



Division - Soil Processes and Properties | Commission - Soil Chemistry

# Combined legume and non-legume residues management improve soil organic matter on an Oxisol in Brazil

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**ABSTRACT:** Understanding soil organic matter (SOM) dynamics in production systems on tropical soils is necessary to guide strategies to increase SOM formation. This study aimed to evaluate soil carbon (C) dynamics by combining applications of different plant residues used on tropical soils. An incubation study was carried out with and without adding millet (*Pennisetum americanum*) residues combined with six crop residues (legumes and non-legumes); and one additional treatment with only millet residue. Higher C-CO<sub>2</sub> fluxes recorded in jack bean, sunflower and velvet bean residues were correlated with high soluble compound contents (49.5, 49.6 and 32.1 %, respectively). Adding millet residues resulted in positive PE for all residue combinations. Soils without millet, except jack bean, had a negative PE. Residues application promoted four times increase in C-POM content (from 1.04 to 4.2 g kg<sup>-1</sup> soil). The C-MAOM content had 2.4 times increase, being more expressive due to its high initial content (from 15.3 to 37.3 g kg<sup>-1</sup> soil). The comparison between the final C contents in the SOM fractions showed a significant increase of 8.8 times for MAOM in relation to POM, demonstrating the larger dimension of this C reservoir in the soil. Both combinations of legumes + non-legumes and non-legume + non-legume contributed significantly to the conversion of C to MAOM. The results give insight into possible management strategies for significant C increments in the more stable SOM fractions, depending on the residue type (quality) and residue combination.

**Keywords:** priming effect, C-CO<sub>2</sub> fluxes, C transfer, mix of residues, Oxisol.

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## INTRODUCTION

Currently, the high demand for food, fiber and bioenergy has required more sustainable production systems as an alternative to land-use change (Molotoks et al., 2021; Lap et al., 2022). Cover crops have been widely used as a strategy within more sustainable systems alternating crops of commercial interest, which can commonly reach two to three annual harvests in tropical regions (Momesso et al., 2021). Benefits of using cover crops include preventing soil erosion, promoting nutrient cycling, improving weed control, and influencing C stocks dynamics in the soil (Garcia-Franco et al., 2015; Momesso et al., 2021).

Decomposition and consequent transfer of carbon from plant residues (left on the surface or incorporated into the soil) to soil organic matter (SOM) is strongly influenced by N and organic compounds (mainly lignin, hemicellulose and cellulose) contents (Berg, 2014; Santos et al., 2014; Chaves et al., 2021). Consequently, the use of legume and non-legume plants as cover crops in succession or associated with other crops (Cocktails or Mix) may contribute differently to the formation and stabilization of SOM (Ghimire et al., 2019; Davi et al., 2022; Silva et al., 2022a) and should be considered in the definition of managements that aim to increase C stocks in soil (Bayer and Mielniczuk, 1997; Silva et al., 2022b). Additionally, climatic conditions in tropical regions strongly affect residue decomposition rate (Ventrella et al., 2016; Ni et al., 2021), which reinforces the importance of a management strategy that adds residues with distinct qualities to the soil to increase SOM stocks.

Because of the varying biochemical compositions of crop residues (C and N contents, C/N ratio, lignin, polyphenols, and soluble extractives content, etc.), different residues added to the soil will have different impacts on the C-CO<sub>2</sub> emissions from the decomposition of the residues or the native SOM, in addition to influencing the amount of C that will be stabilized or mineralized in the soil (Finzi et al., 2015; Ntonta et al., 2022). Legume and non-legume species produce biomass with different elemental compositions and biochemical characteristics, which can directly influence the decomposition process of crop residues (Berg, 2014) and the C-CO<sub>2</sub> emissions resulting from this process. Thus, not all plant material added to the soil will fully be converted into a more stable SOM fraction [e.g., mineral-associated organic matter - MAOM; Cambardella and Elliott (1992)].

Legume species tend to decompose faster because they have a lower C/N ratio when compared to C<sub>4</sub> species. When legume residues are associated with non-legume residues, biomass degradation increases due to the promotion of greater microbial diversity and N availability (Kohmann et al., 2019). Use efficiency of residue-derived C by soil microorganisms will depend on the quality of the residues, the different organic compounds, the availability of nutrients (mainly N) in the soil (Manzoni et al., 2018), and climatic conditions (Ventrella et al., 2016; Ni et al., 2021). In addition, intricate microbial relationships in the soil may trigger the loss of SOM by a process called priming effect (Kuzyakov et al., 2000; Liang et al., 2018; Zhang et al., 2022), which may cause changes in C stocks (Finzi et al., 2015; Ntonta et al., 2022).

Although there are studies indicating significant changes in SOM in production systems with cover crops (Laroca et al., 2018; Davi et al., 2022; Silva et al., 2022a), information on the effect of combining plant-derived residues (legumes and/or non-legumes) on the conversion efficiency of residue into more stable SOM fractions is still incipient for tropical soils. Understanding the dynamics of the decomposition rates and C transfer from different plant residues to SOM can aid in elaborating management plans aimed at more sustainable production systems, especially in tropical soils.

The hypotheses of our study were: i) the combination of legume + non-legume species will promote a reduction in the decomposition of native SOM, consequently reducing the priming effect; and ii) the combination of legume + non-legume species will contribute

more efficiently to the more stable fractions of SOM than the combination of non-legume + non-legume species. This study aimed to evaluate how the addition of a different combination of legume and non-legume residues used as cover crops in tropical soils can influence C-CO<sub>2</sub> fluxes, priming effect, and the conversion of C to SOM fractions.

## MATERIALS AND METHODS

### Incubation experiment

The experiment was carried out in an incubation room under controlled conditions (in the dark at 25 ± 1 °C). The soil was classified as *Latosolo Vermelho-Amarelo caulinitico distrófico* according to the Brazilian Soil Classification System (Santos et al., 2018), which corresponds to an Oxisol (Rhodic Hapludox, in the Soil Taxonomy) (Soil Survey Staff, 2014). This soil had approximately 15 years of *Brachiaria* cultivation (Table 1).

Soil samples were collected in the 0.00-0.20 m layer of unmanaged soil, i.e., no application of fertilizers nor soil corrections. In the laboratory, soil samples were air-dried in a shaded place. When fully dried, the samples were sieved through a 2 mm screen and homogenized before being transferred to the glass containers used for incubation. The soil had an isotopic signature of δ<sup>13</sup>C of -13.12 and -23.48 ‰, in the POM and MAOM fractions, respectively. This signature represents an intermediate value between the natural abundance of <sup>13</sup>C of plant residues with C<sub>3</sub> photosynthetic cycle (δ<sup>13</sup>C from -27.00 ‰ average) and plants with C<sub>4</sub> photosynthetic cycle (δ<sup>13</sup>C from -13.00 ‰ average) (Alves et al., 2005), allowing the evaluation of the contribution of the different residues to soil C.

The treatments were defined by a (2 × 6) + 1 factorial scheme: i) without and with the addition of residues of C<sub>4</sub> species - Millet (*Pennisetum americanum* (L)); and ii) six

**Table 1.** Chemical characterization and soil organic compounds in the studied soil

pH(H <sub>2</sub> O)	3.68
TOC <sup>(1)</sup> (g kg <sup>-1</sup> )	16.36
TN <sup>(2)</sup> (g kg <sup>-1</sup> )	1.54
P (mg dm <sup>-3</sup> )	1.21
K <sup>+</sup> (mg dm <sup>-3</sup> )	26.78
Ca <sup>2+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0.29
Mg <sup>2+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0.17
Al <sup>3+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	2.29
SB <sup>(3)</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0.54
Fe (mg dm <sup>-3</sup> )	226.87
Cu (mg dm <sup>-3</sup> )	0.29
Zn (mg dm <sup>-3</sup> )	1.07
Mn (mg dm <sup>-3</sup> )	16.6
TPS <sup>(4)</sup> (μg g <sup>-1</sup> )	70.57
TL <sup>(5)</sup> (mg g <sup>-1</sup> )	3.05
WSP <sup>(6)</sup> (mg kg <sup>-1</sup> )	23.31
Clay <sup>(7)</sup> (g kg <sup>-1</sup> )	695
Sand <sup>(7)</sup> (g kg <sup>-1</sup> )	292
Silt <sup>(7)</sup> (g kg <sup>-1</sup> )	13

