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Different composition of plant residues as a driver of microbial community structure and soil organic matter composition: A microcosm study

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ABSTRACT

Soil organic matter (SOM) is the main pathway of carbon (C) input to the soil with the decomposition of shoot residues, roots and their exudates. The objective was to evaluate the contribution of different vegetal composition and plant parts of Caatinga species and the effects of introducing a grass in the soil microbial community structure and biochemical composition of SOM. A trial was conducted under controlled conditions (120 days) using, separately, the shoot and roots residues of native species from the herbaceous (HERB) and shrub-arboreal (ARB) strata and a grass (GRASS). *Megathyrsus maximum*, which is native from Africa, but well adapted to the semi-arid conditions of Brazil, was used. Combinations of these species in different proportions were also evaluated: (i) 55 % shrub and trees + 45 % grass (MIX1) and (ii) 75 % shrub and trees + 25 % grass (MIX2). At the end of incubation, soil samples were collected to evaluate the microbial community structure through the phospholipid fatty acids (PLFA). Physical fractioning of SOM into particulate organic matter (POM) and mineral-associated organic matter (MAOM) was also performed, followed by biochemical characterization of these fractions by thermochemolysis analysis. The ARB shoot residue resulted in a 181.5 % increase ($p < 0.05$) in total PLFA biomass in the soil. A significant increase ($p < 0.05$) in the abundance of fungi and bacteria was observed with the incorporation of shoot residues. MAOM was characterized by a higher abundance of aliphatic (31.6 ± 5.0 %) and nitrogen-bearing compounds (21.0 ± 2.0 %), while higher lignin derivatives were observed in POM (18.0 ± 0.6 %). The ground cover provided a diversity of compounds in the SOM, thus regulating the structure of the microbial community. These results highlight the importance of conserving biodiversity, both in natural ecosystems and in agroecosystems in the semi-arid environment.

1. Introduction

Soil organic matter (SOM) is one of the largest terrestrial C reservoirs, having an important role in the global C cycle (Hatten and Liles, 2019). Therefore, the conversion of areas of natural vegetation into intensive cultivation, causes serious environmental problems, including the loss of SOM (Maciel et al., 2020), land degradation and contributes to climate changes. While conventional agricultural practices contribute to food security, they frequently result in the uncontrolled use of farm inputs, increased greenhouse gas emissions, and degradation of natural resources (Lal, 2016).

It is crucial to embrace sustainable soil management techniques, such as improving soil organic matter content in agricultural areas, to enhance soil fertility and productivity while simultaneously reducing greenhouse gas emissions (Kumara et al., 2023). In this regard, sustainable agricultural management practices, such as integrated nutrient management, organic amendment, no-tillage, crop rotation, residue retention, intercropping, biochar, and agroforestry systems are the most recognized for increasing C (Kumara et al., 2023).

Despite the recognized benefits of agroforestry systems, the impacts of introducing exotic grasses on the structure of microbial communities and on soil organic matter are still unknown. Exotic grasses can be

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cultivated in silvopastoral systems to enhance native pastures and increase pasture biomass production. These practices are implemented in livestock farming in certain areas of the Brazilian semi-arid region to improve forage biomass (Araújo Filho et al., 2002). The biomass of herbaceous forage species in the native Caatinga is typically 400 kg ha^{-1}, although this amount can vary significantly due to rainfall fluctuations (Araújo Filho, 2013). Introducing grasses into the system can substantially increase forage biomass, potentially reaching up to 3600 kg ha^{-1} (Pereira Filho et al., 2013).

Diversifying systems with different plant covers and sources of C, as well as introducing residues with different chemical compositions, can be efficient strategies for sequestering C in the soil, especially in regions that are more susceptible to climate change, such as the semi-arid region. To effectively address the challenges of global change by SOM stocks, a comprehensive understanding of SOM formation, persistence, and function is crucial (Lavallee et al., 2020). POM generally consists of lightweight, relatively undecomposed fragments (Lavallee et al., 2020). As a result, this SOM fraction is considered accessible to microbes, relatively unprotected by the soil mineral matrix, and characterized by fast turnover rates (Witzgall et al., 2021). MAOM comprises single molecules or microscopic fragments of organic material that have either leached directly from plant material or undergone chemical transformation by soil biota (Lavallee et al., 2020). POM and MAOM provide important insights into SOM distribution and its responses to environmental changes (Witzgall et al., 2021). They also reveal differences in sources such as plant and/or microbial residues, degrees of physico-chemical protection (Lavallee et al., 2020), and litter quality (Lyu et al., 2023). Lyu et al. (2023) found that both high- and low-quality litter are crucial for SOM formation throughout the entire process of litter decomposition. Therefore, plant species diversity with a large range of C, N, amino sugar and/or lignin content can alter the litter input quality, decomposition rates, and SOM formation. A recent study indicates that plant lignin components (vanillyl, syringyl, and cinnamyl), and microbial necromass play an important role in the formation of POM and MAOM, respectively (Chen et al., 2024).

