 A	11.2 A	52 Ca	51 Bab	45 Bb	35 Ac	0.095A	7.3A
75	84 Aa	75 Ab	63 Ac	48 Ad	0.062 A	11.6 A	68 Aa	58 Ab	55 Ab	37 Ac	0.091A	7.6A

(*) Decomposition rate constants; (§) Half-lives; (¢) means followed by same uppercase letters within each column denote no significant differences among the treatments for sugarcane straw removal and means followed by the same lowercase letters within each line denote no significant differences among the times of straw decomposition at the $p<5\%$ level according to Tukey's test.

Table 4. Model of carbon and nitrogen loss of sugarcane straw over two years of decomposition under removal rates in central-southern, Brazil.

Sugarcane straw removal (%)	Carbon				Nitrogen			
	First year		Second year		First year		Second year	
	Model	R^2	Model	R^2	Model	R^2	Model	R^2
Bom Retiro dry season								
0	$f = 7.90\ e^{-0.11x}$	0.95	$f = 8.94\ e^{-0.21x}$	0.98	$f = 0.06\ e^{-0.06x}$	0.84	$f = 0.11\ e^{-0.18x}$	0.96
25	$f = 6.10\ e^{-0.10x}$	0.95	$f = 6.97\ e^{-0.20x}$	0.94	$f = 0.06\ e^{-0.06x}$	0.81	$f = 0.08\ e^{-0.18x}$	0.87
50	$f = 3.55\ e^{-0.09x}$	0.96	$f = 4.40\ e^{-0.20x}$	0.98	$f = 0.03\ e^{-0.06x}$	0.94	$f = 0.05\ e^{-0.16x}$	0.91
75	$f = 1.53\ e^{-0.10x}$	0.93	$f = 1.78\ e^{-0.19x}$	0.93	$f = 0.01\ e^{-0.05x}$	0.81	$f = 0.02\ e^{-0.14x}$	0.87
Bom Retiro rainy season								
0	$f = 7.40\ e^{-0.11x}$	0.97	$f = 8.97\ e^{-0.25x}$	0.94	$f = 0.06\ e^{-0.04x}$	0.70	$f = 0.08\ e^{-0.11x}$	0.75
25	$f = 5.75\ e^{-0.10x}$	0.95	$f = 7.34\ e^{-0.20x}$	0.91	$f = 0.04\ e^{-0.02x}$	0.32	$f = 0.07\ e^{-0.08x}$	0.91
50	$f = 3.14\ e^{-0.09x}$	0.90	$f= 4.29\ e^{-0.18x}$	0.89	$f = 0.03\ e^{-0.02x}$	0.34	$f = 0.04\ e^{-0.07x}$	0.68
75	$f = 1.60\ e^{-0.12x}$	0.90	$f = 1.98\ e^{-0.26x}$	0.90	$f = 0.01\ e^{-0.05x}$	0.60	$f = 0.02\ e^{-0.10x}$	0.77
Univalem dry season								
0	$f = 5.54\ e^{-0.08x}$	0.95	$f = 7.17\ e^{-0.19x}$	0.89	$f = 0.03\ e^{-0.01x}$	0.25	$f = 0.05\ e^{-0.06x}$	0.59
25	$f = 4.19\ e^{-0.08x}$	0.97	$f = 5.39\ e^{-0.12x}$	0.87	$f = 0.02\ e^{-0.01x}$	0.11	$f = 0.04\ e^{-0.03x}$	0.59
50	$f = 3.00\ e^{-0.08x}$	0.94	$f = 3.85\ e^{-0.14x}$	0.88	$f = 0.02\ e^{-0.01x}$	0.36	$f = 0.03\ e^{-0.03x}$	0.64
75	$f = 1.78\ e^{-0.08x}$	0.91	$f = 2.19\ e^{-0.12x}$	0.89	$f = 0.01\ e^{-0.02x}$	0.42	$f = 0.01\ e^{-0.02x}$	0.32
Univalem rainy season								
0	$f = 6.67\ e^{-0.08x}$	0.97	$f = 8.37\ e^{-0.11x}$	0.83	$f = 0.05\ e^{-0.02x}$	0.49	$f = 0.08\ e^{-0.08x}$	0.71
25	$f = 5.17\ e^{-0.07x}$	0.93	$f = 7.08\ e^{-0.11x}$	0.93	$f = 0.04\ e^{-0.01x}$	0.57	$f = 0.06\ e^{-0.07x}$	0.80
50	$f = 4.39\ e^{-0.07x}$	0.96	$f = 5.63\ e^{-0.13x}$	0.81	$f = 0.03\ e^{-0.02x}$	0.47	$f = 0.05\ e^{-0.06x}$	0.62
75	$f = 1.69\ e^{-0.08x}$	0.95	$f = 2.19\ e^{-0.10x}$	0.89	$f = 0.01\ e^{-0.02x}$	0.43	$f = 0.02\ e^{-0.06x}$	0.77

The straw-C and -N content (Mg ha^{-1}) decreased as the decomposition process proceeded (Fig. 3 and 4). In general, the decay curves were similar regardless of the straw removal rates (Table 4, Fig. 3 and 4), indicating that straw removal did not impact the relative C and N loss (*i.e.*, regardless of initial straw amount left on the soil, the relative C and N loss was similar). However, in absolute values, total straw-C and -N loss during the year were higher under no removal treatment than those quantified under high straw removal rates. This pattern was more evident for C, which losses averaged 4.6 and 6.8 Mg ha^{-1} (first and second year, respectively) under no removal whereas 1.1 and 1.6 Mg ha^{-1} (first and second year, respectively) under 75% of straw removal rate.

The straw-C and -N loss were two- and threefold higher in the first than in the second year of the experiment, respectively (Table 4, Fig. 3 and 4). The sugarcane harvest season did not influence the straw-C loss. However, especially in the second year, the harvest season influences the straw-N loss. In Bom Retiro, straw-N losses were twofold higher in the dry season than the rainy season, whereas in the rainy season was twofold higher than the dry season at Univalem.

The straw-C and -N concentrations (g kg^{-1}) were affected by straw removal rates (Table 1 and 2 Appendix). High straw removal rates were associated with lowest C and highest N concentration. Furthermore, throughout the year the C concentration decreases (9 and 3% in the first and in the second year, respectively) whilst N concentration enriches (85 and 86% in the first and in the second year, respectively).

The initial C:N ratio of sugarcane straw was 111:1, 126:1, 172:1, and 146:1 in dry and rainy seasons of Bom Retiro and Univalem, respectively. In the second year, the mean of initial C:N ratio decline by nearly 23% in relation to the first year. The C:N ratio decreasing over time, and this decrease was similar in the first (~50%) and in the second year (~46%) of the experiment.

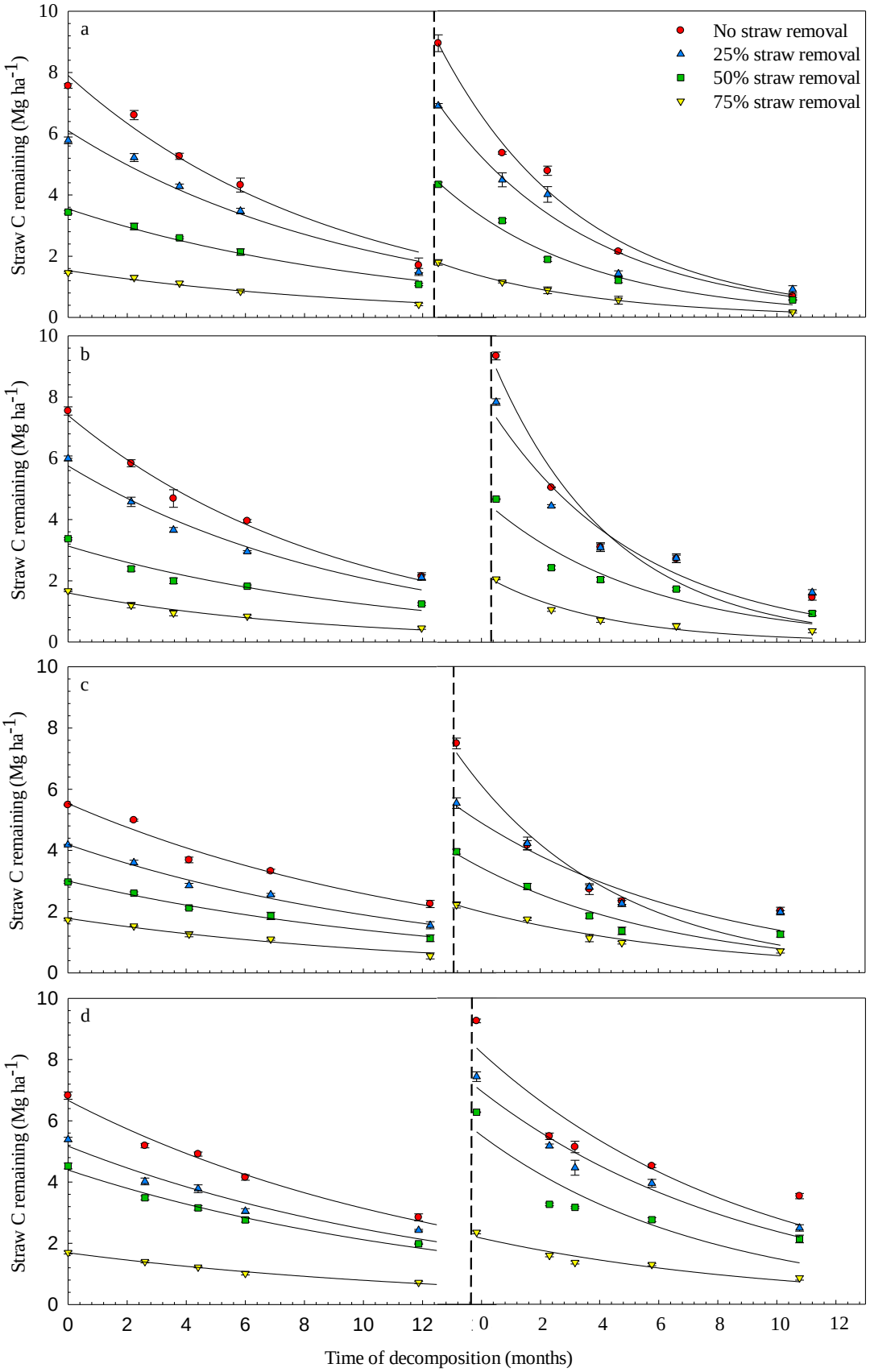

Figure 3. Straw-C remaining (Mg ha^{-1}) of sugarcane straw over two years of decomposition under removal rates in central-southern, Brazil, in Bom Retiro dry season (a), Bom Retiro rainy season (b), Univalem dry season (c) and Univalem rainy season (d). Graphs to the left of the dashed line refer to the first year of sampling and the graphs from the right to the second year of sampling.

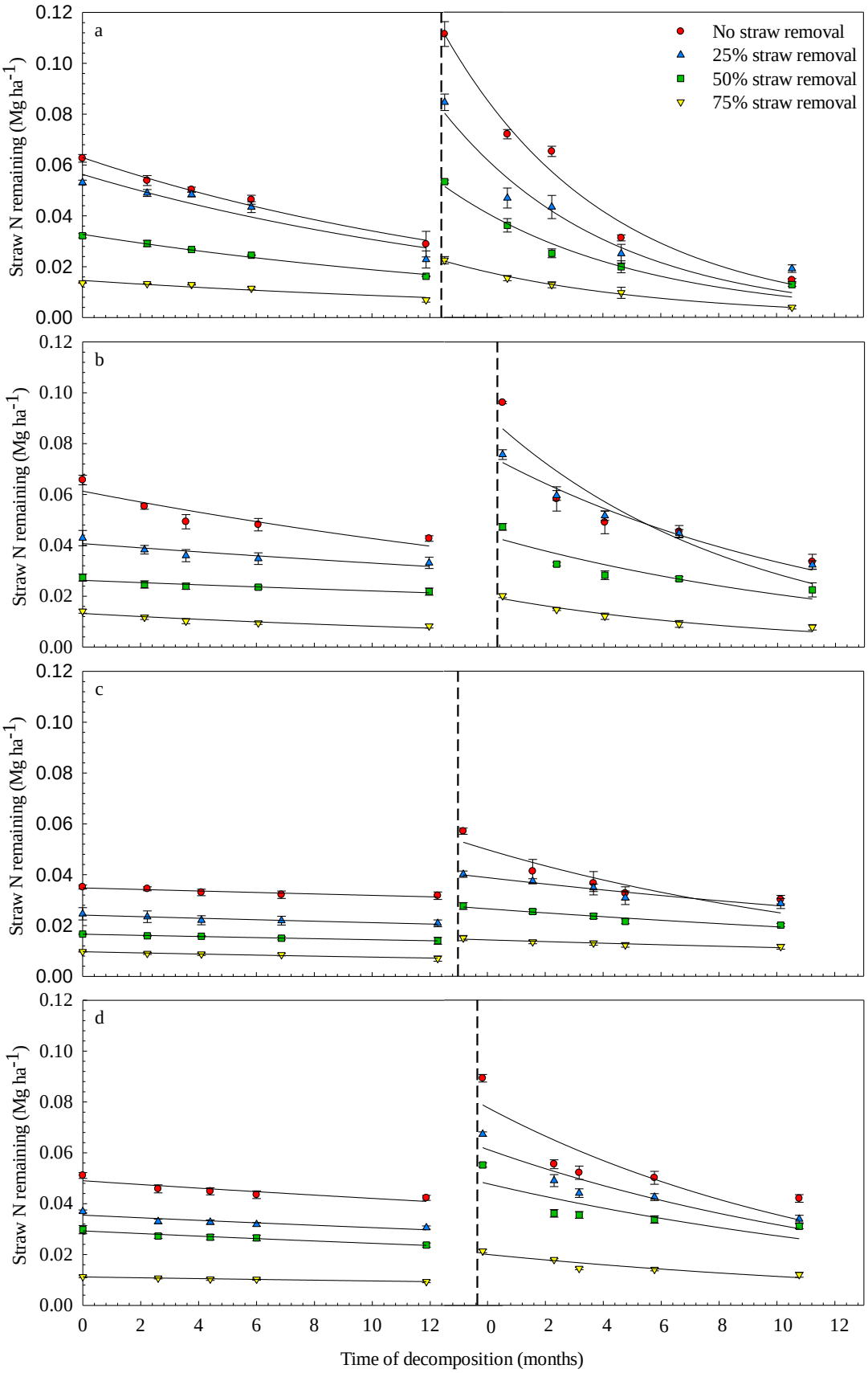

Figure 4. Straw-N remaining (Mg ha^{-1}) of sugarcane straw over two years of decomposition under removal rates in central-southern, Brazil, in Bom Retiro dry season (a), Bom Retiro rainy season (b), Univalem dry season (c) and Univalem rainy season (d). Graphs to the left of the dashed line refer to the first year of sampling and the graphs from the right to the second year of sampling.

2.3.3. Biochemical compound changes during sugarcane straw decomposition

The relative initial sugarcane straw composition was approximately by 50% of cellulose, 40% of hemicellulose and 10% of lignin, independent of the study site (Fig. 5). In general, the relative loss of lignin was not influenced by straw removal rates. The straw removal rates affect more sharply the cellulose relative loss in Bom Retiro sites. In the first year of study, the lowest cellulose loss was observed in 75% and 50% straw removal rates, in dry and rainy seasons, respectively. In the second year of straw deposition, for both seasons, the highest cellulose loss was observed under 50% straw removal rate. In the first year, lower hemicellulose losses were found under no straw removal treatment for dry season sites. For the rainy season sites, the highest hemicellulose loss was verified under 50% and 75% straw removal rates, in the first and second year, respectively.

After the first year of decomposition, the mean of cellulose decreased by 9%, the hemicellulose 6%, and the lignin enriched by 85%. Following the same dynamic pattern, in the end of the second year, the cellulose decreased 17%, the hemicellulose 7% and the lignin increased by 98%. It should be noted that all these values correspond to relative amounts of these straw compounds. In contrast, the absolute values of all biochemical compounds decrease over time, including lignin (Table 3 Appendix).

2.4. Discussion

2.4.1. Straw quantity effects on decomposition

The lowest decomposition was associated with high straw removal. Several authors reported the same pattern of decomposition dynamics (Sousa Jr. *et al.*, 2017, Ramos *et al.*, 2016, Yamaguchi *et al.*, 2017, Zheng & Marschner, 2017). In similar field conditions of this study, Sousa Jr. *et al.* (2017) found the lowest (40%) DM loss in treatments with 75% of sugarcane straw removal (3.5 Mg ha^{-1} left on the soil), while under no removal (14.0 and 21.0 Mg ha^{-1} left on the soil) presented the highest DM losses (65%) after one year of decomposition.