<sup>(1)</sup> Total organic carbon and <sup>(2)</sup> total nitrogen were determined by the sum of C and N contents in POM and MAOM fractions, with the isotope-ratio mass spectrometer (IRMS). <sup>(3)</sup> Sum of bases. <sup>(4)</sup> Total polysaccharides (Lowe, 1993). <sup>(5)</sup> Total lipids of the soil were determined by the Soxhlet continuous extraction method, based on modifications of the methods used by Bull et al. (2000), Naafs et al. (2004) and Nierop et al. (2005). <sup>(6)</sup> Water-soluble phenols (Lowe, 1993). <sup>(7)</sup> For textural analysis, the pipette method was adopted.

crop residues of C<sub>3</sub> species – Brown hemp (*Crotalaria juncea*), Jack bean (*Canavalia ensiformis*), Sunflower (*Helianthus annuus*), Velvet bean (*Mucuna pruriens*), Eucalyptus (*Eucalyptus sp.*), a mix of Eucalyptus + Brown hemp; and an additional treatment with millet residue only, with destructive samples over times (0, 30, 90 and 152 days after incubation - DAI). The treatments were distributed in a randomized block design with four replicates.

The residues of legume species (brown hemp, jack bean, and velvet bean) and non-legume species (sunflower, millet, and eucalyptus) consisted of the aerial part of 60-d-old plants, except for eucalyptus residues. The eucalyptus residues came from a mix of aerial parts of 7-yr-old eucalyptus plants (13.57 % leaves, 12.32 % thin branches, and 74.10 % bark). Following collection, the material was dried in a forced air circulation oven at 65 °C. Then, the dried material underwent biochemical and chemical characterization.

Dry soil samples (70 g) were placed in glass containers (500 mL, with a threaded lid and a rubber stopper in the center). The water content of the soil samples was adjusted to 60 % of the field capacity. The crop residues were manually fragmented into 1 to 2 cm particles and distributed on a polyethylene screen (Ø = 2 mm opening) positioned on the soil surface inside the glass containers. The screen helped in disassembling the experimental units when removing plant residues for evaluations. In each experimental unit, 10 g of residue was added (equivalent to 19.89 Mg ha<sup>-1</sup> of residues), and they were moistened with 10 mL of water after being placed on the soil surface. For the treatments composed of single plant residues, a 10 g residue sample was used; for the treatments combined with the millet, 5 g of millet + 5 g of the other residue were used, with the only exception of the treatment with the mix, in which the proportion of residues of eucalyptus and brown hemp was 1:1. Detailed information about the proportions of the residues used in this study can be found in Silva et al. (2022a). Soil/residue mixture ratios were selected based on preliminary experiments and previous studies (Maluf et al., 2015a,b; Silva et al., 2022b). The containers were opened every two days for 15 minutes to favor gas exchange and avoid reducing O<sub>2</sub> concentration,

### Collection and determination of soil C-CO<sub>2</sub> fluxes

The C-CO<sub>2</sub> fluxes were measured by sampling the atmosphere in the glass containers using syringes (60 mL) with regulating valves at the tip. Air samples were collected at 7, 14, 21, 28, 56, 88, and 128 DAI. At the time of collection, sampling was performed just after the containers were closed, filling four syringes at accumulated times of 0, 1, 2, and 3 hours. After collection, C-CO<sub>2</sub> concentrations and δ<sup>13</sup>C-CO<sub>2</sub> isotopic composition of the gas samples were determined by Cavity Ring-Down Resonant Spectroscopy – CRDS (G2131-i, Picarro, Sunnyvale, CA), which uses, as a standard, the atmospheric CO<sub>2</sub> concentration (410 ppm) and CO<sub>2</sub> isotopic signature of δ<sup>13</sup>C (-8 ‰).

The calculation of the C-CO<sub>2</sub> fluxes was performed according to equation 1:

$$C - CO_2 \text{ fluxes: } [(\Delta Q/\Delta t) \times M \times P \times V] / (R \times T \times A) \quad \text{Eq. 1}$$

in which: C-CO<sub>2</sub> fluxes is the total surface flux of C-CO<sub>2</sub> (mg h<sup>-1</sup> m<sup>-2</sup>); ΔQ/Δt: the slope of the fitted line (mg g<sup>-1</sup>) by t (min); M: the molar mass of C (g mol<sup>-1</sup>); P: pressure inside the chamber, assuming 1 atmosphere (atm); V: chamber volume (L); R: universal gas constant (0.08205 L atm K<sup>-1</sup> mol<sup>-1</sup>); T: air temperature (K); A: basal area of pots (m<sup>2</sup>).

The method of “Keeling plot” (Keeling, 1958) was used to determine the δ<sup>13</sup>C-CO<sub>2</sub> of C-CO<sub>2</sub> fluxes, using a linear regression model fitted to the relationship between the inverse of CO<sub>2</sub> concentrations (x-axis), during the sampled ranges, and δ<sup>13</sup>C-CO<sub>2</sub> of the C-CO<sub>2</sub> concentrations (y-axis).

Then, the fractionation of C-CO<sub>2</sub> fluxes at each sample was performed according to equation 2, proposed by Vitorello et al. (1989):

$$f = \frac{\delta^{13}\text{C-CO}_2\text{ Treat} - \delta^{13}\text{C-CO}_2\text{ Contr}}{\delta^{13}\text{C-CO}_2\text{ Res} - \delta^{13}\text{C-CO}_2\text{ Contr}} \quad \text{Eq. 2}$$

in which:  $f$  represents the proportion of C derived from the residues;  $\delta^{13}\text{C-CO}_2\text{Treat}$  is the isotopic C-CO<sub>2</sub> ratio of the treatments in which the residues were applied;  $\delta^{13}\text{C-CO}_2\text{Contr}$  is the isotopic ratio of the control treatment, without the addition of residues;  $\delta^{13}\text{C-CO}_2\text{Res}$  is the average isotopic ratio of the residues used in the experiment.

The residue-derived C-CO<sub>2</sub> fluxes (C-CO<sub>2res</sub>) and soil-derived C-CO<sub>2</sub> fluxes (C-CO<sub>2soil</sub>) for the total fluxes released from the soil were estimated by equations 3 and 4.

$$C - \text{CO}_2\text{ res} = f \times C - \text{CO}_2\text{ Total} \quad \text{Eq. 3}$$

$$C - \text{CO}_2\text{ soil} = C - \text{CO}_2\text{ Total} - C - \text{CO}_2\text{ res} \quad \text{Eq. 4}$$

The effect of residue addition on the decomposition of native soil organic matter (*Priming effect - PE*) was measured by equation 5 (Blagodatsky et al., 2010):

$$P (\%) = \frac{\text{C-CO}_2\text{ Soil Treat} - \text{C-CO}_2\text{ Soil Contr}}{\text{C-CO}_2\text{ Soil Contr}} \times 100 \quad \text{Eq. 5}$$

in which: C-CO<sub>2 Soil Treat</sub> is the soil-derived C-CO<sub>2</sub> fluxes of the treatments to which the residues were applied; C-CO<sub>2 Soil Contr</sub> is the residue-derived C-CO<sub>2</sub> fluxes of the control treatment, without the addition of residues.

For both C-CO<sub>2</sub> fluxes and PE results, cumulative values were determined after 128 days of incubation of the residues, determining the residue- and soil-derived C-CO<sub>2</sub> fluxes accumulated at the end of the experimental period. The accumulated PE was determined based on accumulated soil-derived C-CO<sub>2</sub> data. Accumulated CO<sub>2</sub> fluxes were determined following equation 6 (Cai et al., 2012):

$$\text{Accumulated C - CO}_2\text{ fluxes} = \sum_{i=1}^n (F_i + F_{i+1})/2 \times (t_{i+1} + t_i) \times 24 \quad \text{Eq. 6}$$

in which:  $F$  represents the C-CO<sub>2</sub> fluxes,  $i$  is the  $i$ th measurement, the term of  $(t_{i+1} - t_i)$  is the interval days between one collection and another, and  $n$  is the total measurement times.