Thus, the objective was to evaluate the contribution of different vegetal composition and plant parts of Caatinga species (herbaceous and arboreous) and the effects of introducing an exotic grass in the soil microbial community structure and biochemical composition of SOM. It is hypothesized that the mixtures of exotic grass and native vegetation species of Caatinga will promote changes in the structure of the microbial communities and the biochemical composition of the SOM.

2. Materials and methods

2.1. Study area and soil and plant sampling

Samples of a Luvisol (IUSS Working Group WRB, 2015) were collected in a pasture area cultivated for more than 10 years with *Megathyrsus maximum* cv. Mombaça, used for experiments with dairy goats (3°40'58.42"S, 40°16'50.5"W and 79 m a.s.l), located in the municipality of Sobral, state of Ceará, Brazil (Fig. 1). The region is dry, very hot and has a semi-arid tropical equatorial climate, classified as BSh (Alvares et al., 2013), with the last 20 years average temperature of 28 °C and annual rainfall of 825.0 mm concentrated between January and June.

The soil was collected in the 0–5 cm layer, which is considered to have the highest microbial activity (Vargas and Scholles, 2000) and is

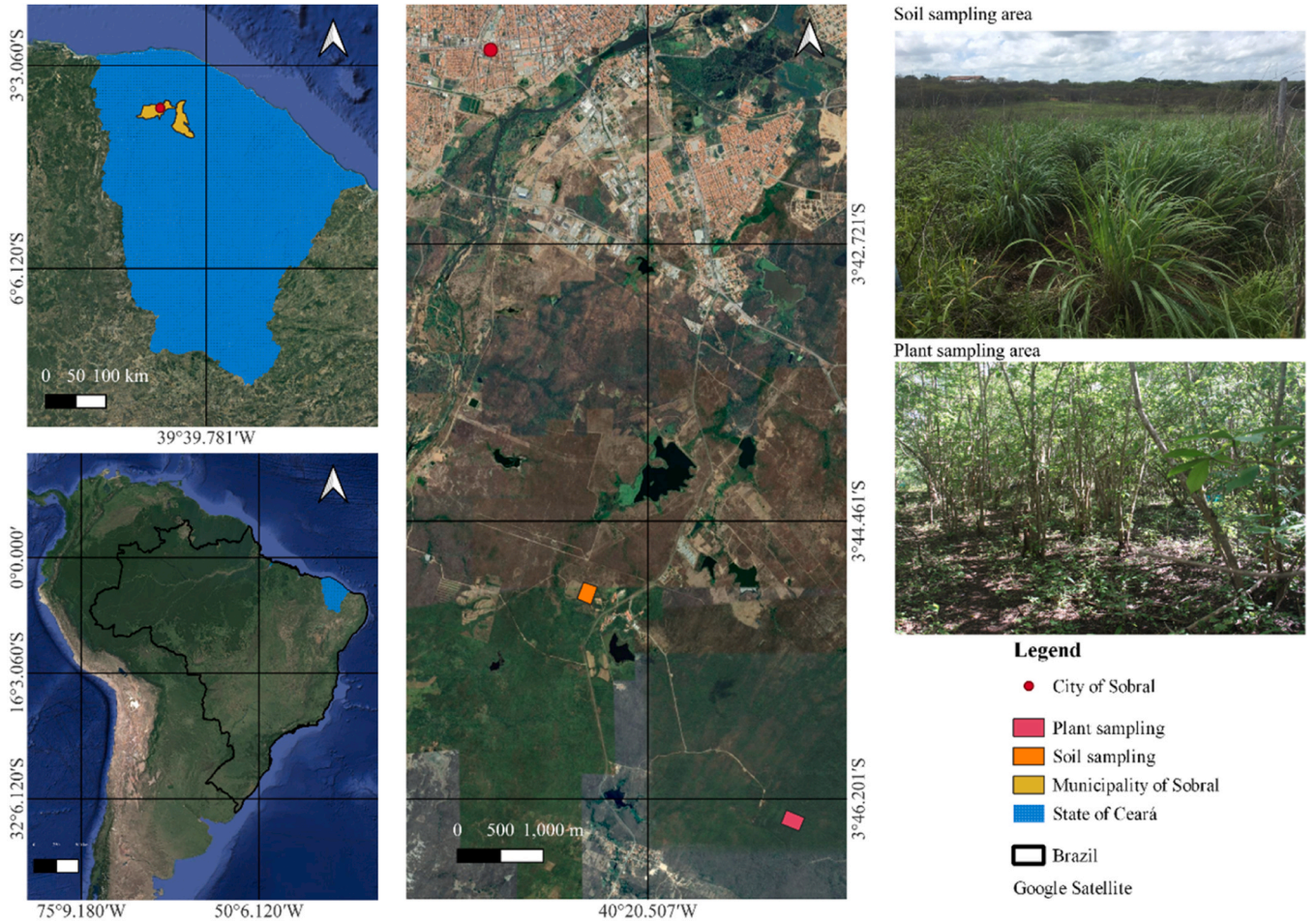

Fig. 1. Location of the study area in the Brazilian Agricultural Research Corporation (Embrapa goats and sheep), Sobral, Ceará, Brazil.

more sensitive to management (Miranda et al., 2020). The samples were air-dried, sieved on a 2-mm mesh, and stored for later use.

Nine native species were collected from the shrub-arboreal and herbaceous strata selected in the silvopastoral system at the Brazilian Agricultural Research Corporation (Embrapa goats and sheep) (3°45'53"S, 40°20'03"W and 100 m a.s.l.). According to a floristic and phytosociological survey carried out in the collection area (Araújo, 2015), the selection of the shrub-arboreal and herbaceous strata was made considering the criteria of greatest density and frequency of species. The selected species of the herbaceous stratum were: *Centrosema sp.*, *Senna obtusifolia* (L.) H. S. Irwin & Barneby, *Alternanthera brasiliana* (L.) Kuntze, and *Hyptis suaveolens* (L.) Poit; and for the shrub-arboreal stratum: *Mimosa tenuiflora* (Willd.) Poir.; *Cordia oncocalyx* Allemão.; *Cenostigma pyramidale* (Tul.) Gagnon & G.P.Lewis.; *Mimosa caesalpiniifolia* Benth.; and *Croton sonderianus* Müll.Arg. In addition to those species, samples from an exotic grass called *Megathyrsus maximum* Jacq. cv. Massai was collected, cultivated in the same area, and technically recommended by the Embrapa Goats and Sheep for the semiarid region for pastoral use, to increase the carrying capacity of the areas and the forage supply in the dry season (Cavalcante et al., 2014).