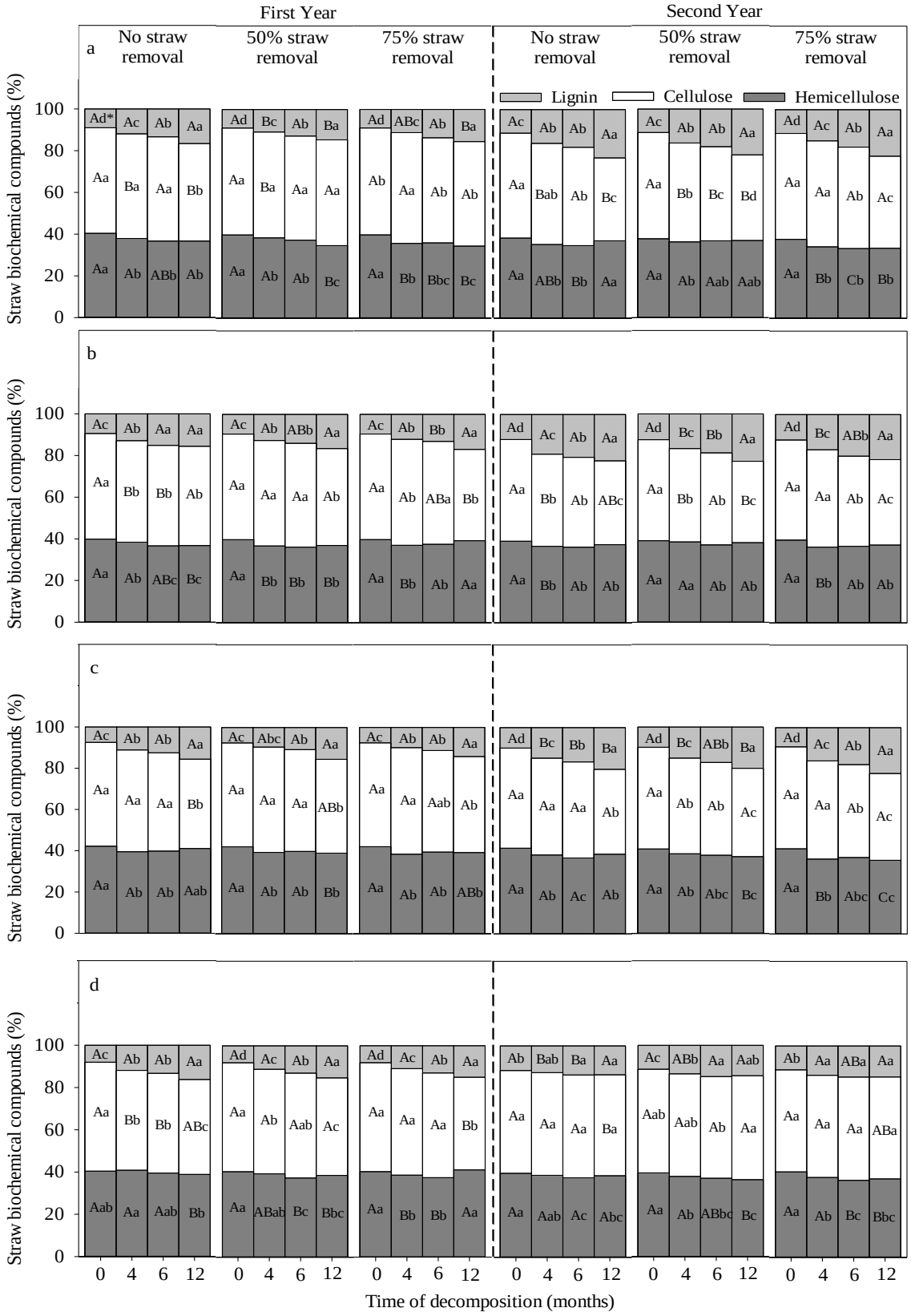

Figure 5. Straw biochemical composition changes (%) over two years of decomposition under increasing sugarcane straw removal rates at the Bom Retiro dry season (a), Bom Retiro rainy season (b), Univalem dry season (c) and Univalem rainy season (d). Graphs to the left of the dashed line refer to the first year of sampling and the graphs from the right to the second year of sampling. The uppercase letters denote significant differences between the treatments for sugarcane straw removal and the lowercase letters denote significant differences between the times of straw decomposition at the $p<5\%$ level according to Tukey's test.

The maintenance of the straw on the soil surface affects primarily the microbial community, the main controlling factor of decomposition process (Rachid *et al.*, 2016, Sun *et al.*, 2013). Soil microorganisms are highly sensitive to management practice changes (Carvalho *et al.*, 2016, Lammel *et al.*, 2015), being affected directly by the increase in the food supply, especially by easily decomposable sources, such as sugars and proteins, cellulose and hemicellulose. In this sense, the high amount of straw, especially in the early stages (labile compounds), enables greater energy gains by the microorganisms, resulting in larger microbial abundance and activity in the soils, and consequently increase the decomposition process (Paredes *et al.*, 2015, Sun *et al.*, 2013). Greater density, richness, and diversity of soil organisms have been reported under the higher amount of sugarcane straw left on the soil surface (Rachid *et al.*, 2016). Larger amount of straw on the soil also provides greater contents of available nutrients to the organisms over the straw decomposition process (Fortes *et al.*, 2012, Kumar & Goh, 1999, Thorburn *et al.*, 2012), which is a key mediator of nutrient recycling.

In addition, the high input of straw improves the soil environmental for a development of abundant and diverse biota, which in turn favors straw decomposition. The straw maintenance reduces the thermal amplitude and preserves soil moisture (Cherubin *et al.*, 2017, Sousa Jr. *et al.*, 2017, Kumar & Goh, 1999). Sousa Jr. *et al.* (2017) verified that soil temperature decreased by up to 7°C and soil moisture remained close to 2% higher in treatments with low sugarcane straw removal (21.0 Mg ha^{-1}). These soil physical changes accelerate growth and increase the activities of soil organisms (Coppens *et al.*, 2006). Therefore, larger amounts of sugarcane straw enhancing the straw decomposition rate, as verified in this study.

The greatest DM loss in the second year could be explained by the "legacy effect". The legacy effect can be defined by the influence of previously added residue in decomposition process and nutrient availability after the second residue with the same or different quality (Zheng & Marschner, 2017). In the first year, the straw was left on the soil surface, whereas in the second year the straw was left over the straw remaining. The decomposition process over the first year likely favored the microbial biomass and increased the availability of N (Carrillo *et al.*, 2012, Zheng & Marschner, 2017). These changes associated with new fresh straw deposition, rich in labile compounds, create a better environment condition to straw decomposition over the second year, increasing the DM loss in relation to the first year. Furthermore, in the second year, there was the higher amount of residue in the soil (new straw over the straw remaining), which increases the decomposition rate in relation to the first straw amendment.

2.4.2. Straw quality effects on decomposition

This study confirmed that straw chemical composition changed significantly over time. The negative exponential model was important to estimate C and N losses throughout the year, as previously reported in studies of crop residue/litter decomposition (Ouellette *et al.*, 2016). The exponential model was better adjusted for the C losses than for N losses. It is in agreement with several studies (Gao *et al.*, 2016, Soong *et al.*, 2015, van Huysen *et al.*, 2016, Zhou *et al.*, 2015) that showed the high association between DM loss and C loss in the decomposition process (Yamaguchi *et al.*, 2017). During the decomposition process, DM loss is associated with C loss as CO_2 emission, by microbial respiration, and as dissolved organic carbon to the soil (Soong *et al.*, 2015). In this present study, we quantified the straw-C loss, but we could not track the final C fate.

The aboveground straw-C is the major responsible for input and output of C in the system (Carvalho *et al.*, 2013). One of the main factors that positively affect the soil C stocks is the C inputs by crop residues left on the soil after the harvest events (Sun *et al.*, 2013). On the other hand, the decomposition dynamics was commonly associated with C mineralization. In the same way that low straw removal (*i.e.*, high C loss) is important to increase soil C stocks, by C input (Carvalho *et al.*, 2016, Thorburn *et al.*, 2012), may also increase absolute emissions of GHG, especially CO_2 emissions, by residue decomposition and microorganisms respiration (Carmo *et al.*, 2013, Jin *et al.*, 2014). Thus, in long term, the unbalanced sugarcane straw removal might reduce the soil organic carbon, with negatives implications for CO_2 emissions (Six *et al.*, 2004).

The straw-N did not present large losses during the decomposition process. Actually, at the beginning of this process, an N enrichment in the straw was observed (Fortes *et al.*, 2012, Robertson & Thorburn, 2007), since the microorganisms immobilize soil N for their own metabolism (Jensen *et al.*, 2005). This is necessary once the straw has a high C/N ratio, varying to 111:1 to 172:1 and 81:1 to 140:1, in the first and in the second year of sampling, respectively. In this sense, the low mineralization of N during the decomposition process, shown the lower contribution to N supply to plants, accounting for only 2.1% of the total N requirements after three years of decomposition (Ferreira *et al.*, 2016).

However, successive cycles of straw deposition on the soil surface should contribute towards a greater accumulation of organic N into the soil (Ferreira *et al.*, 2016, Fortes *et al.*, 2012, Kumar & Goh, 1999, Robertson & Thorburn, 2007). This N input, in long term (~20 to 40 years), may reduce the need for replacement via synthetic nitrogen fertilizers (Meier & Thorburn, 2016, Robertson & Thorburn, 2007, Trivelin *et al.*, 2013), the principal source of N_2O emissions from agriculture (Guzman *et al.*, 2015).

The straw removal rates did not influence the straw C and N contents (Fig. 3 and 4). Nevertheless, it should be noted that different amounts of remaining straw, even with similar decomposition rate, contribute with proportional amounts of C and N loss to their initial mass deposition (Ramos *et al.*, 2016). In this sense, low straw removal rate (*i.e.*, high straw deposition) leads to higher C and N loss than high straw removal rate, in spite of both treatments had the similar loss of C and N contents. This information could be important in studies about the C footprint of bioethanol production since the sugarcane straw removal management is closely linked with nutrient recycling, soil C stocks and GHG emissions (Cherubin *et al.*, 2017).

The C concentration does not change significantly among decomposition process (Table 1 Appendix). However, the relative allocation of this C to the different biochemical compounds (soluble and insoluble) does and may control straw decomposition rate. The biochemical changes observed during the decomposition process were generally similar to other studies (Gao *et al.*, 2016, McKee *et al.*, 2016). As the decomposition advanced readily decomposable components of straw, such as sugars, proteins, hemicellulose, and cellulose are being consumed (Sousa Jr. *et al.*, 2017, Jensen *et al.*, 2005). In contrast, the proportion of straw complex structures, such as tannins and lignin, which was barely degraded by microorganisms, enrich over time.

Cellulose and hemicellulose are structural compounds that are easier to decompose on sugarcane straw (Sousa Jr. *et al.*, 2017, Ramos *et al.*, 2016, Zhou *et al.*, 2015). These compounds are more susceptible to decomposition than other structural compounds (Santos *et al.*, 2012) and therefore, it is easier and faster degraded in the early stage of decomposition (Couteaux *et al.*, 1995, Zhou *et al.*, 2015). In general, the highest hemicellulose loss was observed under high straw removal rate. Opposed to this, the lowest cellulose loss was found in the same removal rate. McKee *et al.* (2016) exhibited the same pattern of our results from biochemical changes, with higher cellulose loss than hemicellulose loss after one year of big bluestem grass litter residue decomposition.

The AUR fraction, commonly associated with lignin content, increased in absolute amounts throughout the year, as also reported by Cotrufo *et al.* (2015), and McKee *et al.* (2016). These authors attribute this increase to the incorporation of insoluble microbial residues (Cotrufo *et al.*, 2015), roots growth (Fortes *et al.*, 2012) or even overestimate due to silica inclusion (Uden *et al.*, 2005), that increase with the decomposition proceeds. The straw washing and the imperceptible presence of sugarcane growth root in the straw suggest that the increase in AUR fraction is due to the incorporation of unhydrolysable microbial residues. Since the AUR is a complex structure that contains not only lignin but also secondary compounds, or insoluble microbial products, produced during decomposition. Therefore, AUR fraction is less easily

degraded by microorganisms, since requiring high energy or even a co-metabolism with other compounds for their decomposition (Cotrufo *et al.*, 2013, Couteaux *et al.*, 1995), suggesting an enrichment of the AUR fraction.

Some authors use indicators to evaluate the effect of a given crop residues management (Jensen *et al.*, 2005, Ramos *et al.*, 2016). The most common indicators used in this type of study are the C:N ratio, the lignin and N content. The lignin and N content did not respond significantly to the removal rates, contrarily the C:N ratio was affected by the removal rates. In this sense, the C:N ratio was a potential predictor of sugarcane straw decomposition. The C:N ratio decreased throughout the year, with reduction nearly by 50 and 46% (first and second year, respectively). Actually, cellulose and hemicellulose were the C-components more significantly affected by the straw removal rates. Therefore, in addition to C:N ratio, cellulose and hemicellulose content would be important predictors of sugarcane straw decomposition.

2.4.3. Edaphoclimatic effects on straw decomposition

In addition to residue quantity and quality, edaphoclimatic conditions also control the decomposition rate, especially in the region/global scale (Robertson & Thorburn, 2007, Sun *et al.*, 2013, Zhou *et al.*, 2015). The greater sugarcane straw decomposition in the first six months of each year may also attribute to climate conditions. In contrast, reduced DM loss in the early stages was verified in dry season sites over the first year of the experiment (Table 3). This result can be explained by the drought that occurred between the months of August/14 and February/15 (681 and 835 mm, in Bom Retiro and Univalem sites, respectively), corresponding the first sixty months of decomposition in dry seasons. In this period, historical rainfall average (20-years data series) was 961 and 971 mm, in Bom Retiro and Univalem (CIIAGRO, 2017), respectively; *i.e.*, rainfall was 29 and 14% lower than historical rainfall average. This period for the sugarcane crop is called a "'grand growth" phase (Fageria *et al.*, 2010), in which the climate conditions have more influence in the decomposition dynamics than microclimate created after the sugarcane growth. Thereby, reduced rainfall greatly decreases the decomposition in this period. In the second year, the rainfall was 17 and 36% higher than the historical average recorded (1122 and 1319 mm in Bom Retiro and Univalem, respectively), and the decomposition was highest in the grand growth phase (first six months).

Moreover, rainy season experiments showed higher decomposition rate than those installed in the dry season (Table 3). In the rainy season, especially during the grand growth phase (corresponding to the spring-summer period), there was the highest temperature, ultraviolet

radiation, and rainfall (Fig. 2). All of these factors significantly contribute to the decomposition of biomass on the soil surface (Sun *et al.*, 2013, Zhou *et al.*, 2015).

Bom Retiro sites (sandy clay loam soil texture) showed higher decomposition rate than Univalem sites (loamy sand soil texture), independent of the harvesting season (Table 3). The differences between Bom Retiro and Univalem sites may be attributed to the higher clay content in Bom Retiro sites, which contributes for C storage and a better soil aggregation, increasing water storage capacity (Hartge & Horn, 2016) and nutrient retention capacity in the soil (Ajayi & Horn, 2016). In this regard, the soil organisms may have a better environment for development, increasing its activity and consequently accelerating the straw decomposition.

2.5. Conclusions

The sugarcane straw removal for bioenergy production should be done with prudence. High straw removal rates could reduce the decomposition and possibly the inputs of C and nutrients in the soil. Thus, straw removal has the potential to alter soil characteristics that are closely linked to the C cycle (C stocks and CO_2 emissions) and nutrient recycling. Sugarcane harvesting in the dry season should be preferred taking into account decomposition rate since during the grand growth phase, might have enough organic residue left on the soil surface to reduce the adverse climatic effects (surface selling by the impact of raindrops, high water evaporation, and thermal variations) on the soil environmental. Harvesting in the rainy season induced accelerated decomposition due to higher soil moisture and temperature observed in this time in Brazil.

The results obtained in this study improve and clarify the knowledge about the complex decomposition process in these evaluated scenarios. In addition, straw decomposition data could be used as inputs (C, N, and lignin in DayCent model, for example) in future simulation studies, varying the scenarios which still have knowledge gaps (use of vinasse, filter cake) and its impacts on long-term soil C and N balance. Finally, based on the results obtained and on literature data, it is possible to provide support for the decision-making for sugarcane producers and government about better sugarcane straw removal management to sustain the production of 2G ethanol or bioelectricity in Brazil.