### C and $\delta^{13}\text{C}$ in soil organic matter (SOM) fractions

To evaluate the contribution of the residues to SOM fractions and the residue's efficiency in transferring C to the soil, physical particle-size fractionation of SOM into particulate organic matter - POM and mineral-associated organic matter - MAOM (Cambardella and Elliott, 1992) was performed at 0 and 152 DAI. The fractionation consisted of dispersing soil particles and organic matter by adding 30 mL of a chemical dispersant (sodium hexametaphosphate - 5 g L<sup>-1</sup>) and stirring the mixture for 15 h at 120 rpm. Then, the samples were sieved through a 53  $\mu\text{m}$  mesh where the coarser fraction (sand) was retained on the sieve, and the finer fraction (<53  $\mu\text{m}$ ), associated with the more reactive soil fractions (silt + clay), was collected in the solution that passed through the sieve. Both fractions were collected in plastic cups and dried in a forced air circulation oven at 60 °C.

After drying, the fractions were weighed and macerated for further analysis. Carbon and N contents, and the <sup>13</sup>C/<sup>12</sup>C ratio (expressed as  $\delta^{13}\text{C}$  ‰ values based on Pee Dee Belemnite - PDB standard) were quantified on a C and N elemental analyzer coupled to an isotope ratio mass spectrometer - IRMS (ANCA GSL 20-20, Sercon, Crewe, UK)

with an accuracy of 0.04 ‰. The organic C associated with the material retained in the 53- $\mu\text{m}$  sieve corresponds to POM-C, and that which passed through the sieve, that is, associated with the silt + clay mineral fraction, corresponds to MAOM-C. Thus, using the  $\delta^{13}\text{C}$  present in the POM and MAOM samples, it was possible to perform the fractionation of residue- and soil-derived C in the fractions, according to equations 2 to 4.

### Determination of total polysaccharides and water-soluble phenols in soil

Total polysaccharides (TPS) and water-soluble phenols (WSP) contents in soil (Lowe, 1993) were determined at each sampling time (0, 30, 90 and 152 days). In the initial soil characterization, total lipid (TL) contents were also quantified (Bull et al., 2000; Naafs et al., 2004; Nierop et al., 2005).

### Statistical analyses

Data of total residue- and soil-derived C-CO<sub>2</sub> fluxes, priming effect, TPS, WSP, C-POM and C-MAOM derived from soil and residues were subjected to the Shapiro-Wilk normality test ( $p = 0.05$ ) and Levene's homoscedasticity test ( $p = 0.05$ ), using the software Statistica 12.0 (Stat soft Inc., Tulsa, USA). When the assumptions of parametric statistics were not met, the variables were transformed by Box-Cox, using the software PAST version 3.19 (Hammer et al., 2001).

The TPS and WSP data were tested by analysis of variance (ANOVA) considering each evaluation time, while residue- and soil-derived C-POM and C-MAOM were tested using measurement taken only at 152 DAI. The accumulated data of total, residue- and soil-derived C-CO<sub>2</sub>, as well as priming effect, were also tested by analysis of variance. Means of TPS, WSP, C-POM and C-MAOM derived from residues and soil, total accumulated residue- and soil-derived C-CO<sub>2</sub>, and priming effect were compared between residues, and the addition or not of millet was tested by the Scott-Knott test ( $p = 0.05$ ) using Sisvar 5.3 software (Ferreira, 2011).

Multivariate analysis between residue biochemical characteristics (acetone-soluble extractives - ASE, soluble lignin - SL, insoluble lignin - IL, and holocellulose - HC) and SOM- associated variables (total C-CO<sub>2</sub> fluxes, C-CO<sub>2</sub>T; residue-derived C-CO<sub>2</sub> fluxes, C-CO<sub>2</sub>R; soil-derived C-CO<sub>2</sub> fluxes, C-CO<sub>2</sub>S; priming effect, PE; the contribution of residues and soil to C in the POM and MAOM fractions: CPOM.R, CMAOM.R, CPOM.S, CMAOM.S, respectively; and soil TPS and WSP contents) were performed to explore general trends in biochemical residue composition and to assess which major SOM-associated variables could be grouped or separated according to plant groups. Principal Component Analysis (PCA) was performed using the stats package. Spearman correlations were also performed between the biochemical characterization variables of the residues and the other variables associated with SOM. For Spearman's correlation, the corrplot package was applied, where probability values of 5 % or less ( $p \leq 0.05$ ) were considered statistically significant based on Student's "t" test. These analyses were carried out using R statistical software (R Development Core Team, 2019).

## RESULTS

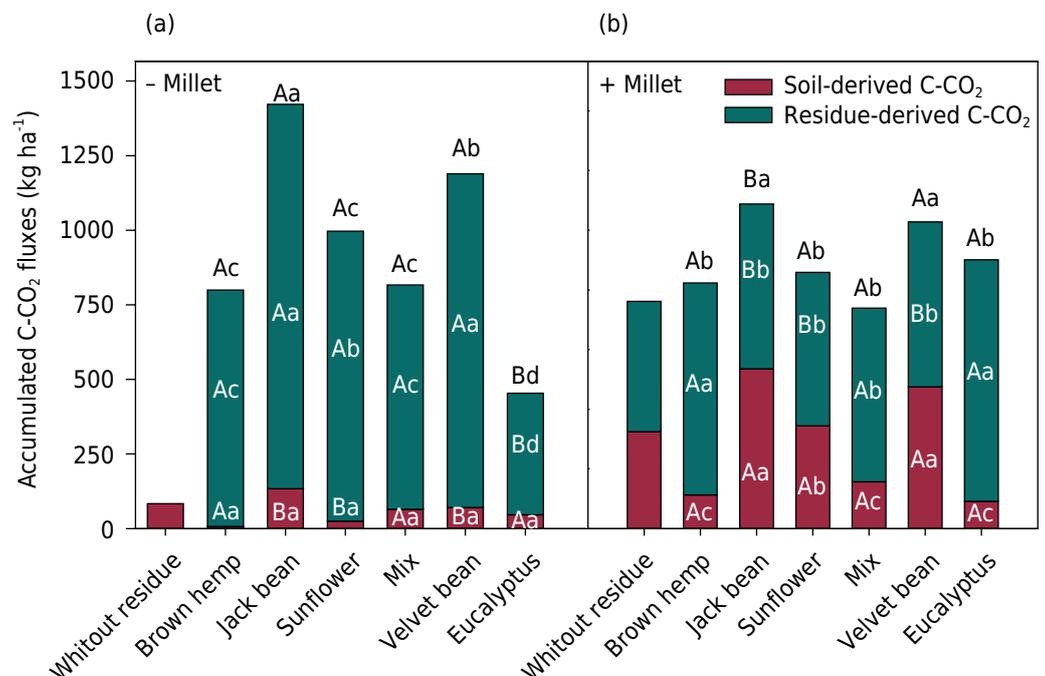
### Accumulated C-CO<sub>2</sub> fluxes and Priming effect (PE)

Data analysis showed a significant interaction between the factors studied (Figure 1). In evaluating the addition or not of millet (first factor) for the total accumulated C-CO<sub>2</sub> fluxes, only the treatment with jack bean residue differed, where the highest average was observed when not combined with millet residue (1,421 kg C-CO<sub>2</sub> ha<sup>-1</sup>; Figure 1a). The ratio between the highest and lowest total C-CO<sub>2</sub> fluxes showed a 2.6 times difference between these observations with the lowest flux being observed in the treatment with eucalyptus residue only (453.21 kg C-CO<sub>2</sub> ha<sup>-1</sup>; Figure 1a). When evaluating the first

factor for soil-derived C-CO<sub>2</sub> fluxes, an 81 times increase was observed between fluxes in treatments without added millet to those combined with it (from 6.61 kg C-CO<sub>2</sub> ha<sup>-1</sup> in brown hemp-only residue to 535.45 kg C-CO<sub>2</sub> ha<sup>-1</sup> in jack bean residue combined with millet). The treatments with jack bean, velvet bean and sunflower residues showed higher values ( $p < 0.05$ ) when combined with millet (535, 474 and 343 kg C-CO<sub>2</sub> ha<sup>-1</sup>, respectively; Figure 1b). As for the residue-derived C-CO<sub>2</sub> fluxes, the treatments with jack bean, velvet bean and sunflower residue had the highest fluxes for the residues alone (1,287; 1,119 and 971 kg C-CO<sub>2</sub> ha<sup>-1</sup>, respectively; Figure 1a), and eucalyptus with higher averages when combined with millet (810 kg C-CO<sub>2</sub> ha<sup>-1</sup>; Figure 1b).