Samples of leaves in full development from shrubs and trees and the aboveground parts (leaves + branches) from herbaceous species, as well as roots up to 2 mm, referred to in a simplified way in this study as shoot and roots, were collected in March, during the rainy season in the region. For the collection of the roots from the shrub-arboreal species, excavation was done in a transect perpendicular to the tree trunk, until the depth of 20 cm, and then right after collecting the roots. The herbaceous species were manually removed from the soil, also collecting the root. The collected samples from the shoot and the roots were washed, and oven-dried at 60 °C, milled in a knife mill, with a 2-mm sieve, and stored for the experiment.

2.2. Incubation experiment

An incubation trial was conducted in laboratory under controlled temperature conditions (25 ± 1 °C) using a B.O.D. (Biological Oxygen Demand) incubator. We used 50 g of soil, kept at a humidity equivalent to 80 % of the field capacity, in glass vials (0.590 L) with a screw cap containing a central septum. All the plant material used in the study was cut to 2 mm and incorporated into the soil. These materials, used in the treatments, are described in Table 1. To the incorporation of the plant materials into the soil, the proportion of 1 % of C was used, and, to compose the sample to be added, the C contents determined individually in each part of the plant and species were considered proportionally (Table 1 – Supplementary material).

The contents of C and N of the different treatments were analyzed using an elemental analyzer (Flash EA 2000, Thermo Fisher Scientific, Bremen, Germany) and are presented in Table 2. The biochemical characterization of the plant mixtures was analyzed by thermochemolysis (Table 3).

The treatments were disposed of in randomized block design in a factorial scheme (5 * 2) + 1 with 4 repetitions totaling 44 experimental units. The first factor was composed of the grass (GRASS), the groups of herbaceous species (HERB) and trees (ARB), and two combinations in different proportions (MIX1 and MIX2), and the second factor represented by the plant parts: shoot and roots. An additional treatment, composed of soil without any addition of plant material was also used. The plant material was mixed in the moist soil and incubated for 120 days and after that, the sub-samples were collected for analysis of phospholipid fatty acids (PLFA), macronutrient contents and for the physical fractioning of SOM, separating it from the particulate organic matter (POM) and organic matter associated to the minerals (MAOM).