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APPENDIXES

Table 1. Straw-C concentration (g kg^{-1}) of sugarcane straw over two years of decomposition under removal rates in central-southern, Brazil.

Sugarcane straw removal (%)	First year					Second year				
	initial	2 months	4 months	6 months	12 months	initial	2 months	4 months	6 months	12 months
	Straw-C (%)									
Bom Retiro dry season										
0	47.74 Aa*	47.46 Aa	47.16 Aab	46.03 ABbc	45.28 ABc	46.98 ABa	47.21 ABa	47.11 Aa	47.08 Aa	44.93 Ab
25	47.80 Aa	47.49 Aab	47.18 Abc	46.86 Acd	46.41 Ad	47.16 Aab	47.65 Aa	47.40 Aa	46.55 ABb	46.59 Ab
50	47.60 Aa	47.08 Aa	46.68 ABab	45.76 BCbc	44.79 ABc	46.70 BCa	47.10 ABa	46.95 Aa	46.91 Aa	46.52 Aa
75	47.14 Aa	46.79 Aa	46.98 Bab	44.89 Cbc	43.88 Bc	46.48 Ca	46.66 Ba	45.99 Ba	45.60 Ba	45.46 Aa
Bom Retiro rainy season										
0	48.30 Aa	47.68 Aa	45.76 ABb	45.06 Ab	42.96 ABc	46.96 ABa	46.95 Aa	46.64 Aab	46.06 Aab	45.58 Ab
25	47.79 Aa	47.18 Aab	46.50 Ab	46.01 Ab	44.40 Ac	47.18 Aa	46.90 Aa	46.73 Aa	45.99 Ab	45.53 Ab
50	48.21 Aa	47.00 ABa	44.88 BCb	44.16 ABbc	42.69 ABc	46.65 Ba	46.58 Aa	46.25 Aa	45.98 Aa	42.85 Bb
75	46.42 Ba	45.45 Bab	43.92 Cbc	42.90 Bcd	40.87 Bd	46.46 Ba	46.41 Aa	46.00 Aab	45.71 Aab	45.48 Ab
Univalem dry season										
0	47.84 Aa	47.71 Aab	46.99 Aab	45.82 Ab	42.93 Ac	46.32 Aa	46.05 Aa	46.00 Aa	45.86 Aa	45.26 Aa
25	48.18 Aa	46.88 Aab	46.18 ABab	45.01 Abc	43.41 Ac	46.58 Aa	46.78 Aa	46.54 Aa	46.29 Aab	45.85 Ab
50	47.98 Aa	47.00 Aab	45.57 Bbc	44.18 Ac	41.73 Ad	45.96 Aa	46.24 Aa	46.24 Aa	46.06 Aa	45.32 Aa
75	47.89 Aa	46.80 Aab	45.45 Bbc	44.62 Acd	43.12 Ad	46.69 Aa	46.53 Aab	46.19 Aab	45.22 Abc	43.87 Ac
Univalem rainy season										
0	47.86 ABa	47.38 Aab	47.04 Aab	46.55 Ab	44.07 Ac	46.84 Aa	46.30 Aab	45.96 Abc	45.39 Acd	44.81 Ad
25	48.32 Aa	47.33 Aab	46.77 Ab	46.18 Ab	44.61 Ac	46.93 Aa	46.40 Aab	46.34 Aab	46.03 Ab	45.60 Ab
50	47.89 ABa	46.93 ABa	46.77 Aa	46.49 Aa	43.99 Ab	46.75 Aa	46.74 Aa	46.48 Aa	45.51 Ab	44.93 Ab
75	47.27 Ba	46.25 Bab	45.32 Bbc	44.64 Bc	41.85 Ad	46.00 Aa	46.08 Aa	46.15 Aa	45.80 Aa	45.61 Aa

*means followed by same uppercase letters within each column denote no significant differences among the treatments for sugarcane straw removal and means followed by the same lowercase letters within each line denote no significant differences among the times of straw decomposition at the $p<5\%$ level according to Tukey's test.

Table 2. Straw-N concentration (g kg^{-1}) of sugarcane straw over two years of decomposition under removal rates in central-southern, Brazil.

Sugarcane straw removal (%)	First year					Second year				
	initial	2 months	4 months	6 months	12 months	initial	2 months	4 months	6 months	12 months
	Straw-N (%)									
Bom Retiro dry season										
0	0.40 Bb*	0.39 Bb	0.45 Bb	0.50 Bb	0.77 Aa	0.58 Ab	0.63 Ab	0.64 Ab	0.69 Ab	0.96 Aa
25	0.44 Ac	0.45 Ac	0.53 Abc	0.59 Aab	0.71 Aa	0.58 Ab	0.50 Ab	0.51 Ab	0.81 Aa	1.01 Aa
50	0.45 Ac	0.46 Ac	0.48 Bbc	0.53 ABb	0.68 Aa	0.57 Ab	0.54 Ab	0.62 Ab	0.77 Ab	1.07 Aa
75	0.44 Ac	0.48 Ac	0.53 Abc	0.61 Ab	0.71 Aa	0.58 Ab	0.63 Ab	0.70 Ab	0.82 Aab	1.08 Aa
Bom Retiro rainy season										
0	0.42 Ac	0.45 Ac	0.48 Abc	0.55 Ab	0.86 Aa	0.48 Ac	0.54 Ac	0.73 ABb	0.77 Ab	1.04 Aa
25	0.34 Bd	0.40 Bcd	0.46 Ac	0.54 Ab	0.70 Aa	0.46 Ad	0.63 Ac	0.78 Ab	0.75 Ab	0.91 Aa
50	0.39 ABc	0.48 Abc	0.54 Ab	0.57 Ab	0.75 Aa	0.47 Ac	0.63 Ab	0.64 Bb	0.71 Ab	1.02 Aa
75	0.39 ABb	0.44 Ab	0.47 Ab	0.48 Ab	0.74 Aa	0.45 Ac	0.65 Ab	0.78 Ab	0.79 Ab	0.98 Aa
Univalem dry season										
0	0.31 Ac	0.33 Ac	0.42 Ab	0.44 Ab	0.61 Aa	0.35 Ac	0.46 Abc	0.62 Aab	0.65 Aa	0.68 Aa
25	0.28 Ac	0.31 Abc	0.36 ABbc	0.39 Ab	0.60 Aa	0.34 Ab	0.42 Ab	0.58 Aa	0.63 Aa	0.66 Aa
50	0.27 Ad	0.29 Acd	0.34 ABbc	0.36 Ab	0.52 Aa	0.32 Ac	0.42 Ac	0.59 Ab	0.74 Aa	0.74 Aa
75	0.27 Ac	0.28 Abc	0.32 Bbc	0.35 Ab	0.56 Aa	0.32 Ac	0.36 Ac	0.55 Ab	0.57 Ab	0.73 Aa
Univalem rainy season										
0	0.36 Ad	0.42 Acd	0.43 Abc	0.49 Ab	0.66 Aa	0.45 Ac	0.47 ABbc	0.47 Abc	0.50 Aab	0.53 Ba
25	0.33 ABd	0.39 ABc	0.41 Ac	0.48 Ab	0.56 ABa	0.43 ABb	0.44 Bb	0.46 Ab	0.50 Ab	0.63 ABa
50	0.32 Bd	0.37 ABc	0.40 Ac	0.45 Ab	0.53 Ba	0.41 Bc	0.52 Ab	0.52 Ab	0.56 Aba	0.66 Aa
75	0.31 Bd	0.35 Bcd	0.38 Ac	0.46 Ab	0.55 ABa	0.42 ABc	0.52 Ab	0.49 Abc	0.50 Ab	0.64 ABa

*means followed by same uppercase letters within each column denote no significant differences among the treatments for sugarcane straw removal and means followed by the same lowercase letters within each line denote no significant differences among the times of straw decomposition at the p<5% level according to Tukey's test.

Table 3. Straw-biochemical content changes (Mg ha^{-1}) of sugarcane straw over two years of decomposition according to removal rates in central-southern, Brazil.

Sugarcane straw removal (%)	First year									Second year								
	0	4	12	0	4	12	0	4	12	0	4	12	0	4	12	0	4	12
	Cellulose			Hemicellulose			Lignin			Cellulose			Hemicellulose			Lignin		
	Mg ha^{-1}																	
Bom Retiro dry season																		
0	6.84	4.80	1.23	5.46	3.63	0.96	1.22	1.14	0.43	7.87	4.00	0.42	6.02	2.91	0.39	1.77	1.33	0.25
50	3.12	2.32	0.91	2.49	1.76	0.62	0.56	0.49	0.26	3.90	1.56	0.36	2.90	1.19	0.32	0.86	0.54	0.19
75	6.84	1.05	0.35	1.07	0.71	0.24	0.24	0.22	0.11	1.60	0.77	0.12	1.19	0.52	0.09	0.36	0.23	0.06
Bom Retiro rainy season																		
0	6.79	3.97	1.71	5.35	3.13	1.32	1.27	1.05	0.56	7.94	2.28	0.90	6.38	1.89	0.84	1.96	0.98	0.50
50	3.04	1.80	0.96	2.40	1.31	0.77	0.57	0.45	0.34	3.93	1.52	0.56	3.18	1.31	0.54	1.00	0.57	0.32
75	1.57	0.84	0.32	1.23	0.61	0.29	0.29	0.20	0.12	1.72	0.53	0.23	1.42	0.42	0.21	0.44	0.20	0.12
Univalem dry season																		
0	5.07	3.17	1.52	4.25	2.54	1.44	0.75	0.71	0.55	6.36	2.24	1.23	5.44	1.83	1.15	1.30	0.71	0.61
50	2.74	1.82	0.85	2.29	1.41	0.73	0.41	0.34	0.29	3.47	1.50	0.82	2.88	1.24	0.72	0.69	0.48	0.38
75	1.60	1.16	0.42	1.34	0.86	0.37	0.24	0.22	0.13	1.95	0.92	0.45	1.63	0.70	0.38	0.37	0.31	0.24
Univalem rainy season																		
0	6.27	3.64	2.12	4.92	3.15	1.84	0.98	0.92	0.77	7.82	4.33	2.81	6.39	3.45	2.27	1.88	1.13	0.80
50	4.15	2.56	1.42	3.26	2.05	1.19	0.65	0.58	0.47	5.23	2.57	1.72	4.23	2.01	1.28	1.21	0.71	0.51
75	1.58	1.05	0.56	1.24	0.81	0.52	0.25	0.23	0.19	2.00	1.12	0.67	1.68	0.88	0.52	0.47	0.32	0.20

3. DIFFUSE REFLECTANCE INFRARED FOURIER TRANSFORM (DRIFT) SPECTROSCOPY TO ASSESS SUGARCANE STRAW DECOMPOSITION

ABSTRACT

An improved understanding about sugarcane straw decomposition is essential towards a sustainable removal rate for bioenergy production. The quantity and quality (chemical composition) of crop residue are the primary factors that control its decomposition dynamics in the field. Advanced spectroscopic techniques can be useful tools to trace more efficiently straw decomposition process. Thus, we conducted a field study at two sites in central-southern Brazil to investigate the use of DRIFT spectroscopy to assess sugarcane straw decomposition over a year, and compare the results with the traditional wet chemical methodology. In-field straw decomposition was evaluated under four removal rates (*i.e.,* no removal, 25, 50, and 75% of straw removal). Straw samples were collected after 4, 6, 12 months and analyzed using DRIFT and Van Soest method. Results showed that spectrum peaks were similar regardless sites and sampling time. After one year, the spectra showed the decrease in the absorbance between 1200 to 1100 cm^{-1}, which is associated with labile C, and the increase in more recalcitrant C (1800 to 1600 cm^{-1}) over time. In a regional scale, the straw chemical composition differed between the sites, especially by the climatic and soil characteristic. On the other hand, in a local scale, the chemical composition alteration was induced significantly by time of decomposition whereas straw removal rates had no effect. The DRIFT specific peak was strongly correlated with the wet chemical data. In this sense, in order to trace straw cellulose and hemicellulose changes we suggested the use of 896, 987, 1173, and 1447 cm^{-1} bands, whereas to trace lignin changes, the absorbance at 1510 cm^{-1} seems to be an efficient predictor. The application of DRIFT analysis to follow decomposition process was sensitive and a potential methodology to detect sugarcane straw chemical changes induced by the edaphoclimatic conditions and time of decomposition.

Keywords: Spectroscopic technique; Van Soest; Cellulose; Hemicellulose; Lignin; Straw sensing

3.1. Introduction

Straw decomposition is an essential process that connects plant residues to the soil and atmosphere, regulating nutrient recycling, soil organic matter turnover, source of food for the soil organisms, and soil CO_2 flux to the atmosphere (Cotrufo *et al.*, 2010; Cotrufo *et al.*, 2015; Gao *et al.*, 2016; Soong *et al.*, 2015). In this sense, litter decomposition has the key role to sustain several ecosystem functions and services (Cotrufo *et al.*, 2010). Diverse aspects affect directly or

indirectly the decomposition rate, such as those related to climatic and soil conditions as well as aspects associated with the quantity and quality of organic material left on the soil surface (Gao *et al.*, 2016; Zhang *et al.*, 2008; Zheng & Marschner 2017). Despite numerous factors that affect the straw decomposition, some studies considered the chemical composition (e.g., C:N ratio, N, and lignin content) of residue as its primary driver. The straw quality controls more significantly the early stages of the decomposition process, especially in local scale (Cotrufo *et al.*, 2015; Cyle *et al.*, 2016; Fortes *et al.*, 2012; Johnson *et al.*, 2007).

Traditionally, chemical composition changes of organic residues are assessed through the wet chemical fractionation, also known as Van Soest method (Van Soest *et al.*, 1991). This method is based on sequential extractions of chemical components (cellulose, hemicellulose, and lignin) using alkaline and acid reagents. The wet chemical method presents suitable accuracy to characterize the litter/residue composition into lability fractions (Van Soest *et al.*, 1991), being considered the reference method in decomposition studies of a wide diversity of litter and crop residues (Chen *et al.*, 2010; Sousa Jr. *et al.*, 2017; Gao *et al.*, 2016; Liu *et al.*, 2016; Soong *et al.*, 2014). However, this method does not provide direct structural information (e.g. presence of C=O stretch, C-O bonds of polysaccharides, COO^- stretch of aromatics) since each fraction is defined based on its resistance to different digests (acid and alkaline). Moreover, the use of this technique generates chemical residues that can be harmful to the human health and environment (Van Soest & Wine 1968). Thus, alternative methods have been researched to overcome these limitations.

The Fourier Transform Infrared (FTIR) spectroscopy has been successfully applied to distinguish chemical components in several organic materials during decomposition process (Duboc *et al.*, 2012; McKee *et al.*, 2016). The FTIR is a simple and quick method that measures light transmitted or reflected from a sample (Chen *et al.*, 2010; Ouellette *et al.*, 2016). More recently, the Diffuse Reflectance Infrared Fourier Transform (DRIFT) spectroscopy, a rapid, nondestructive and semi-quantitative method (Gholizadeh *et al.*, 2013; Ouellette *et al.*, 2016), has also been tested. The DRIFT is a surface localized FTIR spectroscopy (Accardo *et al.*, 2014) that allows obtaining chemical and structural information for the samples. After the infrared radiation reaches a sample surface the light can be adsorbed or reflected from the surface, or it can penetrate in the sample. Then, the radiation is scattering. Diffuse reflectance occurs if the scattering centers are randomly oriented (Anbalagan *et al.*, 2010). Finally, this diffuse reflectance is collected and relayed to the infrared detector, and the absorption, by chemical groups, is revealed.

The DRIFT presents some key advantages relative to traditional FTIR technique, such as: direct measurement without pretreatments of the sample (pelleting samples for transmittance

mode); applicable to various complex samples, has higher resolution of the spectra; and reduced water interference in the bands (Haberhauer & Gerzabek 1999; Ouellette *et al.*, 2016; Spaccini *et al.*, 2001).

In studies that include measurements of chemical compounds using DRIFT spectroscopy is common to use the spectral peak heights (Ding *et al.*, 2006) and the spectral peak area (Spaccini *et al.*, 2001), e.g., to describe residue decomposition dynamics (Duboc *et al.*, 2012; Ouellette *et al.*, 2016; Soong *et al.*, 2014). Moreover, DRIFT spectroscopy data can be linked with measurements of dry mass (DM) loss over time (Haberhauer & Gerzabek 1999) and correlated with chemical composition changes quantified by different methods (Chen *et al.*, 2010; McKee *et al.*, 2016). Thus, DRIFT can be a useful tool to the better understanding of the phases and dynamics of litter and crop residues decomposition (Ouellette *et al.*, 2016; Spaccini *et al.*, 2001; Zaccheo *et al.*, 2002).