In studying the second factor (residues) within the levels of the first factor (addition or not of millet), greater differences were observed among treatments. For the total cumulative C-CO<sub>2</sub> flux without adding millet (Figure 1a), the treatment with only jack bean residues had the highest total flux. Conversely, the treatment with only eucalyptus residues showed the lowest total flux. The soil-derived C-CO<sub>2</sub> fluxes for the residues without millet did not differ ( $p < 0.05$ ). The residues-derived C-CO<sub>2</sub> fluxes without adding millet had a pattern similar to that of total C-CO<sub>2</sub> flux. However, jack bean and velvet bean residues, which had higher average fluxes, were similar. Despite having low soil-derived C-CO<sub>2</sub> emissions, the brown hemp residue treatment, similar to the eucalyptus and mix treatments, did not significantly contribute to residues-derived C-CO<sub>2</sub> fluxes.

As for the addition of millet for total C-CO<sub>2</sub> fluxes (Figure 1b), jack bean and velvet bean residues exhibited the highest fluxes ( $p < 0.05$ ). Similar to the pattern observed for total fluxes, jack bean and velvet bean residues showed the highest soil-derived C-CO<sub>2</sub> fluxes, which differed from the other treatments. For the residues-derived C-CO<sub>2</sub> fluxes, brown hemp and eucalyptus had the highest values (711 and 810 kg C-CO<sub>2</sub> ha<sup>-1</sup>, respectively; Figure 1b), contrasting with that observed for soil-derived C-CO<sub>2</sub> fluxes in these same treatments that showed the lowest contributions.

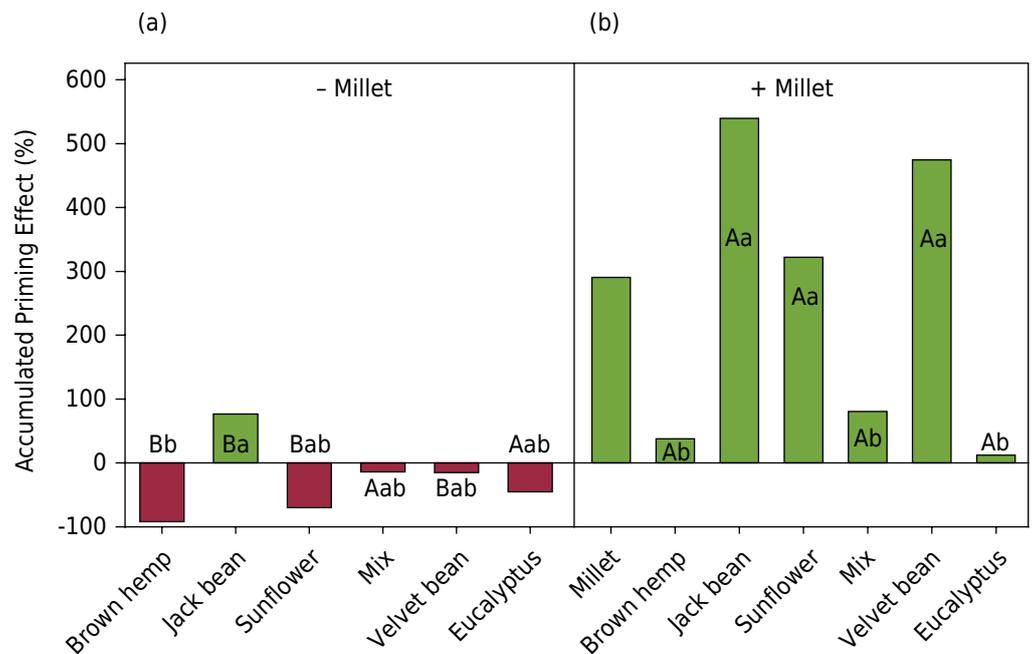


**Figure 1.** Accumulated C-CO<sub>2</sub> fluxes, Soil-derived C-CO<sub>2</sub> fluxes (brown bars) and Residue-derived C-CO<sub>2</sub> fluxes (green bars) (a) without and (b) with the addition of millet residues and different crop residues of brown hemp, jack bean, sunflower, mix (eucalyptus + brown hemp), velvet bean and eucalyptus after 128 days of incubation. Uppercase letters represent the effect of adding or not adding millet, and lowercase letters represent the comparative effect among residues within the millet factor by Scott-Knott test ( $p < 0.05$ ).

Accumulated soil-derived C-CO<sub>2</sub> fluxes resulted in distinct PEs among the treatments with and without millet, with a variation of 631.79 % between the most negative PE (-92.08 % in the treatment with brown hemp residue only) and the most positive one (539.72 % in the treatment with jack bean residue combined with millet). Analyzing the addition or not of millet, only the treatments with mix and eucalyptus residues showed significantly similar accumulated PE values. The other residues differed ( $p < 0.05$ ) when compared to the addition or not of millet. The addition of millet residues resulted in a scatter plot with a pattern showing a possible decomposition of soil organic matter native (Figure 2b), where all treatments presented positive PE values with higher averages observed for the treatments with jack bean, velvet bean and sunflower residues (539, 474 and 321 %). For the treatments without millet, most of them had negative PE (Figure 2a), except for the treatment with jack bean residue, which showed an average positive PE value that was significantly similar to the other treatments, even those having negative PE, except for the treatment with brown hemp residue. This pattern indicates a possible degradation of organic material in the soil, which may increase the contribution of C to SOM.

### Contents of total polysaccharides (TPS) and water-soluble phenols (WSP) in soil

In summary, there was a wide variation in total polysaccharide (TPS) content throughout the evaluation times (Table 2), with contents ranging from 70.56 to 307.31  $\mu\text{g g}^{-1}$  of soil. For the treatments without adding millet, the average TPS content increased to 2.8 times the initial content in the treatment with brown hemp residue. With the addition of millet, the increase was up to 3 times more in the brown hemp and eucalyptus treatments (comparing between 0 and 152 days). However, adding millet to jack bean and velvet bean residues resulted in the highest average TPS contents in the soil at 90 DAI, with increases up to 3.2 and 4.4 times, respectively, followed by decreases at the last evaluation time.



**Figure 2.** Accumulated Priming Effect (APE; %) in soil (a) without and (b) with the addition of millet residue and different crop residues of brown hemp, jack bean, sunflower, mix (eucalyptus + brown hemp), velvet bean and eucalyptus after 128 days of incubation. Blue bars represent positive APE, while red bars represent negative APE. Data were Box-cox transformed ( $y+1$ ),  $\lambda = 0.4$ . Uppercase letters represent the effect of adding or not adding millet, and lowercase letters represent the comparative effect among residues within the millet factor by Scott-Knott test ( $p < 0.05$ ).

**Table 2.** Total polysaccharides (TPS) content in soil for the different times of decomposition (0, 30, 90 and 152 days) of the residues

Residues (-millet)	Time (days)				Mean
	0	30	90	152	
Polysaccharides					
μg g <sup>-1</sup>					
Without residue	70.56 Aa	144.51 Aa	181.65 Aa	221.15 Aa	154.47
Brown hemp	70.56 Aa	167.07 Aa	165.95 Aa	200.79 Aa	151.09
Jack bean	70.56 Aa	161.00 Aa	164.94 Ba	178.41 Ab	143.73
Sunflower	70.56 Aa	151.31 Aa	161.13 Aa	176.14 Ab	139.78
Mix	70.56 Aa	160.37 Aa	165.13 Aa	177.47 Ab	143.38
Velvet bean	70.56 Aa	157.22 Aa	169.12 Ba	173.07 Ab	142.49
Eucalyptus	70.56 Aa	157.79 Aa	175.60 Aa	171.21 Bb	143.79
Mean	70.56	157.04	169.07	185.46	
Residues (+Millet)					
Millet	70.56 Aa	169.96 Aa	162.10 Ac	185.80 Aa	147.11
Brown hemp	70.56 Aa	145.07 Aa	158.60 Ac	210.58 Aa	146.20
Jack bean	70.56 Aa	166.95 Aa	226.59 Ab	182.27 Aa	161.59
Sunflower	70.56 Aa	148.68 Aa	152.56 Ac	193.78 Aa	141.39
Mix	70.56 Aa	116.86 Ba	104.60 Bd	201.52 Aa	123.38
Velvet bean	70.56 Aa	136.39 Aa	307.31 Aa	181.82 Aa	174.02
Eucalyptus	70.56 Aa	139.42 Aa	161.20 Ac	210.73 Aa	145.48
Mean	70.56	146.19	181.28	195.21	

Uppercase letters represent the effect of adding or not adding millet, and lowercase letters represent the comparative effect between residues within the millet factor by Scott-Knott test ( $p < 0.05$ ).