In Brazil, the crescent interest to use part of sugarcane straw as raw material for bioenergy production (e.g., cellulosic ethanol and bioelectricity) has raised important agronomic and environmental concerns (Carvalho *et al.*, 2016; Cherubin *et al.*, 2017). Consequently, several studies recently published aimed to understand the straw removal management effects on straw decomposition dynamic in the field (Sousa Jr. *et al.*, 2017; Dietrich *et al.*, 2017; Ramos *et al.*, 2016; Pimentel *et al.*, 2017) However, none of these studies tested advanced techniques as DRIFT to evaluate sugarcane straw decomposition dynamics in Brazil or other places.

Therefore, we conducted a field study at two contrasting sites within central-southern Brazil, to investigate the use of DRIFT spectroscopy as a method to evaluate straw chemical changes over one year of decomposition process under natural conditions. Additionally, the present study compared DRIFT spectroscopy data with data obtained by traditional wet chemical methodology (Van Soest *et al.*, 1991) to validate this alternative technique. Our hypothesis was that the use of DRIFT technique will allow the identification of specific peaks related to the straw biochemical compounds (aliphatic and aromatic), being an effective alternative to the traditional method (Van Soest *et al.*, 1991) to study the sugarcane straw decomposition and improve the characterization of straw at different stages of decomposition.

3.2. Material and Methods

3.2.1. Study sites location and experimental design

The field experiments were conducted in two sites located in central-southern Brazil, near to Capivari, southeast region of São Paulo state, at the Bom Retiro mill (Lat. 22°59'42"S; Long. 47°30'34" W) and near to Valparaiso, northwest region of São Paulo state, at the Univalem mill (Lat. 21°14'48" S; Long. 50°47'04" W). We collected the whole dataset with previous authorization of farmer-owned or company's managers. This study did not involve endangered or protected species, so no formal permissions were needed from regulatory agencies. The soil was classified as Rhodic Kandiudox at the Bom Retiro site and as Kanhaplic Haplustults at the Univalem site (USDA 2014). The soil clay content at 0-0.3 m of soil depth was 331 and 116 g kg^{-1} in Bom Retiro and Univalem site, respectively.

The climate in Bom Retiro site is subtropical (Cwa type - Köppen-Geiger classification), with rains concentrated in the summer (October to April) and a dry season in winter (May to September). At the Univalem site, the climate is tropical (Aw type), with the dry season in the winter (April to September). During the experiment conduction in Bom Retiro site the mean annual temperature was 23.3 °C and an annual precipitation was 1375 mm, and in Univalem site the mean annual temperature was 23.9 °C and the annual precipitation was 1533 mm.

The field experiments were installed in the dry season (August 2014) under a randomized complete block design with split-plots and four replications. Straw removal rates served as the main plot factor and time of sampling as the split-plot factor. Four sugarcane straw removal rates were studied, as follows: no removal (~14 Mg ha^{-1} of DM left on the soil surface), 25 (~11 Mg ha^{-1}), 50 (~7 Mg ha^{-1}) and 75% (~3.5 Mg ha^{-1}) of straw removal. These treatments were proposed to simulate the straw removal in sugarcane commercial areas in Brazil. For more details about site location, climate conditions during the year and the experiment design, see the chapter 2 of this Thesis (Pimentel *et al.*, 2017).

At each site, the straw amount respective to each treatment was added into the metallic frames (experimental unit) with 0.25 m^2 and heights ranging from 0.07 to 0.14 m, according to the treatment, which was placed in the inter-row position within sugarcane field. Before the straw added into the frames, the soil surface was cleaned and the straw was placed in direct contact with the soil. In order to prevent fresh litter inputs or outputs, the frames were covered by a metal net (0.05 x 0.05 m).

3.2.2. Wet chemical analyses of the sugarcane straw

The total remaining straw contained into the frames was collected after 1, 2, 4, 6, 8 and 12 months of decomposition. The straw was washed to remove any mineral impurities and then dried at 65°C for 72 hours and weighed to quantify the DM loss. A sub-sample of 100 g of straw was finely ground using a mill and sieved (100 μm) for wet chemical analysis by Van Soest *et al.* (1991) method and spectroscopic analysis by DRIFT. The chemical characterization using wet method was performed only for the samples collected after 2, 4, 6 and 12 months of decomposition in Univalem site and after 4, 6 and 12 months in Bom Retiro site.

For the wet chemical analysis, the biomass was sequentially separated in an Ankom200 Fiber Analyzer (Ankom Tech., Fairport, NY, 2000) into the neutral detergent fiber (NDF), the acid detergent fiber (ADF) and sulfuric acid (73%) that refers to the acid unhydrolyzable residue (AUR). Briefly, NDF mainly consists of hemicellulose, cellulose, lignin, and insoluble minerals, while ADF is composed of cellulose, lignin, and insoluble minerals. The hemicellulose content was calculated as the difference between the NDF insoluble fraction and the ADF fraction. In addition, the AUR fraction is commonly associated with lignin content.

3.2.3. Diffuse Reflectance Infrared Fourier Transform (DRIFT) spectroscopy

The straw samples (100 μm) were oven dried at 65 ºC for 24 h, then stored in a desiccator at room temperature until the analyses. We collected the DRIFT spectra directly from the sample powder by Fourier-Transform Infrared Spectrometer (Alpha, Bruker Optics Inc., Billerica, MA, USA). For DRIFT spectra, the data were recorded into a range from 4000 to 600 cm^{-1} as the reflectance spectra were obtained in apparent absorbance spectra [log(1/reflectance)] units, with 2 cm^{-1} of resolution and 24 scans were co-added per spectrum. The background spectrum was measured using a gold-coated sample holder. Atmospheric interferences (water vapor and CO_2) corrections, background subtraction and spectral averages were carried out using OPUS/Mentor software.

Spectra were pre-processed using a combination of Savitzky-Golay smoothing (polynomial of second-order at 15 points of the window), mean-centering and standard normal deviation. More details for pretreatment analyses see Rinnan *et al.* (2009).

For further evaluations, the spectral range of interest was limited between 1900 to 800 cm^{-1} since this interval presents the fingerprint features of most important biochemical groups, as

reported for diverse types of residues (Artz *et al.*, 2008; Chen *et al.*, 2010; Duboc *et al.*, 2012; McKee *et al.*, 2016).

3.2.4. Statistical analyses

A principal component analysis (PCA) was performed to identify changes in straw chemical composition during the decomposition process, measured through DRIFT spectra. The first derivative spectra were calculated from the DRIFT's spectra. All principal components were computed from the covariance matrix since all the data used for each principal components analysis had the same scale (Calderon *et al.*, 2006; Soong *et al.*, 2014).

Particularly the wavelength regions 1900 to 860 cm^{-1} were taken for detailed mathematical treatment in order to correlation the wet and spectroscopy evaluations. The MagicPlot 2.7.2 software was used to fitting 12 individual Gaussian curves to a combined set of overlapping curves in the original spectrum, after the baseline correction (Figure 1 Appendix). All multiple peaks fitting have reached a coefficient of determination $R^2 \geq 0.995$. The area from the sum of individual curves was considered 100% and each curve after fitting was expressed as a percent fraction. The results obtained were used to identify curves (bands) in which had shifts in the relative area correlated to biochemical compounds by Pearson's correlation matrix, using the Statistica13 software.

3.3. Results and Discussion

3.3.1. Characteristic bands in the DRIFT absorption spectrum

A typical DRIFT spectrum of sugarcane straw is showed in Figure 1. The initial peaks represent recalcitrant and intermediate compounds in relation to the ability of the organisms to decompose them. The ester carbonyl band (C=O), from fatty acids, is represented at 1735 cm^{-1}, the small band at 1630 might be associated with lignin and xylan, and the stretch of aromatics (C=C and/or COO^-) at 1530 cm^{-1}, *i.e.*, recalcitrant compounds (Fig. 1, Table 1). Besides these bands, from peak 1447cm^{-1} more labile compounds are observed in sugarcane straw. The bands at 1447, 1369, 1319 and 896 cm^{-1} could be associated with celluloses and hemicelluloses. The region between 1150 to 1036 cm^{-1} contains bands that can be assigned to several carbohydrates (C–O), and/or polysaccharides (Fig. 1, Table 1).

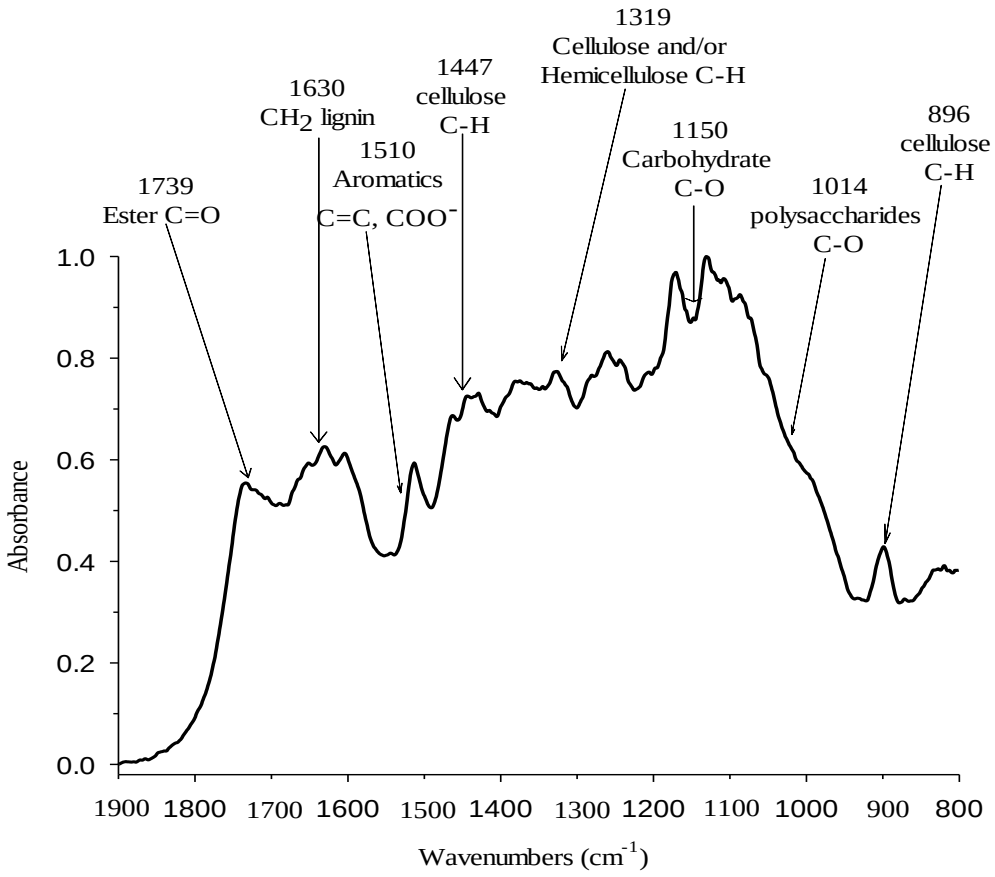

Figure 1. The 1900 to 800 cm^{-1} region of the DRIFT spectrum of sugarcane straw with "fingerprint" bands of biochemical compounds.

Table 1. Assignment of the principal descriptive infrared absorption bands in residues samples.

Wavenumbers (cm^{-1})	Base group	Reference
1739	C=O stretch	McKee *et al.* 2016
1620 and 1510	C=C and/or COO^- stretch of aromatics	Chen *et al.* 2010
1630 and 1463	CH_2 deformation stretching in lignin	Chen *et al.* 2010
1447	C–H in cellulose	McKee *et al.* 2016
1427 and 1114	C–H in cellulose, hemicelluloses, or lignin	McKee *et al.* 2016
1379and 1319	CH_2 in cellulose and hemicelluloses	McKee *et al.* 2016
1739 and 1369	C–H in hemicelluloses	McKee *et al.* 2016
1150 and 1036	C-O bonds of polysaccharides	Chen *et al.* 2010
896 and 987	C–H in cellulose	Chen *et al.* 2010

Overall, the position and relative intensity of peaks in the spectrum of sugarcane straw were similar regardless sites and sampling time. The greater variation was observed between initial and final sampling times (Fig. 2). Although sugarcane straw samples came from different sites and decomposition times, small changes in the spectra were detected, making the visual comparison a difficult task. This result is consistent with those reported by Ouellette *et al.* (2016) who verified the importance of the visual inspection of the spectrum but to compare the treatments they used the relative peak data rather than examination inferences.

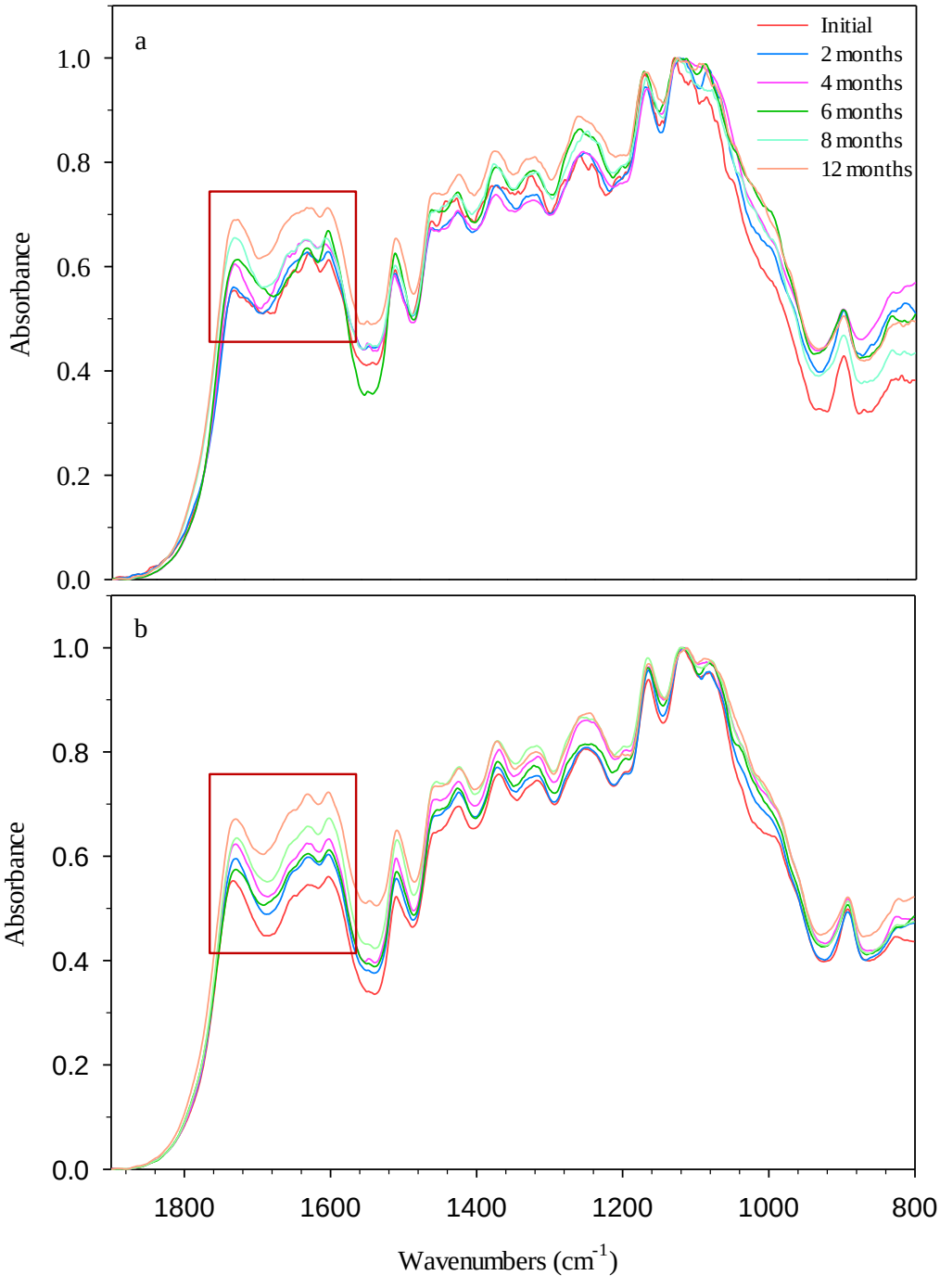

Figure 2. The 1900 to 800 cm^{-1} region of the DRIFT spectrum of Bom Retiro (a) and Univalem sites (b). The red squares indicate the main region which the absorbance increases over the decomposition time.

Despite that, visual examination of the spectrum (Fig. 2) showed the decrease in the absorbance between 1100 and 800 cm^{-1} after one year of decomposition. These bands were associated with carbohydrates and polysaccharides (Fig. 1, Table 1) (Chen *et al.* 2010). The reduction in polysaccharide bands can be attributed to microbial consumption of this easily decomposable C fraction (Kuo *et al.* 1997). The same pattern was described by Ouellette *et al.* (2016) in a lab-incubation study about cover crop and corn stover decomposition in the USA.

DRIFT data also revealed higher concentration of recalcitrant C, linked with lignin and aromatic compounds (1800 to1600 cm^{-1}, Fig. 1) in the late phase of decomposition. It likely occurred due to preferential microbial consumption of more labile C fractions in the early phases (Zaccheo *et al.* 2002). The intermediate decomposable C fraction by microorganisms, such as hemicellulose and cellulose (1500 to 1200 and 1000 to 800 cm^{-1}, Fig. 1), also increased the absorbance over time, but to a smaller extent than recalcitrant compounds (1800 to 1600 cm^{-1}).

Straw chemical composition over the decomposition process differed between the sites (Fig. 3). The first two PCs explained together about 92% of the total variability observed in the dataset. While PC1 did not allow to separate sample groups since all samples corresponding a sugarcane straw, two different clusters could be identified based on PC2, one with negative scores related to samples from Univalem site and another cluster with positive scores related to samples from Bom Retiro site. This finding indicated that, on the regional scale, the main driver of chemical composition changes during decomposition was the contrasting edaphoclimatic conditions, as reported by Robertson & Thorburn (2007).

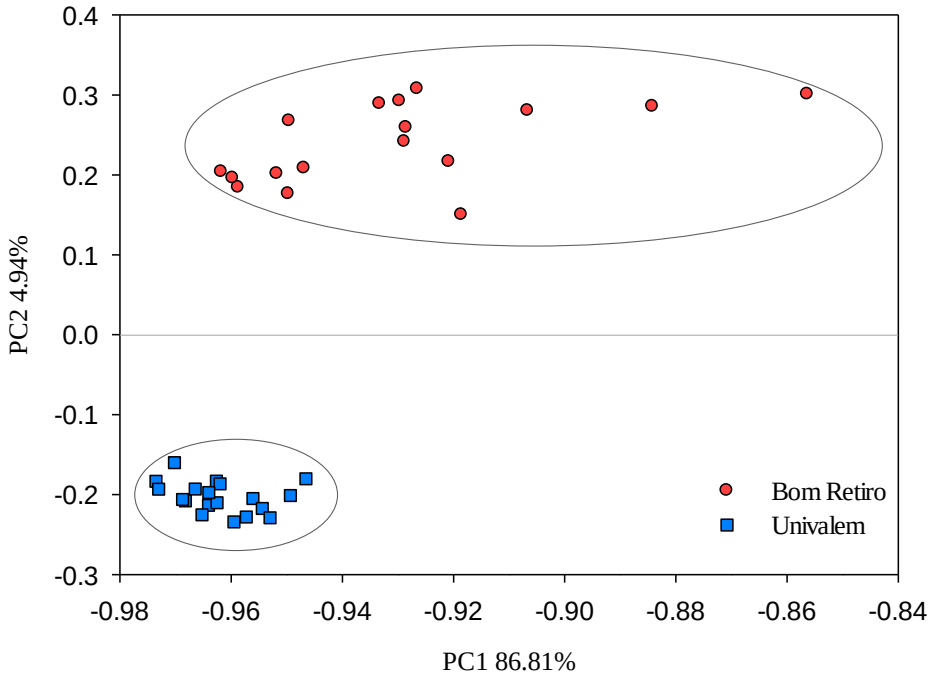

Figure 3. Scores scatter of PC1 (86.81%) *vs* PC2 (4.94%) of the first derivative DRIFT (1900 to 800 cm^{-1}) spectrum of sugarcane straw samples.

Although the mean annual temperature is very similar between the study sites (23.3 and 23.9 °C in Bom Retiro and Univalem, respectively), in Bom Retiro, the accumulated rainfall was 10% lower than in Univalem site during the year of study conduction. The highest temperature and rainfall contribute to increase the straw decomposition rate and consequently affect the chemical composition of the remaining straw (Sun *et al.* 2013; Zhou *et al.* 2015). Furthermore, the contrasting soil texture (*i.e.*, loamy sand soil in Univalem and sandy clay loam soil in Bom Retiro) observed in the sites may be contributed to the different pattern of straw changes between the study sites. In the same way, Spaccini *et al.* (2001) just verified specific maize-derived band changes in soils with low clay content, and conclude that the evaluation of chemical changes by DRIFT spectroscopy was dependent on the soil texture.

In a local scale, we verified that the chemical composition changes were more significantly induced by the time of decomposition than by the straw removal rates (Fig. 4). The first two PC explained about 94 and 98% of the total variability in Bom Retiro and Univalem site, respectively. In both sites, chemical composition changes allowed grouping the samples from the early (*i.e.*, up to four months of decomposition) in the positive PC2 score side and samples from late stages of decomposition (*i.e.*, after four months of decomposition) in the negative PC2 score side.

In the early stage of straw decomposition, easily C decomposable fractions, such as sugars and proteins, cellulose and hemicellulose predominate in the straw composition. As the decomposition proceeds these compounds are being consumed, and in the straw begins to predominate complex structures, such as lignin, which was barely degraded by microorganisms, concentrate over time (Sousa Jr. *et al.* 2017).

The PC2 and PC3 represent only about 3 and 1% of the total variability in Bom Retiro and Univalem sites, respectively (Figure 2 Appendix). Thus, diffuse distribution of the samples in PC3 axis did not allow identifying clusters based on the straw removal rates. In a study conducted in the same area, but using the wet chemical analysis to evaluate the chemical changes, Pimentel *et al.* (2017) showed that the relative lignin loss was not influenced by straw removal rates whereas cellulose and hemicellulose were subtly affected by treatments, in accordance with the DRIFT data. Ouellette *et al.* (2016) reported the same pattern, in which only the time of decomposition was sensitive to detect changes in the DRIFT spectra of the different cover crop residues.

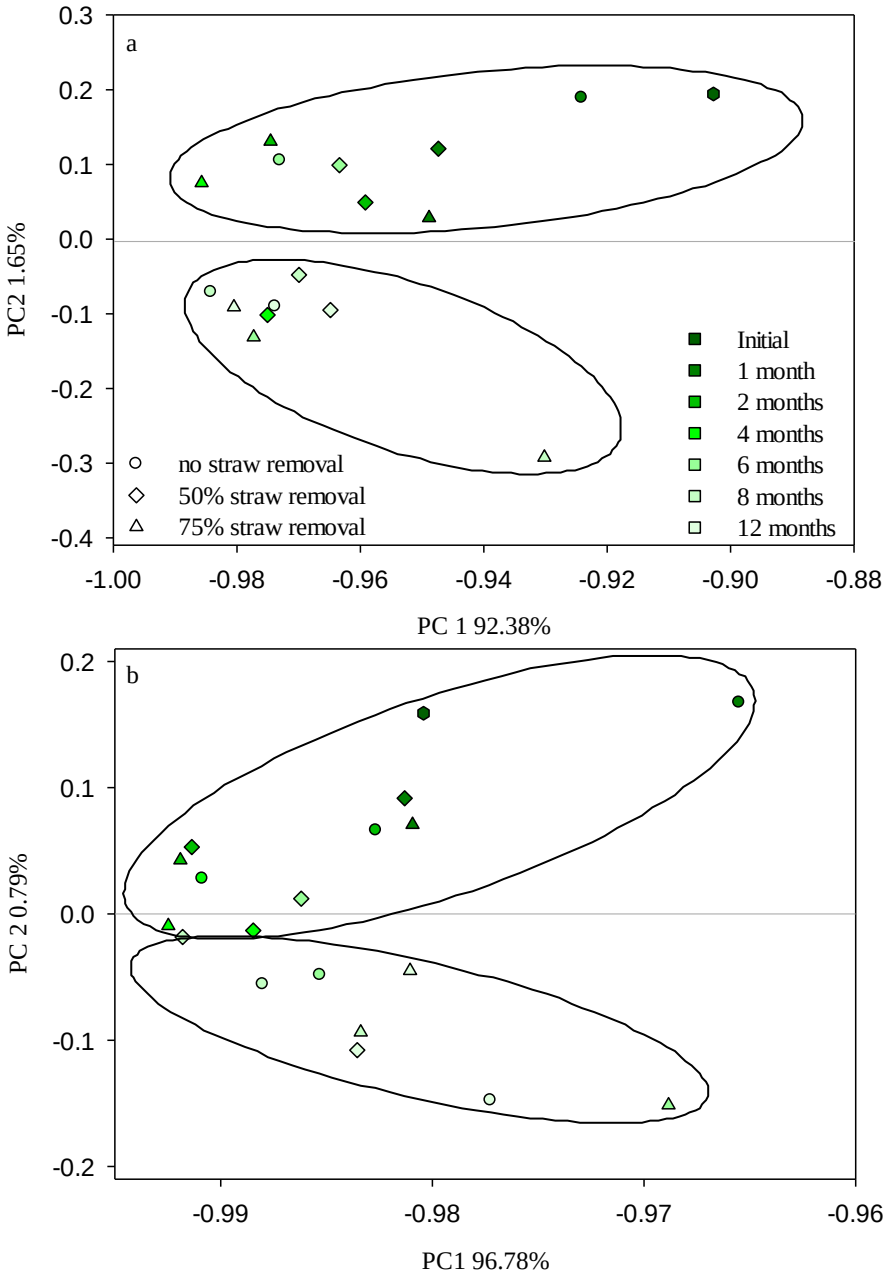

Figure 4. Principal component analysis of the first derivative DRIFT spectrum (1900 to 800 cm^{-1}) of sugarcane straw samples: scores scatter of PC1 (92.38%) vs PC2 (1.65%) in Bom Retiro site (a), scores scatter of PC1 (96.78%) vs PC2 (0.79%) in Univalem site (b).

3.3.2. DRIFT analysis *vs* wet chemical data

As determined by wet chemical analysis (operationally defined), the sugarcane straw was composed approximately by 44% of cellulose, 36% of hemicellulose and 7% of lignin, independent of the study site (Table 2). After the first year of decomposition, average cellulose and hemicellulose contents decreased by 26 and 24%, whereas the lignin was enriched by 52% (Table 2). These changes observed during the progress of the decomposition by wet chemical

methodology followed the same pattern reported in recent studies conducted under similar edaphoclimatic conditions (Sousa Jr. *et al.* 2017; Ramos *et al.* 2016).

Table 2. Sugarcane straw mass remaining and straw chemical compounds analyzed by wet chemical fractionation. Data are averages with standard error in parentheses (n=4).

Month	Mass remaining (Mg ha^{-1})	Hemicellulose (%)	Cellulose (%)	AUR/Lignin (%)
		Bom Retiro – no straw removal		
0	15.84 (0.14)	34.48 (1.06)	43.20 (0.38)	7.69 (0.17)
4	11.16 (0.17)	32.58 (0.52)	43.02 (0.44)	10.25 (0.18)
6	9.39 (0.42)	28.69 (0.63)	39.02 (0.60)	10.46 (0.28)
12	3.75 (0.50)	25.59 (0.47)	32.60 (0.47)	11.58 (0.36)
		Bom Retiro – 50% straw removal		
0	7.23 (0.09)	34.48 (1.06)	43.20 (0.38)	7.69 (0.17)
4	5.57 (0.09)	31.64 (0.59)	41.61 (0.11)	8.86 (0.39)
6	4.67 (0.22)	28.91 (0.32)	38.55 (0.43)	9.85 (0.47)
12	2.41 (0.07)	25.93 (0.36)	37.71 (0.19)	10.85 (0.22)
		Bom Retiro – 75% straw removal		
0	3.11 (0.04)	34.48 (1.06)	43.20 (0.38)	7.69 (0.17)
4	2.43 (0.10)	29.20 (0.45)	43.24 (0.52)	9.06 (0.16)
6	1.90 (0.09)	26.44 (0.79)	36.92 (0.80)	9.96 (0.15)
12	0.97 (0.10)	24.84 (0.38)	36.10 (1.41)	11.09 (0.35)
		Univalem – no straw removal		
0	11.47 (0.04)	37.05 (0.47)	44.24 (0.48)	6.57 (0.17)
2	10.46 (0.06)	35.40 (0.33)	42.70 (0.09)	6.83 (0.03)
4	7.85 (0.22)	32.30 (0.71)	40.39 (0.51)	9.10 (0.19)
6	7.25 (0.11)	29.57 (0.41)	35.41 (0.80)	9.32 (0.25)
12	5.23 (0.18)	27.53 (0.75)	29.13 (1.87)	10.48 (0.44)
		Univalem – 50% straw removal		
0	6.18 (0.08)	37.05 (0.47)	44.24 (0.48)	6.57 (0.17)
2	5.54 (0.09)	34.63 (0.51)	41.88 (0.81)	6.33 (0.02)
4	4.65 (0.05)	30.32 (0.68)	39.22 (0.83)	7.31 (0.24)
6	4.21 (0.27)	29.63 (0.37)	36.66 (0.80)	7.95 (0.17)
12	2.72 (0.28)	26.89 (0.81)	31.26 (0.92)	10.61 (0.85)
		Univalem – 75% straw removal		
0	3.62 (0.07)	37.05 (0.47)	44.24 (0.48)	6.57 (0.17)
2	3.26 (0.03)	34.61 (0.65)	43.37 (0.59)	6.49 (0.08)
4	2.78 (0.17)	31.10 (0.53)	41.64 (0.23)	7.91 (0.03)
6	2.47 (0.08)	29.26 (1.00)	36.36 (0.27)	8.31 (0.63)
12	1.28 (0.22)	28.32 (0.80)	33.55 (1.01)	10.16 (0.35)

Despite the significant straw DM losses (average 71 and 58% in Bom Retiro and Univalem, respectively) and reduction in the cellulose and hemicellulose fractions over time (Table 2), the DRIFT spectra did not show abrupt changes after one year (Fig. 2). The DM loss is not only associated with the straw mineralization (chemical changes) but also with physical mass fragmentation and leaching (Cotrufo *et al.* 2015), which may have contributed to this subtle change in DRIFT spectra.

Significant linear correlations (Table 3) were found between most of the DRIFT specific peak areas (Figure 1 Appendix) and wet chemical data (Table 2). There were strong correlations (varying from 0.5 to 0.73) between DRIFT spectral bands and the wet chemical fractions for cellulose and hemicellulose (Table 3), especially at the 896, 987, 1173, and 1447 cm^{-1} bands (Chen *et al.* 2010; McKee *et al.* 2016). McKee *et al.* (2016) also found the high correlation between cellulose and hemicellulose determined using these two methodologies for big bluestem residue after one year of decomposition. These results suggest that those bands could be used to trace straw cellulose and hemicellulose dynamics in the sugarcane straw decomposition process.

Table 3. Pearson correlation coefficients between relative area of multiple peak fitting from DRIFT data and wet chemical data from sugarcane straw under different stages of decomposition (n=23).

Multiple peak fitting from DRIFT data		Wet Chemical fractions		
Wavenumbers of center peak (cm^{-1})	Base group*	Hemicellulose	Cellulose	AUR/lignin
896	Cellulose	**0.51**	0.29	**-0.72**
987	Cellulose	0.32	**0.69**	**-0.47**
1150	Polysaccharides	**0.42**	**0.73**	**-0.52**
1173	Cellulose, hemicellulose	0.30	**0.73**	-0.40
1270	C=O stretch	0.09	0.07	0.09
1319	Cellulose, hemicellulose	0.12	-0.20	-0.08
1369	Hemicellulose	-0.18	0.10	0.03
1447	Cellulose	**0.50**	0.21	**-0.61**
1510	Lignin	**-0.66**	**-0.60**	**0.72**
1630	Lignin	0.26	0.14	-0.20
1739	C=O stretch	0.06	0.35	-0.36

*Base groups were selected according to McKee *et al.* (2016) and Chen *et al.* (2010). Bold values denote significant correlation between DRIFT data and wet chemical fractions at the $p < 5\%$ level.