At 30 DAI, there was a difference only in the treatment with mixed residues, in which the highest mean was only in the mixture without millet ( $160.37 \mu\text{g g}^{-1}$ ). There was no difference between the residues when evaluated separately with or without millet ( $p < 0.05$ ).

At 90 DAI, the differences were higher among the factors. Adding millet to velvet bean and jack bean residues resulted in the highest average contents ( $307.31$  and  $226.59 \mu\text{g g}^{-1}$  of soil, respectively) compared with their respective treatments without adding millet. The treatments with residue mixes also differed from those with and without millet, with the highest average content observed when only one residue was present, but without millet ( $165.13 \mu\text{g g}^{-1}$  of soil). Assessing the treatments with and without millet separately revealed that the treatments with residues alone (without the addition of millet) did not differ from each other ( $p < 0.05$ ). However, the treatment with velvet bean residue combined with millet showed higher average TPS content, which differed from the other residues. The mix, in turn, presented the lowest average content when combined with millet.

At the last evaluation time (152 days), only the treatment with eucalyptus residue differed between the addition or not of millet, with the highest average observed when combined with millet ( $210.73 \mu\text{g g}^{-1}$  of soil). Evaluating the differences between single residue treatments without millet, the control treatment (without residue) was statistically similar to brown hemp residue ( $p < 0.05$ ), with higher mean contents ( $221.15$  and  $200.79 \mu\text{g g}^{-1}$  of soil), than those recorded in the other treatments without millet. For treatments with millet residue, although mean contents varied between  $181.82$  and  $210.73 \mu\text{g g}^{-1}$  of soil, the means did not differ from each other.

There was a wide variation in the water-soluble phenols (WSP) contents over the evaluation times (Table 3). The contents ranged from  $23.32$  to  $438.89 \text{ mg kg}^{-1}$  soil. There was a

**Table 3.** Water-soluble phenols (WSP) content in the soil at different decomposition times (0; 30, 90 and 152 days) of the residues

Residues (-Millet)	Time (days)				Mean
	0	30	90	152	
<b>Water-soluble phenols</b>					
mg kg <sup>-1</sup>					
Control	23.32 Aa	106.55 Ab	186.60 Ac	58.58 Bc	93.76
Brown hemp	23.32 Aa	78.40 Ab	157.77 Ad	60.24 Bc	79.93
Jack bean	23.32 Aa	173.91 Aa	222.01 Bb	203.63 Bb	155.72
Sunflower	23.32 Aa	172.28 Aa	438.89 Aa	281.46 Ba	228.99
Mix	23.32 Aa	74.86 Ab	129.58 Ad	51.51 Bc	69.82
Velvet bean	23.32 Aa	158.46 Aa	136.97 Bd	31.69 Bc	87.61
Eucalyptus	23.32 Aa	80.65 Ab	104.12 Be	44.12 Ac	63.05
Mean	23.32	120.73	196.56	104.46	
<b>Residues (+Millet)</b>					
Control	23.32 Aa	84.30 Ab	194.17 Ab	150.41 Ac	113.05
Brown hemp	23.32 Aa	70.48 Ab	176.75 Ab	96.01 Ad	91.59
Jack bean	23.32 Aa	107.22 Ba	324.56 Aa	276.51 Ab	182.90
Sunflower	23.32 Aa	120.03 Ba	306.67 Ba	317.70 Aa	191.93
Mix	23.32 Aa	74.19 Ab	136.97 Ac	102.61 Ad	84.27
Velvet bean	23.32 Aa	117.96 Ba	206.31 Ab	283.74 Ab	157.83
Eucalyptus	23.32 Aa	61.04 Ab	162.43 Ac	73.68 Ad	81.12
Mean	23.32	90.75	215.41	185.81	

Uppercase letters represent the effect of adding or not adding millet, and lowercase letters represent the comparative effect between residues within the millet factor by Scott-Knott test ( $p < 0.05$ ).

considerable increase in mean WSP content of up to 18.8 times the initial content for the treatments without millet. By adding millet, the increase was up to 13.6 times. The increments were most expressive in the treatments with sunflower residue alone and sunflower combined with millet. For most treatments, the pattern revealed that WSP contents in the soil peaked at 90 DAI, except for the soil under sunflower and velvet bean residues combined with millet, where these showed a pattern of continuous increase in WSP contents in the soil until the last evaluation day.

At 30 DAI, the soils under jack bean, sunflower, and velvet bean residues showed significant difference ( $p < 0.05$ ) with and without the addition of millet, with the highest mean WSP contents observed in the soils with only one residue, without millet (173.91, 172.28 and 158.46 mg kg<sup>-1</sup> soil, respectively). The differences among residues followed the same pattern where treatments with jack bean, sunflower, and velvet bean were similar and differed from the other residues in the single residue treatments as well as those combined with millet.

At 90 DAI, WSP contents in the soils under jack bean and velvet bean residue showed an opposite pattern to that observed at 30 DAI, where they had higher mean WSP contents when combined with millet, as well as eucalyptus. The soils of the treatments under only sunflower residues maintained the highest levels when millet was not present. In studying the residues within the first factor, the treatment with sunflower residues maintained the same pattern at all evaluation times, always showing the highest means, both alone and combined with millet, differing from the other residues.

At the last evaluation time, the higher average WSP contents were observed in almost all treatments with added millet, differing from treatments without added millet, except

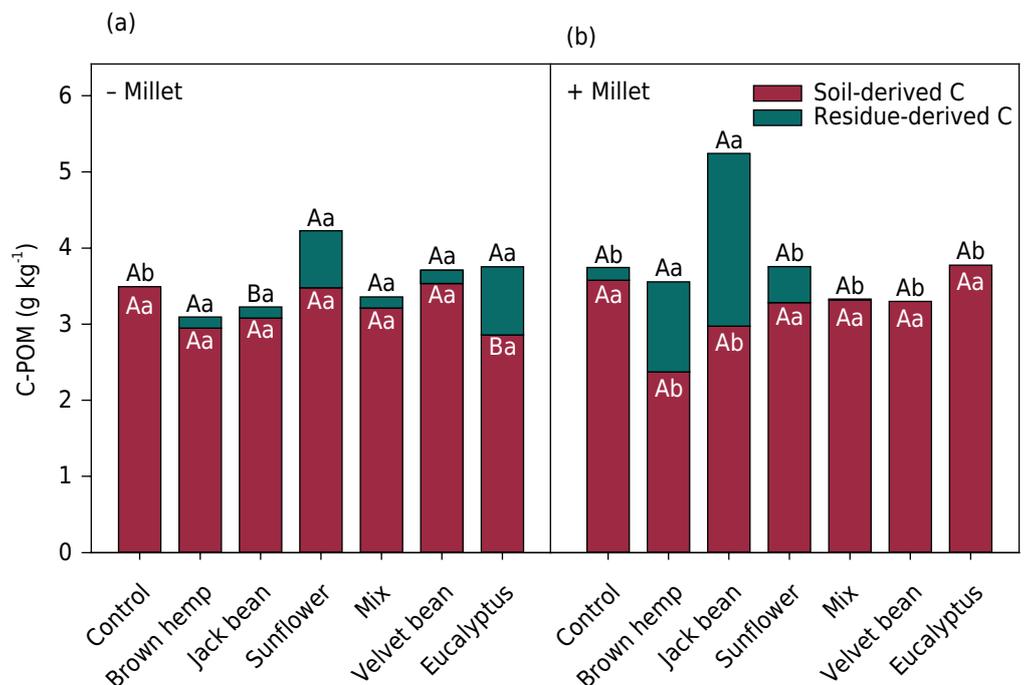
for the treatment with eucalyptus residue that showed no significant difference between the addition or not of millet.