The lignin fraction determined by wet chemical fractionation increased over one year of decomposition, similar to reported by Sousa Jr. *et al.* (2017). This straw lignin enrichment likely is associated with the incorporation of unhydrolysable compounds created during the decomposition process, mainly by the microorganisms residues (McKee *et al.* 2016). Consistently, during this period, the greatest net increase in the DRIFT absorbance was observed at 1800 to 1600 cm^{-1} spectral bands (Fig. 2). In the same way, the net increase in DRIFT absorbance could have been due to microbial protein amide (bands at 1660 and 1540 cm^{-1}) created over time, as reported by McKee *et al.* (2016).

The absorbance at 1630 and 1510 cm^{-1} were associated with lignin fraction in our study (Table 3) since these bands were previously found in the pure lignin spectra (Reeves *et al.* 2008). However, the absorbance at 1630 cm^{-1} did not correlate with the lignin fraction, just the 1510 cm^{-1} band was positively and highly correlated (0.72) with the lignin content obtained by wet chemical fractionation (Table 3). In this sense, in order to trace straw cellulose and hemicellulose changes we suggested the use of 896, 987, 1173, and 1447 cm^{-1} bands, whereas to trace straw lignin changes over time, the absorbance at 1510 cm^{-1} seems to be an efficient predictor.

Some variations could affect the DRIFT results, such as the orientation of samples surface, particles sizes, dilute or not the samples, device parameters (Parikh *et al.* 2014) as well as spectrum pretreatment (Rinnan *et al.* 2009). These variables could shift the intensity and exact peak center, which hinders the direct comparison among results from several references.

Nevertheless, our results showed the strong correlations between DRIFT spectral bands and the wet chemistry methodology, suggested that the DRIFT has a promising potential to be used for assessing sugarcane straw decomposition dynamics, as previously reported in studies with different residues by Zaccheo *et al.* (2002); McKee *et al.* (2016); and Ouellette *et al.* (2016). It is worth mentioning that the total time required for DRIFT analysis was about 10 min per sample (added to the preparation time of the straw sample) against one week required for wet chemical analysis (Chen *et al.* 2010). In addition, disregarding the cost to buy the equipment, the analysis using DRIFT spectroscopy is much cheaper than wet chemical analysis.

3.4. Conclusions

The application of DRIFT analysis to follow decomposition process was sensitive to detect straw chemical changes induced by the edaphoclimatic conditions, especially by the soil texture, and by the time of decomposition. Furthermore, the spectral bands specific for aliphatic (*i.e.*, 896, 987, 1173, and 1447 cm^{-1} bands) and aromatic (*i.e.*, 1510 cm^{-1}) regions was correlated

significantly with the cellulose, hemicelluloses and lignin data obtained by the wet methodology that is considered as a reference in studies of decomposition.

This research indicates that DRIFT spectroscopy technique can be an effective and non-destructive tool to evaluate the chemical composition changes during the sugarcane straw decomposition process and provide basic information to assist a sustainable straw removal management to bioethanol production in Brazil.

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APPENDIXES

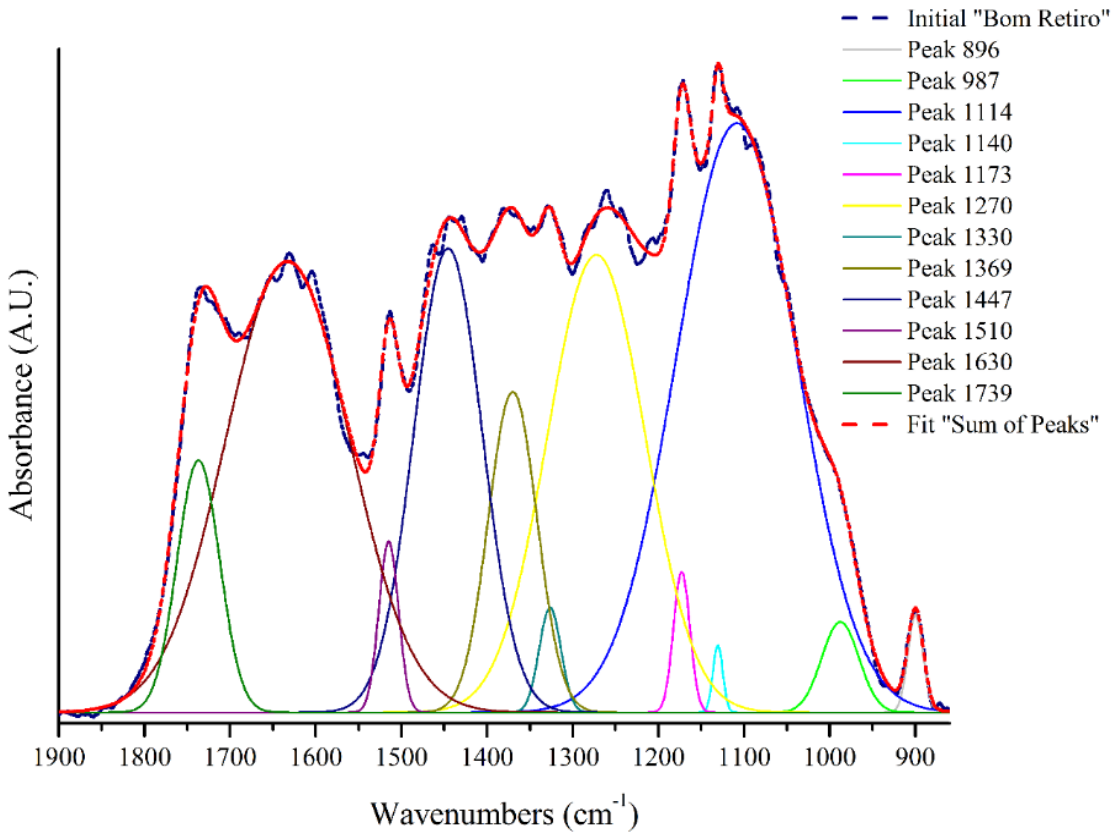

Figure 1 Appendix. Example of the peak deconvolution in the 1900 to 860 cm^{-1} region of the sugarcane straw DRIFT spectrum in initial time of decomposition in Bom Retiro. Briefly, the original spectra was decomposed in twelve Gaussians' curves, which the centers are 896, 987, 1114, 1140, 1173, 1270, 1330, 1369, 1447, 1510, 1630 and 1739 cm^{-1}. The area of each curve was adjusted to generate a composited curve ("Sum of Peaks" – dashed red line) to overlap the original spectrum area ("Initial Bom Retiro" – dashed blue line).

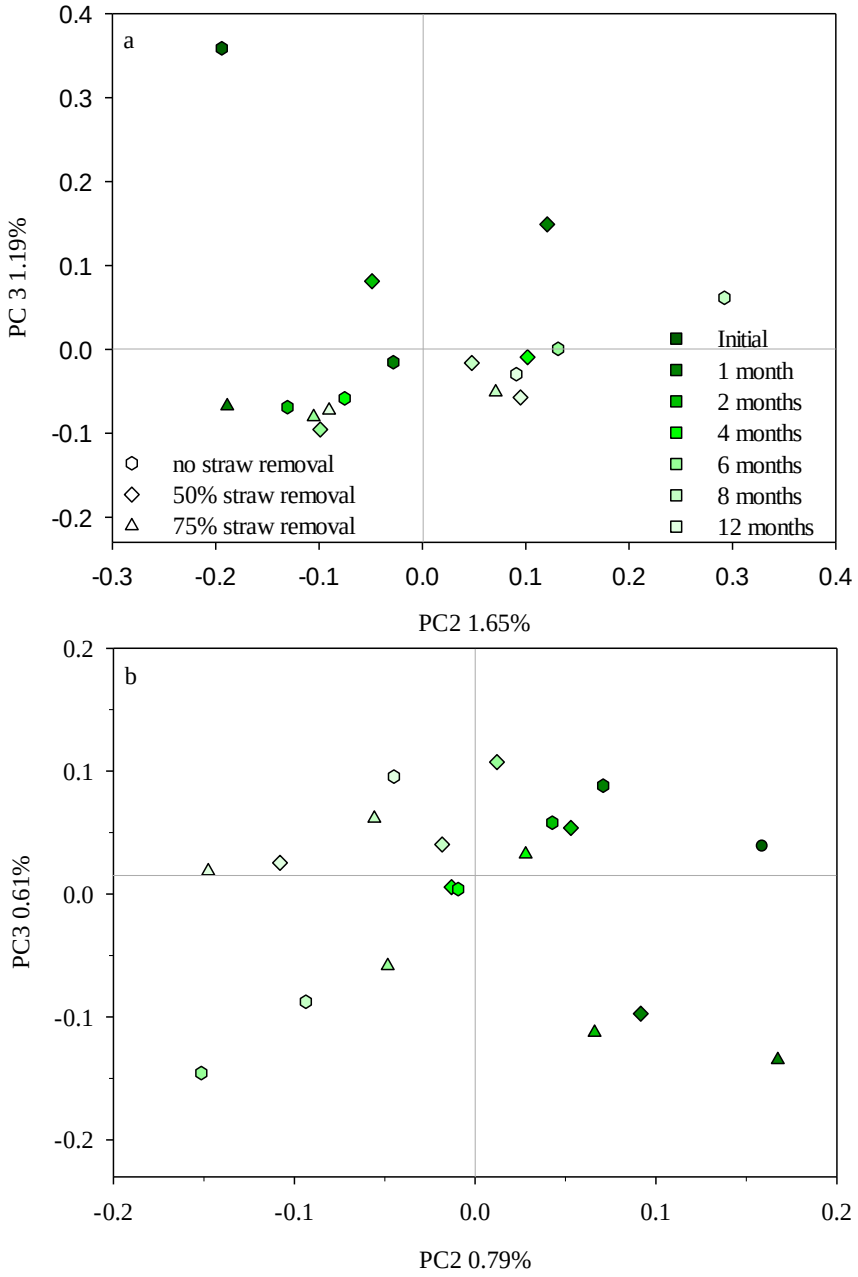

Figure 2 Appendix. Principal component analysis of the first derivative DRIFT spectrum (1900 to 800 cm^{-1}) of sugarcane straw samples. Scores scatter of PC2 (1.65%) *vs* PC3 (1.19%) in Bom Retiro site (a), scores scatter of PC2 (0.79%) *vs* PC3 (0.61%) in Univalem site (b).

4. SOIL BACTERIAL COMMUNITY CHANGES IN SUGARCANE FIELDS UNDER STRAW REMOVAL IN BRAZIL

ABSTRACT

One of the main drivers of the straw decomposition dynamics is the bacterial community, which it has been used as a very sensitive indicator of land management. However, there is a gap to follow in the field-scale the effect of the bacterial structure changes over the straw decomposition process in sugarcane areas. In this sense, we conducted a field study at two sites in Brazil, to investigate how rates of sugarcane straw removal are associated with soil bacterial community changes over one year of decomposition. Four sugarcane straw removal rates were tested: no removal (~17.5 Mg ha^{-1} of dry mass left on the soil surface), 50% (~9.0 Mg ha^{-1}), 75% (~4.5 Mg ha^{-1}) and 100% of straw removal. The soil bacterial community structure was evaluated by the Terminal Restriction Fragment Length Polymorphism (T-RFLP). Time of decomposition, associated with changes from the season and from the straw composition, was the driver of soil bacterial community changes. Also, the litter quality explained 23.2% of the total bacterial variation, where hemicellulose accounting for 17.2% of this variation. In this sense, the straw composition changing the microbial community is one of the factors that determine the rates of straw that can be removed in sugarcane areas. The rates of straw removal had a lower effect on the soil bacterial community when compared to the straw quality. Moreover, the bacterial community structure was similar among high levels of straw removal (75 and 100%) and among lower levels of straw removal (0 and 50%). Finally, the soil texture is one more factor to be included in the metrics to establish the rates of straw removal in sugarcane areas in Brazil.

Keywords: T-RFLP; Hemicellulose; Bioenergy; Straw quality; Bacterial community; Sugarcane

4.1. Introduction

Nowadays almost all sugarcane plantations in Brazil are mechanizing harvested without burning (*i.e.*, green harvesting system) (Oliveira *et al.*, 2017). Therefore, about 10-20 Mg ha^{-1} of sugarcane straw are left on the soil surface every year (Carvalho *et al.*, 2016). The straw maintenance in sugarcane fields has an essential role in diverse soil properties and processes, improving soil quality and consequently the crop performance (Carvalho *et al.*, 2016, Cherubin *et al.*, 2017). However, the sugarcane straw has become an attractive source of biomass for bioelectricity and second generation (2G) ethanol production in Brazil (Carvalho *et al.*, 2016). In

this sense, field studies about the environmental suitability of straw removal in sugarcane areas are mandatory before using crop residues as a source of biomass for large-scale bioenergy production in Brazil (Oliveira *et al.*, 2017).

It is well known that some factors regulate the straw decomposition, such as the microbial community, climate, and straw quality (Garcia-Palacios *et al.*, 2013). The decomposition is a complex and dynamic process governed by these interactive factors (Wall *et al.*, 2008). However, the interactions among these various controlling decomposition factors, especially the straw quality and the edaphoclimatic conditions on the soil bacterial community remain unclear.

Soil biota contributes for provisioning essential ecosystem services, such as nutrient recycling through decomposition process of organic residues. The soil biota comprises macroinvertebrates, fungi, and bacteria communities that together were responsible for many soil functions that contribute to the soil quality (Zhao *et al.*, 2016). In the early stages of decomposition, crop residues are physically fragmented (Wall *et al.*, 2008), followed by chemical degradation through extracellular enzyme, such as cellulase and ligninase that are mainly produced by the soil microorganisms.

Soil microorganisms are highly sensitive to management practice changes (Rachid *et al.*, 2016). Thus, they are often used to evaluate land use and management practice effects on soil quality (Mendes *et al.*, 2015, Rachid *et al.*, 2016, Zhao *et al.*, 2016). Bacteria already have been known to contribute less actively in the decomposition process than fungi. However, recently, several studies, which use modern techniques, have shown that bacteria are equally or even more important than fungi in the decomposition dynamics, especially in the early stages of decomposition (Kim *et al.*, 2014, Snajdr *et al.*, 2011, Stursova *et al.*, 2012).

The use of the bacterial marker gene (*i.e.*, 16S rRNA) in molecular techniques, such as Terminal Restriction Fragment Length Polymorphism (T-RFLP), provides valuable information regarding shifts in the structure of soil microbial community (Mendes *et al.*, 2015). Accordingly, this tool can be used to evaluate the effects of the management practices, such as the straw removal to bioenergy production, in the soil microorganisms (Lammel *et al.*, 2015). Recently, some studies have been conducted in Brazilian sugarcane fields to evaluate straw management effects in the soil bacterial community (Pitombo *et al.*, 2016, Rachid *et al.*, 2016). However, to our knowledge, there are no published field-scale studies that assessed bacterial structure changes over the straw decomposition process in these fields.

In this sense, we conducted a field study at two contrasting sites within central-southern Brazil, the main sugarcane-producing region of the world, to investigate how rates of sugarcane straw removal are associated with soil bacterial community changes over one year of

decomposition. Our main hypotheses are that i) the straw removal rates changes the soil environmental and affect the bacterial community; ii) straw chemical changes during decomposition processes induce changes in bacterial community structure; and iii) the edaphoclimatic conditions also influenced the soil bacterial community structure in sugarcane areas under straw removal in Brazil.

4.2. Material and Methods

4.2.1. Study sites location and experimental design

The field experiments were conducted in two sites located in central-southern Brazil, near to Capivari, southeast region of São Paulo state, at the Bom Retiro mill (Lat. 22°59'42"S; Long. 47°30'34" W) and near to Valparaiso, northwest region of São Paulo state, at the Univalem mill (Lat. 21°14'48" S; Long. 50°47'04" W). The soil was classified as Rhodic Kandiudox at the Bom Retiro site and as Kanhaplic Haplustults at the Univalem site (USDA, 2014). The soil clay content at 0-0.1 m of soil depth was 326.5 and 114.7 g kg^{-1} in Bom Retiro and Univalem site, respectively.

The climate in Bom Retiro site is subtropical (Cwa type - Köppen-Geiger classification), with rains concentrated in the summer (October to April) and a dry season in winter (May to September). At the Univalem site, the climate is tropical (Aw type), with the dry season in the winter (April to September). During the experiment conduction in Bom Retiro site the mean annual temperature was 23.2 °C and an annual precipitation was 1663 mm, and in Univalem site the mean annual temperature was 24.0 °C and the annual precipitation was 1765 mm.

Sugarcane has been cultivated in experimental areas since 1977. However, the green harvesting management, without burning, began only in the last ten years. The field experiments started in August 2014 and repeated in August 2015 under a randomized complete block design with split-plots and four replications. Straw removal rates served as the main plot factor and time of sampling as the split-plot factor. Four sugarcane straw removal rates were studied, as follows: no removal (~17.5 Mg ha^{-1} of dry mss left on the soil surface), 50% (~9.0 Mg ha^{-1}), 75% (~4.5 Mg ha^{-1}) and 100% of straw removal. These treatments were proposed to simulate the straw removal in sugarcane commercial areas in Brazil. For more details about site location, climate conditions during the year and the experiment design, see chapter 2 of this Thesis (Pimentel *et al.,* 2017b).

At each site, the straw amount respective to each treatment was added into the metallic frames (experimental unit) with 0.25 m^2 and heights ranging from 0.07 to 0.14 m, according to the treatment. Each metallic frame corresponded to an experimental unit, *i.e.*, 64 metallic frames were installed in the field, at each site. Frames were placed in the inter-row position within sugarcane field. In order to prevent fresh litter inputs or outputs, the frames were covered by a metal net (0.05 x 0.05 m).

4.2.2. Soil and straw sampling

Soil and straw samples were collected in the initial time and after 4, 8, and 12 months of decomposition. Soil samples were collected from the 0-0.05 m layer, using PVC tubes with 5 cm of diameter disinfected with 70% ethanol. In each metallic frame, three sampling points were selected diagonally, and these samples were pooled to obtain one composite sample per point. Soil cores were frozen immediately after collection and stored at -80 °C, for further molecular analysis.

In addition to soil sampling, the total remaining straw contained into the frames was also sampled. Sugarcane straw was dried at 65°C for 72 hours and weighed to quantify the dry mass (DM) loss. A sub-sample of straw was finely ground and the biochemical compounds were analyzed by Van Soest *et al.* (1991) methodology. Cellulose and hemicellulose were determined by neutral detergent fibers and acid detergent fibers, and lignin in acid detergent. In addition, the total C and N content from soil and from sugarcane straw were determined using an elemental analyzer (Leco CN-2000®, St. Joseph, Michigan).

4.2.3. DNA extraction and Bacterial community fingerprinting using T-RFLP

Total DNA was extracted from 0.4 g of soil using the PowerSoil DNA Isolation kit (MoBio, Carlsbad, USA) according to the manufacturer's instructions. The purity of the DNAs was checked by gel electrophoresis in 1% agarose. Terminal Restriction Fragment Length Polymorphism (T-RFLP) fingerprinting was used to characterize the bacterial community structure in all samples. The domain Bacteria 16S rRNA gene was amplified using the primers 8-FM (5'-6AGAGTTTGATCMTGGCTCAG-3') and 926r (5'-CCGTCAATTCCTTTRAGTTT-3') (Schutte *et al.*, 2009). The forward primer was labeled with 6-FAM (6-carboxy fluorescein) at the 5' end. The reaction mix was composed of 1 µl of DNA template, 4 µl dNTP, 0,1 µl of each primer, 5 µl PCR Buffer 1X, 6 µl of MgCl2 (50 mM), 0,2 µl (5 L) of Platinum Taq DNA

Polymerase (Fermentas, Brazil) in a final volume of 50 μl. The amplifications were performed with the following cycling conditions: 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 53 °C for 30 s, and 72 °C for 30 s with a final extension step at 72 °C for 10 min. Negative controls without DNA were run in all amplifications.

The PCR amplicons were purified by precipitation with 200 μl of isopropanol (0.6 v/v), during 2 h at ambient temperature. After this time, the samples were centrifuged at 4000 rpm for 90 min, and the supernatant was discarded. Ethanol was added to the samples (250 μl) and once again they were centrifuged at the same spin and time, and the supernatant was discarded. The purified product was resuspended in 50 μl of sterilized ultrapure water for 24 h and quantified on 2% agarose gel.

After purification, an aliquot of 10 μl was digested by the restriction enzyme Hhal (Fermentas, São Paulo, Brazil) at 37 °C for 3 h. The digestion product was precipitated by 3M sodium acetate and 125 mM EDTA in absolute ethanol, dried, and the DNA resuspended in a mix of formamide and the marker LIZ 600 (Applied Biosciences, Foster City, Calif.). Samples were loaded into an ABI-3100 automatic sequencer, analyzed by the Gene Mapper software (Applied Biosciences, Foster City, Calif.), and the results exported for further statistical analysis with the software R.

Peaks shorter than 50 bp or that had a standardized fluorescence <1% of the total fluorescence for that sample, were removed from the further analysis (Culman *et al.*, 2008). T-RFLP is a sensitive estimator of the abundance of dominant bacterial groups, and in determining community structure within a sample. In this sense, our study is focused on the dominant bacterial groups.

4.2.4. Statistical analyses

For T-RFLP analysis, the area of distinct peaks of T-RF was used to verify the bacterial variation of samples and also to correlate with treatments. We verified the T-RF's by ordering analysis (Non-metric Multidimensional Scheduling NMDS). ANOSIM test was performed to verify the significant separation of the different groups of bacterial NMDS analysis. We fitted each environmental vector onto the ordination and verified their correlation by PERMANOVA analyses. The edaphoclimatic conditions covered in this study were the soil C e N content, daily temperature, and accumulated precipitation. The sugarcane straw removal rates were associated with the experimental treatments: no removal, 50, 75 and 100% of sugarcane straw removal.

Statistical analysis was performed using the "vegan", "PCNM", "MASS" and "vioplot" packages in the statistical program R 3.2.1 (The R Foundation for Statistical Computing).

4.3. Results

4.3.1. Bacterial community structure

At the local scale, (*i.e.*, Bom Retiro and Univalem site), we observed that the time of decomposition was the higher factor which explains the bacterial community structure variation (Table 1). In Bom Retiro and Univalem sites, the variance in bacterial community structure was 40.7% and 29.2% explained by the time of decomposition, respectively. The straw removal was also correlated with bacterial community structure, but only in Univalem site. At the regional scale, we found that the evaluated factors (edaphoclimatic conditions, sugarcane straw removal rates and time of decomposition) together explained 40.4% of bacterial community variation. In which the time of decomposition explained 33.5%, sugarcane straw removal rates 6.6%, and edaphoclimatic conditions explained only 0.3% (Table 1). However, the edaphoclimatic conditions did not explain significantly the bacterial structure, e.g., the bacterial community was very similar regarding the distinct sites. In this sense, from now on the data (Bom Retiro and Univalem data) was analyzed together, *i.e.*, at the regional scale.

Table 1: PERMANOVA analysis of bacterial community with respect to the sugarcane straw removal rates, the edaphoclimatic conditions and the time of decomposition.

Evaluated factors	— Region scale —		Local scale			
			— Bom Retiro —		— Univalem —	
	r^2	p value	r^2	p value	r^2	p value
Straw removal	0.066	0.015*	0.045	0.488	0.116	0.025*
Edaphoclimatic conditions	0.029	1.000	0.000	1.000	0.000	1.000
Time of decomposition	0.335	0.001**	0.407	0.001**	0.292	0.001**

p values **<0.001, *<0.5.

We observed that the samples were separated, with very low overlap (ANOSIM – R^2 = 0.54; p-value = 0.001), in two clusters: samples from the early stages of decomposition (*i.e.*, up to four months of decomposition) and samples from late stages of decomposition (*i.e.*, after four months of decomposition) (Fig. 1a). On the other hand, the straw removal rates were high overlap between the different groups. Even so, there was a subtle and significant separation (ANOSIM - R^2 = 0.04; p-value = 0.012) accordingly the removal rates; e.g., samples associated

with high (75 and 100% of straw removal) and samples associated with low (50% and no straw removal) sugarcane straw removal rates (Fig. 1b). Finally, edaphoclimatic conditions could not separate the bacterial community (ANOSIM - R^2 = -0.004; p-value = 0.586; Fig. 2).

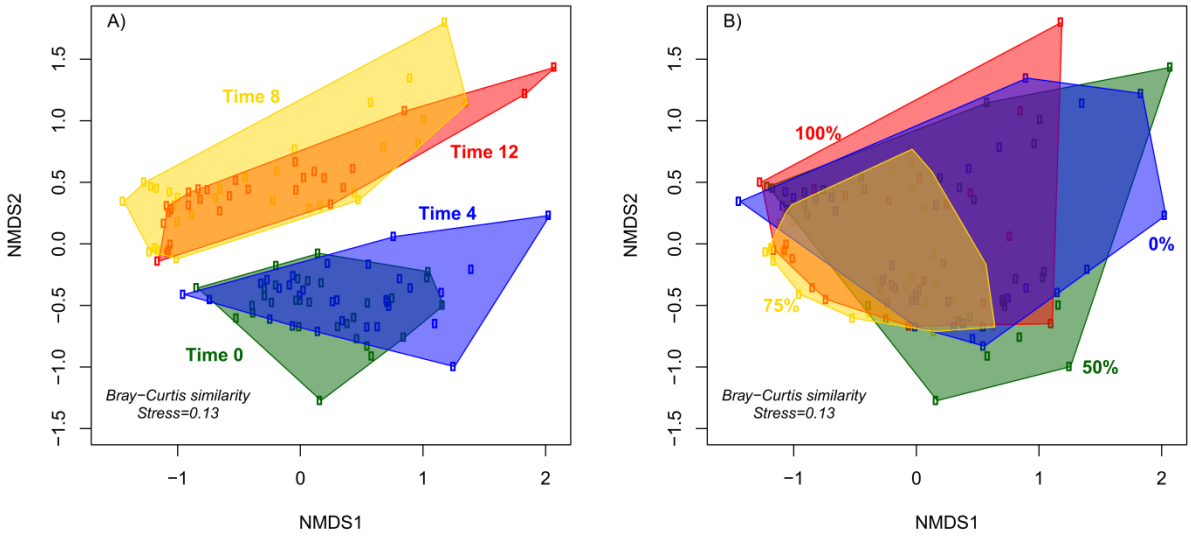

Figure 1: Non-metric Multidimensional Scaling (NMDS) of bacterial community by the time of decomposition (A), and the sugarcane straw removal rates (B).

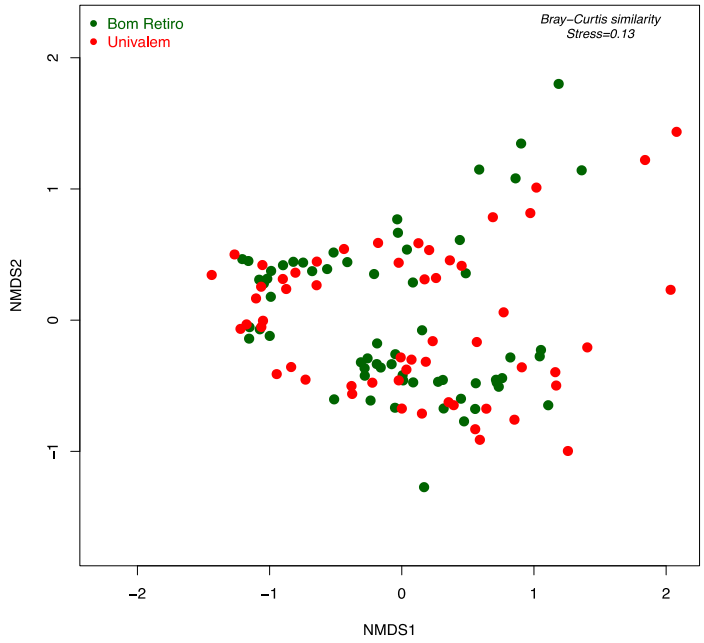

Figure 2: Non-metric Multidimensional Scaling (NMDS) of bacterial community. The colors of points indicate each edaphoclimatic conditions, as samples from Univalem (red points) and sample from Bom Retiro (green points).

4.3.2. Biochemical composition of sugarcane straw and soil bacterial community structure

Besides the straw quantity explained only 6.6% of the bacterial variation, we observed that the straw composition across the time was also correlated with the bacterial variance (23.2%). At the regional scale, the hemicellulose content explained 17.2% of this variation, cellulose 5.0%, and lignin explained only 1.0% (Table 2). In addition to these biochemical compounds, the straw C and N content significantly affected the bacterial structure over time. In a smaller dimension, explaining only 2.7 and 3.2% of the variation attributed to the straw C and N content, respectively (Table 2). At the local scale, the correlations followed a similar pattern, where hemicellulose explains 20.2% in Univalem and 17.1% in Bom Retiro of bacterial variance. We also observed that, in Bom Retiro site, the cellulose explained the bacterial variation, by 7.4%, respectively (Table 2).

Table 2. PERMANOVA analysis of bacterial community with respect to the sugarcane straw composition.

Straw quality g kg^{-1}	Region scale		Local scale			
			Bom Retiro		Univalem	
	r^2	p value	r^2	p value	r^2	p value
Hemicellulose	0.172	0.001**	0.171	0.001**	0.202	0.001**
Cellulose	0.050	0.001**	0.074	0.002*	0.033	0.076
Lignin	0.010	0.274	0.013	0.635	0.021	0.284
Total C	0.027	0.005*	0.154	0.533	0.025	0.176
Total N	0.032	0.007*	0.028	0.127	0.025	0.178

p values **<0.001, *<0.01.

The relationship between straw compounds and bacterial community structure through the time is shown in Figure 3. The straw C, hemicellulose, and cellulose were related to the samples at the early stages of decomposition (up to four months of decomposition), whereas, the straw N and lignin were significantly associated with the samples in the late stage of the decomposition process (after four months of decomposition).

Among the evaluated straw compounds, hemicellulose was the most important regulator of the bacterial community variation over time, explaining alone 74% of the total attributed to the straw quality effect (Table 2). During the decomposition process, the straw hemicellulose content decreased approximately by 32 to 24 g kg^{-1} (Fig. 4). At the same time that the straw hemicellulose was decreasing, the structure of the bacterial community was changing

(Fig 4). The bacterial community was clearly separated into two groups accordingly to the straw hemicellulose content (early and late stage of the decomposition process).

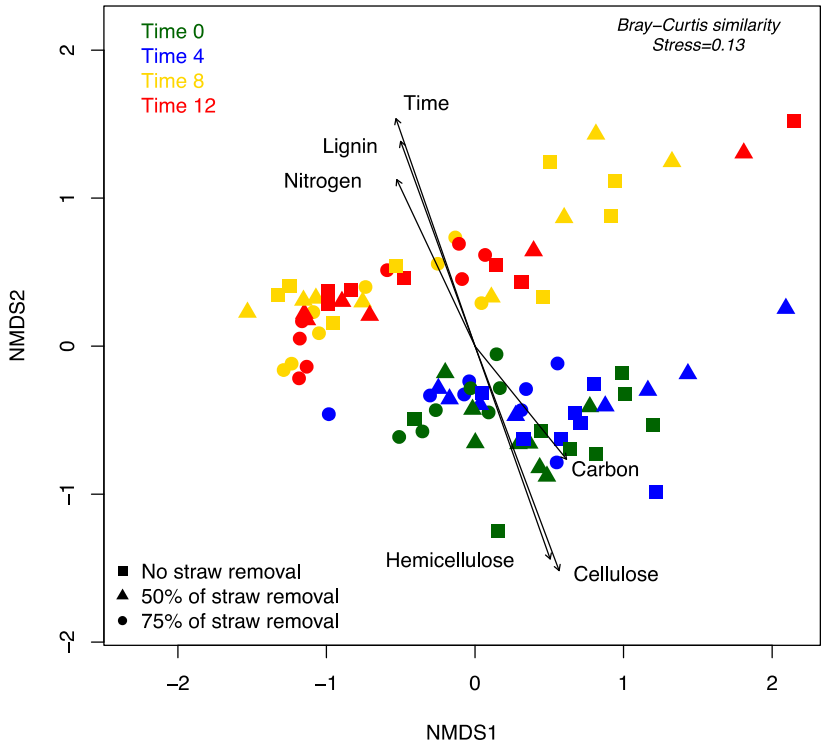

Figure 3. Non-metric Multidimensional Scaling (NMDS) of bacterial community by time of decomposition and the sugarcane straw removal rates. The fitted variables of straw quality are indicated by arrows ($p<0.05$).