### Transfer of C from residues to POM and MAOM fractions

In summary, the contributions of residue- and soil-derived C were higher in MAOM than in POM, with a proportional tenfold increase. In POM fraction, the contribution of residue-derived C was from 0.01 to 2.30 g kg<sup>-1</sup>. Soil-derived C showed values ranging from 2.30 to 3.70 g kg<sup>-1</sup>. The residue-derived C in the MAOM fraction ranged from 2.15 to 22.50 g kg<sup>-1</sup>. For soil-derived C, the values ranged from 13.80 to 37.40 g kg<sup>-1</sup>.

There was a significant interaction between the factors studied (millet versus residue) for soil- and residue-derived C in POM and MAOM fractions. In comparing the effect of adding or not millet on residue-derived C in the POM fraction (Figures 3a and 3b), a difference ( $p < 0.05$ ) was observed only for the treatment with jack bean residue, with higher contribution when combined with millet. The variation between these was 2.12 g kg<sup>-1</sup>, corresponding to 15 times increase when combined with millet. In the treatments without millet (Figure 3a), there was no difference between the residues ( $p < 0.05$ ). For the treatments with added millet (Figure 3b), the highest mean contributions ( $p < 0.05$ ) were observed in brown hemp and jack bean (1.2 and 2.3 g kg<sup>-1</sup>, respectively).

As for the soil-derived C in the POM fraction (Figures 3a and 3b), only in the treatment with eucalyptus residue was a difference ( $p < 0.05$ ) observed between the addition or not of millet. The highest contribution was observed when eucalyptus was combined with millet (3.7 g kg<sup>-1</sup>), with 1.3 times increase compared to the treatment without added millet. In the treatments without the addition of millet, there was no significant difference between residues ( $p < 0.05$ ) for soil-derived C (Figure 3a). For the treatments with added millet (Figure 3b), brown hemp and jack bean showed the lowest contributions ( $p < 0.05$ ) to soil-derived C for the POM fraction.



**Figure 3.** Carbon (g kg<sup>-1</sup>) contribution to particulate organic matter fraction (C-POM) derived from soil (brown bar) and residues (green bar) in treatments without millet (a) and with millet (b) and with the addition of brown hemp, jack bean, sunflower, mix (brown hemp + eucalyptus), velvet bean and eucalyptus residue at the last evaluation time at 152 days. Uppercase letters represent the effect of adding or not adding millet, and lowercase letters represent the comparative effect among residues within the millet factor by Scott-Knott test ( $p < 0.05$ ).

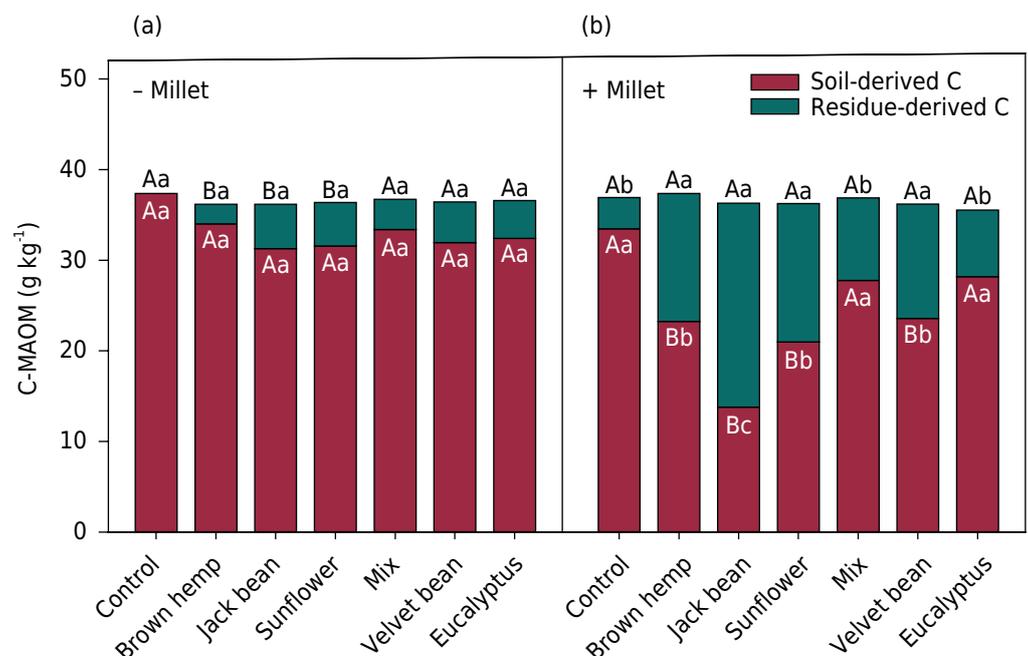
The residue-derived C was more present in the MAOM fraction (Figures 4a and 4b) by showing significant differences ( $p < 0.05$ ) between jack bean, sunflower, and brown hemp residues. The higher contributions occurred when the residue was combined with millet (22.5, 15.3, and 14.3 g kg<sup>-1</sup> soil, respectively). Comparing the residues without added millet (Figure 4a), there was no difference ( $p < 0.05$ ) among the residues for C contribution to MAOM fraction. Among the treatments combined with millet (Figure 4b), jack bean, sunflower, brown hemp, and velvet bean showed a similar contribution of C to MAOM ( $p < 0.05$ ).

The treatments that showed the highest contributions of residue-derived C to the MAOM fraction (jack bean, sunflower, brown hemp, and velvet bean) had the lowest contributions of soil-derived C when combined with millet (Figure 4b). Among treatments without added millet (Figure 4a), there was no difference ( $p < 0.05$ ) for soil-derived C contribution.

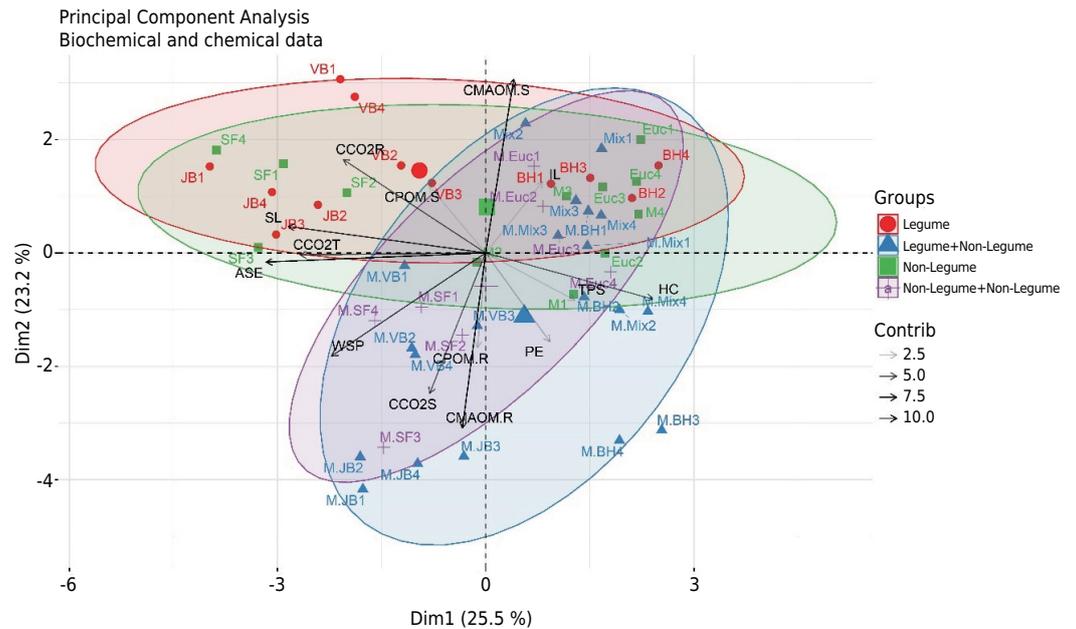
### Multivariate analysis

Principal components analysis considered the first two dimensions, which have a cumulative Eigenvalue of 48.7 % (Figure 5). The results obtained regarding the pattern of variables and their groupings were complementary to the results already described in the previous topics, presenting some important relationships between variables and treatments.