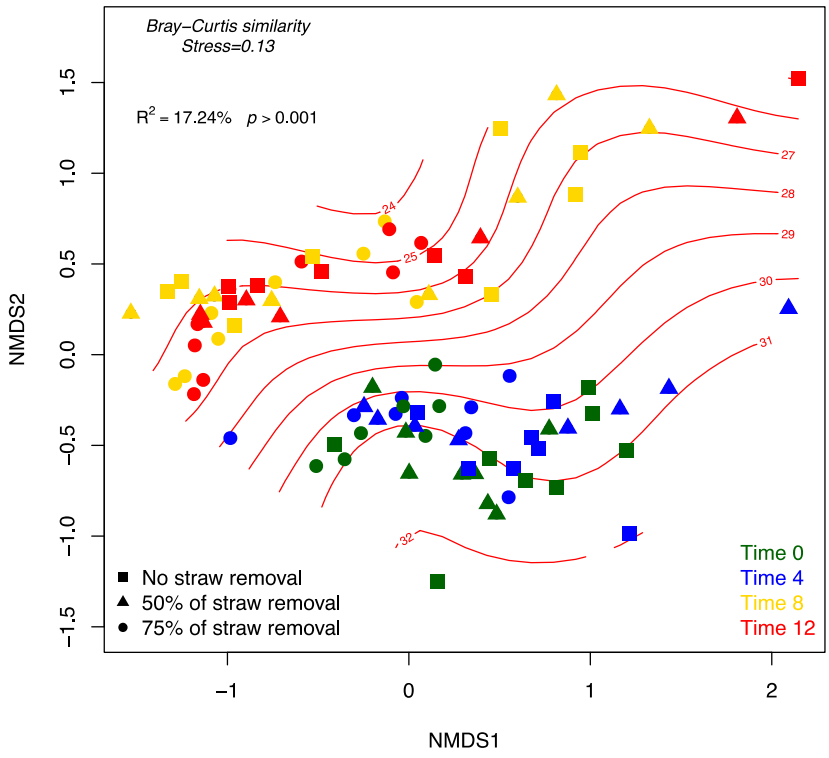

Figure 4. Non-metric Multidimensional Scaling (NMDS) of bacterial community. The symbols indicate the straw removal rates and the colors indicate the time of decomposition. The red lines show the quantity of hemicellulose in sugarcane straw.

4.4. Discussion

In both sites or locally, time of decomposition was the most important factor explaining variations in the soil bacterial community structure (Table 1, Fig. 1a). The time may be associated with intrinsic changes from dry to rainy season, cycles of increase or decrease in temperature throughout the year and changes in straw composition due to the decomposition dynamics. Cleveland *et al.* (2014) and Baumann *et al.* (2009) also reported that time is the principal factor explaining the bacterial variation during the litter decomposition. The dry and rainy seasons induce drying or wetting cycles to the soil environmental, as reported by Mendes *et al.* (2015). In which turn, these cycles of soil microclimate were associated with temporal variations in the bacterial community structure over the year (Lauber *et al.*, 2013) since the temperature and rainfall regulate the characteristics of soil and of straw, and changing the C cycles (Zhou *et al.*, 2016).

The chemical composition of the residual straw also was related with changes in the bacterial community structure (Table 2, Fig. 3). During the decomposition process readily degradable components of straw, such as sugars, proteins, hemicellulose, and cellulose are consumed in the early stages (Sousa Jr. *et al.*, 2017). In contrast, the proportion of complex structures, such as tannins and lignin, which are less biochemically available to microorganisms, enriches over time in the residual straw. Studying the effects of straw quality on the decomposition dynamics in the same area, Pimentel *et al.* (2017b) showed the significant decrease of C, hemicellulose, and cellulose and an enrichment of N, and lignin content in the residual straw over decomposition. These results indicate a strong linkage between the straw quality and the bacterial community, as reported by other studies (Barreiro *et al.*, 2016, Lu *et al.*, 2015, Sauvadet *et al.*, 2016, Zhou *et al.*, 2016).

Higher amounts of more available C in early stages of decomposition stimulate the development of opportunistic bacterial taxa and occurrence of high functional redundancy in the microorganism community (McGuire & Treseder, 2010, Sauvadet *et al.*, 2016, Zhou *et al.*, 2016). In contrast, decreasing litter quality in the late stage of decomposition usually stimulates oligotrophic microbial communities that are well adapted to use less biochemically available compounds to obtain energy and decreasing the functional redundancy (McGuire & Treseder, 2010, Zhou *et al.*, 2016). Cleveland *et al.* (2014) found that litter quality explained 16% of the variation in the soil bacterial community composition, while Zhou *et al.* (2016) found that litter composition explains about 19% of this variation. In the present study, the quality of residual straw explained 23.2% of such variation.

Moreover, the bacterial community was clearly changed between early and late stages of decomposition (Fig. 3). It confirms our previous study that found notably biochemical modifications in the sugarcane residual straw after four months of decomposition (Pimentel *et al.*, 2017a). These findings reinforce that the quality of residual straw is one of the main drivers of bacterial community changes over time.

Among the biochemical components studied, hemicellulose content was the main cause of this variation, accounting for 17.2% of the total bacterial community changes. Hemicellulose is a structural compound that is easier to decompose on sugarcane residual straw (Sousa Jr. *et al.*, 2017). This compound is the most susceptible to decomposition (Santos *et al.*, 2012) and therefore, is faster degraded in the early stage of decomposition (Zhou *et al.*, 2015). Over this one-year study, the hemicellulose content decreased and the soil bacterial community structure changed (Fig. 4), showing that the bacterial community was strongly affected by hemicellulose changes in the residual straw. Locally, the cellulose content was also a significant driver of the bacterial community in Bom Retiro site, explaining 7.4% of the total variation. In this site was also observed a higher residual straw decomposition rate than in Univalem site (Pimentel *et al.*, 2017b).

The soil microbial community structure is influenced by the agricultural practices (Navarro-Noya *et al.*, 2013), highlighting the crop residue management (Pitombo *et al.*, 2016, Rachid *et al.*, 2016). The straw removal is supposed to influence the soil bacterial community directly or indirectly. Directly, the straw increases the food supply, especially in the early stages of decomposition, by the high concentration of easily decomposable sources which usually enhances the bacterial development (Lu *et al.*, 2015, Navarro-Noya *et al.*, 2013). In addition, the input of sugarcane straw influenced indirectly the bacterial structure, by improving the soil environmental, e.g. reducing soil temperature, increasing soil moisture, aeration, water holding capacity and infiltration (Cherubin *et al.*, 2017, Sousa Jr. *et al.*, 2017, Lu *et al.*, 2015).

However, bacterial community was only subtly affected by the sugarcane straw management in this study (Table 1, Fig 1b). In the ANOSIM test analysis (Fig. 1b), was observed a sparse separation between high rates (75 and 100%) and low rates of straw removal (50% and no straw removal). This bacterial response to straw management was less pronounced than we expected. The more reasonable explanation can be the long-term (> 10 years) of green harvesting adoption in these fields without straw removal management (*i.e.*, the total straw is left on the soil at each harvest event). The sugarcane cropping with successive green harvesting increase the soil C and N stocks (Oliveira *et al.*, 2017). In this sense, larger soil C e N pools supply bacterial community needs, making it more resilient to short-term reduction in crop residue inputs. In

addition, short-term sugarcane removal rates have little influence on soil chemical attributes that were directly associated with the soil microorganisms. However, Rachid *et al.* (2012) in studies comparing the effect of burning and unburning sugarcane harvest in the soil microorganisms, found strong discrepancy regarding the bacterial community and associated this difference with the soil chemical attributes.

In addition, straw yield normally varies among the annual sugarcane cycles in response to climate, soil, variety and management conditions. Therefore, over the years, the soil bacterial community possibly was already established and adapted to seasonal changes in the crop residue inputs. Although bacteria can quickly response to any soil management change, two years of straw removal was not enough to alter greatly the soil bacterial community structure. Possibly, in the long-term, straw removal rates have more pronounced effect on the soil bacterial structure.

The soil types and the climatic conditions may also explain the low effect of straw removal on the soil bacterial community. Rachid *et al.* (2016) not found significant differences in bacterial community structure within the levels of sugarcane straw in the same tropical conditions (Oxissol, with mean annual temperature was 23° C, annual precipitation was 1635 mm). These conditions were very close to Bom Retiro site conditions (Oxissol, with temperature was 23.2 °C, precipitation was 1663 mm). In both studies, the straw removal rates did not affect the soil bacterial structure. However, in Univalem site more noticeable effects of straw removal rates in the soil bacterial community were presented (Table 1). In this sense, especially the soil texture and precipitation could explain the effect of sugarcane straw management on soil bacterial community structure.

Currently, Brazil has two commercial 2G ethanol mills in operation, three demo mills and 20 projects in the pipeline (Kutas, 2016). Moreover, a substantial investment by the private sector and government is a strong market signals that sugarcane 2G-ethanol supplies are likely to increase dramatically in the next years (Oliveira *et al.*, 2017).

In this scenario, studies to support the establishment of economically feasible and environmentally friendly rates of straw removal are imperative in Brazil. In our assessment, the rates of straw removal had a lower effect on the soil bacterial community when compared to the straw quality. In this sense, is essential to take into account the straw composition for evaluate the microbial community change as one of the factors to determine the rates of straw that can be removed in sugarcane areas. Finally, based on the possible effect of this attribute in the soil microbial responses in our assessment, soil texture and precipitation should be also included in the metrics to establish the rates of straw removal in sugarcane areas in Brazil.

4.5. Conclusions

The time of decomposition associated with the straw biochemical changes, especially the hemicellulose content, is the most important factor that explaining variations in the soil bacterial community structure. One of the factors to determine the rates of straw that can be removed in sugarcane areas is the straw composition for evaluate the microbial community change, due the strong linkage between them. The indiscriminate sugarcane straw removal for bioenergy production should be avoided since it alters the soil bacterial community, even in the short-term. Finally, other soil parameters and climatic characteristics are needed to be included in the metrics to establish the rates of straw removal in sugarcane areas in Brazil, such as the soil texture and rainfall. Based on these results, the knowledge about the complex decomposition process could be improved, enhancing the sustainability of bioenergy production in Brazil.

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5. FINAL REMARKS

The use of crop residues for energy purposes is one of the principal alternatives to increase bioenergy production in the next few years. In Brazil, there are two mills producing ethanol 2G from sugarcane straw, one in São Miguel dos Campos (AL) and other in Piracicaba (SP). Both of these mills are still operating below their total capacity. This low production is a reflection of the current economic crisis and especially of technological problems. Once these problems are solved, the 2G ethanol and the bioelectricity production should increase close to the estimated capacity for each mill. From this moment, the straw removal from the sugarcane commercial fields should be a regular management practice. However, as showed in all chapters, the sugarcane straw removal for bioenergy production should be done with prudence, since the straw removal rates affected the straw decomposition dynamics and consequently can change some soil properties, such as the nutrient recycling and C cycle.

In Chapter 2, we showed how complex is the straw decomposition dynamics and the possible effects of the straw removal on the environment. We concluded that high straw removal rates reduce the decomposition and consequently the inputs of C and nutrients in the soil; *i.e.*, the straw removal has the potential to alter soil characteristics. Sugarcane harvesting in the dry season should be preferred taking into account decomposition rate since during the initial sugarcane growth, might have enough organic residue left on the soil to reduce the adverse climatic effects (surface selling by the impact of raindrops, high water evaporation, and soil thermal variations) on the soil environmental. Harvesting in the rainy season induced accelerated decomposition, especially due to higher soil moisture and temperature.

In Chapter 3, we used the DRIFT spectroscopy as a method to evaluate straw chemical changes over the straw decomposition process, and we compared this technique with the traditional wet chemical methodology. We concluded that the application of DRIFT analysis to follow decomposition dynamics was sensitive to detect straw chemical changes induced by the edaphoclimatic conditions, especially the soil texture, and by the time of decomposition. Furthermore, we found that the spectral bands specific for alicyclic and aromatic regions were correlated significantly with the cellulose, hemicelluloses and lignin data obtained by the traditional methodology used in Chapter 2, which is considered as a reference in studies of decomposition. This result indicated that DRIFT spectroscopy is an efficient alternative method to evaluate the chemical changes over the sugarcane straw decomposition dynamics.

In Chapter 4, we investigated how rates of sugarcane straw removal were associated with soil bacterial community changes over one year of decomposition. We concluded that rates

of straw removal had a lower effect on the soil bacterial community when compared to the straw quality. The time associated with the straw biochemical changes, especially the hemicellulose content, is the main regulator of the bacterial variation over the year. In this sense, is essential to take into account the straw composition for use changes in the microbial community as one of the factors to determine the rates of straw that can be removed in sugarcane areas.

Overall, our study showed that the straw removal for 2G-ethanol and bioelectricity production affects the straw decomposition dynamics in commercial sugarcane areas in Brazil. The decomposition process was driven by the soil bacterial community, which changes over the time. Until the first four months, the bacterial community structure was different from the bacterial community found in the last four months of the study. Through the decomposition process, the microorganisms modified the straw quality and, as a consequence, the soil bacterial community itself also showed a significant shift in their structure. In the same way, the straw quality changes induce modifications in the soil bacterial structure, e.g., is a complex and cyclic interaction factors associated with the decomposition dynamics (Fig. 1).

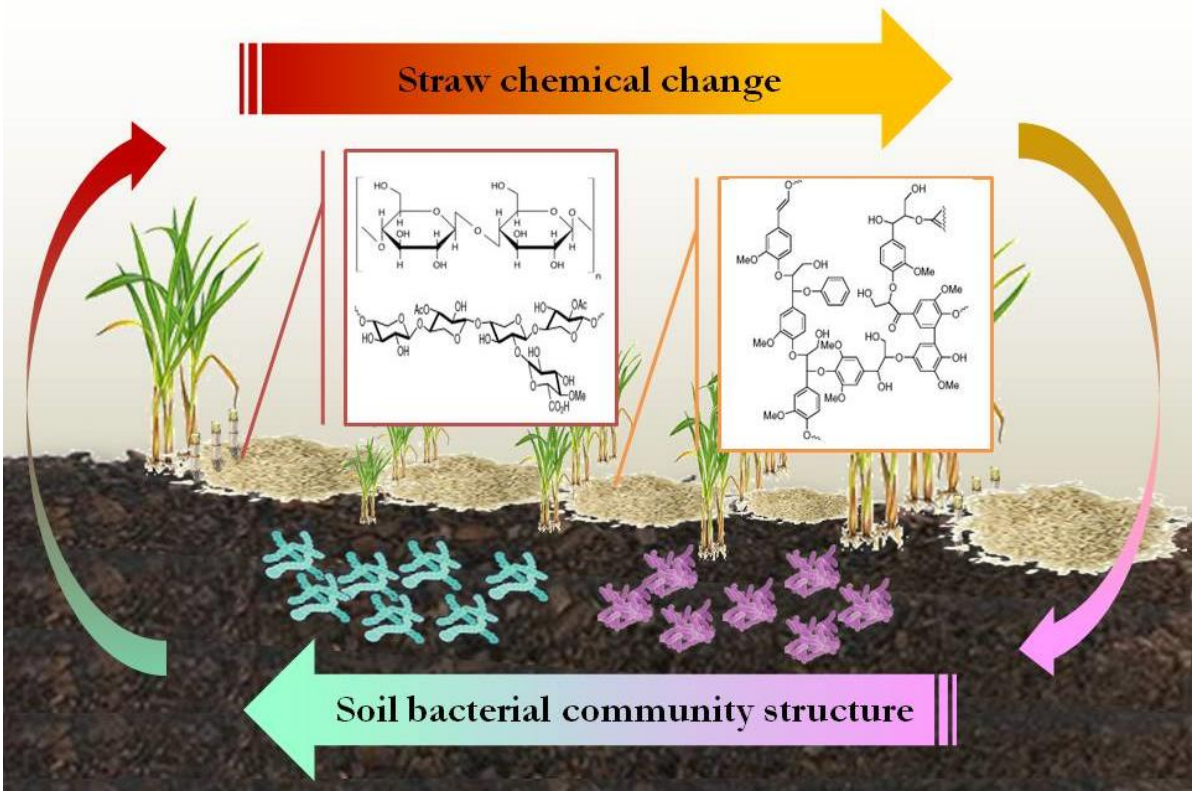

Figure 1. Schematic representation of the sugarcane straw decomposition dynamics according the results obtained in Chapter 2, 3, and 4.

The present study also had practical implications, which could be used to support decision-making for the sugarcane straw removal management. The straw removal affected the bacterial community, even subtly. Possibly, high straw removal levels affect the soil biological

quality, with implications in the straw decomposition process and consequently in the nutrient recycling, in the mitigation of greenhouse gases emissions, and in the soil organic matter formation. However, in all chapters, the local differences among the sites were showed. These variations were especially associated with soil attributes, such as soil texture, and climatic conditions, especially the rainfall, and are essential factors to determine when and how much sugarcane straw can be sustainable removed. In this sense, it seems incorrect to recommend general (regional) amounts of sugarcane straw that can be removed to the field without taking account the site-specific conditions.