In analyzing the large groupings represented by the ellipses, the isolated residues (legume and non-legume) showed similar grouping patterns in the PCA as a function of the same variables. The same pattern was observed for the combinations (legume + non-legume and non-legume + non-legume). Jack bean (JB) and sunflower (SF) residues formed a cluster with ASE and SL. This clustering reflected the behavior of the most intense decomposition of these residues and their contributions to the SOM fractions, since high ASE and SL contents are important indicators for residue decomposition. In addition to this clustering, the variables associated with rapid decomposition and



**Figure 4.** Carbon (g kg<sup>-1</sup>) contribution to mineral-associated organic matter fraction (C-MAOM) derived from soil (brown bar) and residues (green bar) in treatments without millet (a) and with millet (b) and with the addition of brown hemp, jack bean, sunflower, mix (brown hemp + eucalyptus), velvet bean and eucalyptus residue at the last evaluation time at 152 days. Uppercase letters represent the effect of adding or not adding millet, and lowercase letters represent the comparative effect among residues within the millet factor by Scott-Knott test ( $p < 0.05$ ).



**Figure 5.** Principal component analysis (PCA) of the quality parameters of the residues (ASE, acetone-soluble extractives; SL, soluble lignin; IL, insoluble lignin; HC, holocellulose) grouped into principal components, through linear combinations of these with the other variables (CCO<sub>2</sub>T, total cumulative C-CO<sub>2</sub> fluxes; CCO<sub>2</sub>R, residue-derived C-CO<sub>2</sub> fluxes; CCO<sub>2</sub>S, soil-derived C-CO<sub>2</sub> fluxes; PE, priming effect; CPOM. R, the contribution of residues to C-POM; CMAOM. R, the contribution of residues to C-MAOM; CPOM. S, the contribution of soil to C-POM; CMAOM. S, the contribution of soil to C-MAOM; TPS, total polysaccharides contents; WSP, water-soluble phenols contents). The projection shows that most variations in the data are explained by PC1 (25.5 %) and PC2 (23.2 %).

lability of JB and SF residues were associated with cumulative C-CO<sub>2</sub>T fluxes, with these coming mainly from the residue-derived fluxes (C-CO<sub>2</sub>R).

The PE showed an opposite pattern to that of C-CO<sub>2</sub>R. In the treatments where the highest positive PE values were observed, they also resulted in the lowest C-CO<sub>2</sub>R emissions, with higher contributions of C-CO<sub>2</sub>S to C-CO<sub>2</sub>T. These treatments were represented mainly by the combined residues. In turn, the clusters related to the combined residues showed a significant contribution of the variable CMAOM. R, proving the higher contribution of the residues to the MAOM fraction.

The IL and HC variables, which are indicative of residue recalcitrance, showed higher values associated with treatments with residues more resistant to decomposition (Eucalyptus, Brown hemp, Mix, Millet and their combinations). This resistance to the decomposition of these residues could be seen due to the low fluxes of residue-derived C-CO<sub>2</sub> and the low contributions of residue-derived C to the SOM fractions.

In addition to the principal component analysis (PCA), Spearman correlation analysis was also performed to assess the veracity of the correlations between the studied variables. The correlation between ASE and SL (0.78) was positive, indicating an association between these more labile compounds. The C-CO<sub>2</sub>T fluxes also showed a high positive correlation with C-CO<sub>2</sub>R (0.72), suggesting a higher contribution to the total fluxes from the residues rather than those from the soil, which was also justified by the high correlation between C-CO<sub>2</sub>T fluxes with ASE (0.57). The ASE still showed a significant positive correlation with WSP contents (0.62), indicating a possible contribution of extractive compounds to WSP contents in the soil. Negative correlations were less expressive, with the most significant one between CMAOM. S and CMAOM. R contents (-0.99), indicating antagonism between the contributions from residues and soil to the most stable WSP fraction. Another significant negative correlation was between ASE and HC contents (-0.69), indicating the opposite behavior between these compounds

where ASE tends to be more labile in the residue decomposition process and HC tends to be more recalcitrant.

## DISCUSSION

The process of decomposition of residues can be divided into three stages (rapid or intensive - initial decomposition, intermediate decomposition or reduced, and slow or stabilized decomposition), which depend on the residue's chemical components (soluble compounds, cellulose, hemicellulose, and lignin-like compounds) (Hadas et al., 2004; Shahbaz et al., 2017).

In our study, the partitioned C-CO<sub>2</sub> fluxes allowed us to assess the decomposition rate of residues (residue-derived C-CO<sub>2</sub>) or the native SOM (soil-derived C-CO<sub>2</sub>). The highest initial residue-derived fluxes of C-CO<sub>2</sub> were observed in jack bean, sunflower and velvet bean alone without millet input. These residues showed the highest contents of soluble compounds (49.47, 32.09 and 49.56 %, respectively), with a correlation between their biochemical composition and a higher initial decomposition rate (Silva et al., 2022b) and consequent emission of residue-derived C-CO<sub>2</sub>. In the early stages of decomposition, soluble compounds are the first ones to be metabolized (Baumann et al., 2009; Majumder and Kuzyakov, 2010; Clemente et al., 2013). However, there was a significant reduction in residue-derived C-CO<sub>2</sub> fluxes after 14 days, a period similar to that reported by other authors who found exponential reductions in the decomposition of the residues after 20 days (Loss et al., 2012; Andrade et al., 2015; Maluf et al., 2015a).

The derived-soil C-CO<sub>2</sub> fluxes with the addition of millet residues indicate a possible decomposition of the native SOM already at seven days after the addition of the residues. Millet residues may require more energy for decomposition due to their higher contents of insoluble lignin and holocellulose than others organic compounds, which are easier to be decomposed by soil microorganisms and can be provided by some native SOM fractions (Kuzyakov, 2010).

The addition of residues combined or not with millet showed a reduction pattern of PE over incubation time. A similar pattern was observed by Qiu et al. (2016), which identified a positive PE when corn residues were added to the soil, with subsequent decreases down to negative PE values over time. Plant residue quality is an important point regarding the PE of SOM because the residue quality represents the availability of labile compounds or the abundance of complex ones (Bertrand et al., 2006; Wang et al., 2015; Schmatz et al., 2017). Despite the decrease over time, cumulative values revealed a large positive PE in the treatments with millet addition, indicating higher degradation of native SOM. As for the single residues, the pattern of cumulative data indicates higher microbial activity under the residues favoring decomposition and possible faster contributions to the SOM fractions. Active soil microorganisms tend to shift their use of substrate to fresh organic materials (more labile, with higher N availability) containing readily used energy from the C, as microorganism uses less energy to degrade fresh organic materials than more recalcitrant materials (Saar et al., 2016).

Large oscillations were observed in TPS and WSP contents in the soil (Tables 2 and 3). The TPS are easily hydrolyzable carbohydrates originating from soil microorganisms, cover crop exudates, and present on large portions of crop residues (Liu et al., 2005). This explains the significant increase in TPS contents in the soil over the incubation period, attaining significant increases when residues were combined with millet (Table 3). In contrast, Jolivet et al. (2006) evaluated the conversion of forest into corn production fields and observed a rapid and considerable decrease in soil TPS content, revealing the importance of the initial biochemical composition of the residues for the availability of the compounds in the soil. In the present study, the combination of legume and non-legume residues favored a greater biochemical diversity and higher increments of TPS in the

soil. These TPS increments may also favor greater soil stability and contributions to more stable SOM, since polysaccharides are good binding agents in the soil, contributing to the formation of more stable aggregates (Liu et al., 2005).

Under natural conditions, TPS tends to be easily degraded by soil microorganisms because of its high lability (Brandão, 2009). Accordingly, TPS contents are more easily influenced by soil management when compared to the more recalcitrant fractions of SOM (Piccolo et al., 1996). Therefore, the availability of these compounds in the soil promotes a substrate favorable to microbial development (Pavinato and Rosolem, 2008), so a continuous supply of organic residues (from plants or animals) is necessary for agricultural soils as organic residues are easily degradable and leached by rainwater or irrigation (Martins, 2008).

On the other hand, the contributions of WSP to the soil by sunflower residues (alone or in combination with millet) were significant, which might be explained by the higher presence of this compound in sunflower residues (Table 3; Ye et al., 2015).

The release of WSP from plant residues may be associated with leaching mechanisms, with phenols being the last substances to be released, according to the following sequence:  $Ca > P > Mg > N > K > \text{polyphenols}$  (Gama-Rodrigues et al., 2007). However, these authors state that, if the plant residue has high C contents, it tends to have low concentrations of soluble polyphenols and, consequently, lower release rates of these compounds during decomposition. Under the conditions of the present study, no factors favored the release of these compounds from the plant materials added to the soil (irrigation is required for a greater transfer of these compounds to the soil); as a result, the concentrations of these compounds are attributed to the degradation of plant materials by microorganisms.

Our results opposed our hypothesis, which states that adding mixed residues (legume + non-legume) and millet to the soil would promote a reduction in decomposition rates of native SOM, consequently reducing the priming effect. However, the addition of millet led to a trend of higher PE, indicating a higher recalcitrance of this material, initially promoting a positive PE, as well as a positive cumulative PE for all treatments with millet (Figure 2b). Without the addition of millet, cumulative PE (Figure 2) and PE measured over time were generally negative, indicating higher residues degradation and less access of microorganisms to native soil C.

Initial C-CO<sub>2</sub> fluxes derived from the single residues indicate a higher susceptibility of the legume residues (jack bean and velvet bean) to microbial degradation. In this initial phase, more labile and easily degradable compounds are released, contributing to the mineralization of this C or incorporation into SOM fractions. However, when legumes and non-legumes are associated, because they have diverse biochemical and chemical compositions, the C-CO<sub>2</sub> fluxes tend to reduce after this initial phase of decomposition. When analyzing the cumulative data of C-CO<sub>2</sub> fluxes, individual residues tend to emit more C-CO<sub>2</sub> to the atmosphere than when combined with millet, where soil-derived C-CO<sub>2</sub> emissions are intensified, corroborating a positive PE.

Combinations of legume and non-legume cover crops favor the reduction of C-CO<sub>2</sub> fluxes to the atmosphere and the initial degradation of native SOM; however, such combinations result in a gradual transfer of organic compounds to the soil during the decomposition period of the residues. Therefore, it is necessary to observe these variables in the field to understand the dynamics of residue- and soil-derived C-CO<sub>2</sub> fluxes throughout the decomposition process, in addition to the changes occurring in the native SOM, which represents the long-term SOM store.

The maintenance of organic C in the soil is governed by the balance between C input (via plants) and C output (via microbial decomposition of SOM) (Jastrow et al., 2007). The main sources of C input to the soil are crop residues (recently incorporated or partially

decomposed) and labile C, e.g., through the rhizodeposition of growing crops (Chen et al., 2014; Datta et al., 2015).

The POM fraction is considered more sensitive than MAOM in response to soil management, being more responsive to variations in plant material input and decomposition rates promoted by soil tillage practices (Bayer et al., 2002; Salton et al., 2011). However, in our study under incubation experiment conditions of 152 days, the MAOM fraction proved more responsive to residues input, and this is possible due to more suitable conditions for microbial activity (temperature and humidity), which may have favored faster decomposition of particulate materials and consequently a greater C transfer effect to the MAOM fraction. Chaudhary et al. (2014), when studied incubated corn residues labeled with  $^{13}\text{C}$  observed a higher contribution of C to the MAOM fraction (approximately 76 % of TOC), similar to what was observed in our study.

Contributions of residue-derived C were higher in MAOM fraction when combined with millet, especially in the treatments with jack bean ( $22.53 \text{ g kg}^{-1}$ ), sunflower ( $15.28 \text{ g kg}^{-1}$ ), brown hemp ( $14.13 \text{ g kg}^{-1}$ ), and velvet bean ( $12.66 \text{ g kg}^{-1}$ ) (Figure 4b). This may be explained by the redistribution of more labile compounds from SOM to the more stabilized fraction (Chaudhary et al., 2014), since the treatments that obtained the highest contributions had high contents of soluble compounds and lower contents of lignified compounds.

In the POM fraction, significant contributions were observed among jack bean, brown hemp, and sunflower residues combined with millet (respectively  $1.88$ ,  $1.18$ ,  $1.52 \text{ g kg}^{-1}$ ) (Figure 3b). The presence of C-POM is desirable to ensure soil biological activity and to favor the flux of C to the more stable fractions (C-MAOM). The absence of enough labile organic compounds to meet the microbial demands will cause a stimulus to C oxidation processes associated with more stable fractions, resulting in losses of SOM stocks (Causarano et al., 2008; Salton et al., 2011).

Contrary to our second hypothesis, in which the addition of mixed residues (legumes + non-legumes), either by cover crops succession or in association (mix), would promote significant increases in terms of C content in the most stable organic matter fractions (MAOM), the treatment with millet residue combined with sunflower (non-legume + non-legume) showed a similar pattern as the combination of millet and jack bean (legume + non-legume) treatment, indicating that not only the legume + non-legume combination favors considerable C increments, but also the non-legume + non-legume combination (for these specific plant residues).

The high contribution of the treatment with millet residue combined with sunflower, which was similar to the combination of millet with jack bean, is directly related to the quality of the residue. Sunflower and jack bean residues showed close contents of ASE, HC, C, N, and higher content of SL. The relationships between these variables were essential for the decomposition of these residues and the significant increases in the C-MAOM fraction from both mixes (Maluf et al., 2015b; Silva et al., 2022a).

Our study shows evidence that a management strategy that gives priority not only to the combination of legume and non-legume cover crops but also non-legume + non-legume cover crops favors a higher conversion efficiency of C in the mineral-associated fraction (C-MAOM). However, further studies under field conditions should be carried out to observe long-term effects of plant residue addition in crop successions and to investigate the effects of soil tillage, since the incorporation or permanence of residues on the surface may result in different decomposition dynamics from those observed in the incubation experiment. Furthermore, residue input to the soil influences the dynamics of the existing microbial communities associated with the decomposition rates of the residues, so it is also important to investigate the soil microorganisms involved in the decomposition process.

## CONCLUSIONS

Biochemical diversity of crop residues added to the soil has the potential to improve nutrient cycling and the stocks of C and N, as well as changing the dynamics of microbial communities. The initial C-CO<sub>2</sub> fluxes derived from jack bean, velvet bean and sunflower residues indicate a higher susceptibility of these residues to microbial degradation, contributing more labile compounds to the SOM fractions. However, when associated in mix, the C-CO<sub>2</sub> fluxes tend to be lower for having diversified biochemical and chemical compositions, suggesting a lower microbial activity on these residues, and consequent slower decomposition.

Adding mixed residues (legume + non-legume or non-legume + non-legume) to the soil through cover crops succession or in association (Mix) promotes significant increases in the C content of the most stable soil organic matter fraction. These increases can be faster, through a possible redistribution of more labile compounds from SOM to the more stable fraction at the early stage of decomposition, or slower, depending on the biochemical composition of the residues.

Addition of millet combined with legume residues (jack bean and velvet bean) and with non-legume (sunflower) favors a reduction in residue-derived C-CO<sub>2</sub> flux and a high priming effect (positive PE) on native SOM; nonetheless, it results in a significant C contribution to the MAOM fraction.

Brown hemp, despite being a legume, showed different decomposition patterns when compared to the other legumes, indicating greater resistance to decomposition due to the high C/N ratio, high HC content, and low ASE content. Sunflower, even as a non-legume, was shown to be more degradable than brown hemp due to its lower C/N ratio and higher ASE and SL contents.

Management strategies that prioritize the combination of legumes and non-legumes cover crops favor a higher efficiency of C conversion to the MAOM fraction. The combination of jack bean and velvet bean with the addition of millet, as well as the sunflower crop showed similar contributions to the most stable SOM fraction, being good options for SOM increments.

## APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at [https://www.rbcjournal.org/wp-content/uploads/articles\\_xml/1806-9657-rbcs-46-e0220077/1806-9657-rbcs-46-e0220077-suppl01.pdf](https://www.rbcjournal.org/wp-content/uploads/articles_xml/1806-9657-rbcs-46-e0220077/1806-9657-rbcs-46-e0220077-suppl01.pdf)

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