2.3. Physical fractioning of SOM

The physical fractioning of SOM was done according to (Cambardella and Elliott, 1992), in which 10 g of a 2-mm mesh sieved soil were dispersed in 30 mL sodium hexametaphosphate solution (5 g L^{-1}) in a horizontal shaker for 15 h. After the agitation, the suspension was sifted through in a 53-μm sieve and afterward washed several times with deionized water. From the material retained on the sieve, the POM fraction was obtained, and whatever passed through constituted the MAOM fraction. Both fractions were taken to a greenhouse at 60 ºC in disposable cups until the complete evaporation of water. After the drying process, the material of each treatment was weighed, macerated in an agate mortar, and passed through a 100-mesh sieve. Next, the contents of C and N of each POM and MAOM fraction were determined in an elemental analyzer (Flash EA 2000, Thermo Fisher Scientific, Bremen, Germany).

Table 1
Description of the treatments used in the soil incubation test with the shoot and roots of herbaceous, shrub-arboreal and grass species.

Treatments	Description
Soil (S)	Soil (< 2 mm)
Grass shoot ($GRASS_{pa}$)	Soil + shoot of *M. maximum*.
Herbacious species shoot ($HERB_{pa}$)	Soil + shoot of *Centrosema*, *S. obtusifolia*, *A. brasiliana* and *H. suaveolens*.
Shoot of shrub and tree species (ARB_{pa})	Soil + shoot of *M. tenuiflora*, *C. oncocalyx*, *C. pyramidale*, *M. caesalpiniifolia* and *C. sonderianus*.
Combination of the shoot of herbaceous, shrubby-arboreal and grassy species ($MIX1_{pa}$)	Soil + shoot of plants in the proportion of 45 % of grass + 27.5 % of herbaceous stratum + 27.5 % of shrubby-arboreal stratum species
Combination of the shoot of herbaceous, shrubby-arboreal and grassy species ($MIX2_{pa}$)	Soil + shoot of plants in the proportion of 25 % of grass + 37.5 % of herbaceous stratum + 37.5 % of shrubby-arboreal stratum
Grass roots ($GRASS_r$)	Soil + roots of *M. maximum*.
Roots of herbaceous species ($HERB_r$)	Soil + roots of *Centrosema*, *S. obtusifolia*, *A. brasiliana* and *H. suaveolens*.
Roots of shrubby-arboreal species (ARB_r)	Soil + roots of *M. tenuiflora*, *C. oncocalyx*, *C. pyramidale*, *M. caesalpiniifolia* and *C. sonderianus*.
Combination of the roots of herbaceous, shrubby-arboreal and grass species ($MIX1_r$)	Soil + roots of plants in the proportion of 45 % of grass + 27.5 % of herbaceous stratum species + 27.5 % of shrubby-arboreal stratum
Combination of the roots of herbaceous, shrubby-arboreal and grass species ($MIX2_r$)	Soil + roots of plants in the proportion of 25 % of grass + 37.5 % herbaceous stratum species + 37.5 % of shrubby-arboreal stratum species

2.4. Analysis of biochemical composition of POM and MAOM fractions

The biochemical composition was evaluated by the thermochemolysis technique in samples of POM and MAOM, according to the methodology adapted by Hatcher et al. (1995). For the analysis 200 mg of POM and 100 mg of MAOM were weighed and then placed directly in glass vials, where 200 μL of tetramethylammonium hydroxide solution (TMAH - 25 % in methanol) was added, followed by evaporation under N^2 flow. The vials were sealed after the complete evaporation of the solvent and, later they were taken to a muffle furnace at 300 °C for 30 min. After cooling, 1 mL of dichloromethane was added to the vials to extract the compounds, and then shaken for 10 s on a Vortex-type shaker. Using a glass syringe and needle, the solution was removed from the vial and transferred to a 10 mL test tube using a PTFE syringe filter. The extraction was repeated by adding 1 mL of dichloromethane to the vial containing the samples 2 more times.

After extraction, 50 μL of internal standard (methyl nonadecanoate - 19:0) was added to the tubes containing the dichloromethane extracts. The test tubes were dried again under N_2 flux at 38 °C and later on, the extracts were resuspended with 100 μL of dichloromethane (three times), and then transferred to GC-vials for chromatographic analysis (Section 2.6.).

Table 2
Total contents of C and N and ratios C:N and lignin:N from the shoot and roots of *M. maximum* (GRASS) and from groups of herbaceous plants (HERB), trees (ARB) and their combinations in different proportions (MIX1 and MIX2).

Plant groups	Plant components	C	N	P	K	Ca	Mg	C:N	Lignin:N
		—%—			g kg^{-1}				
GRASS	Shoot	40.1	1.2	1.01	18.7	2.6	2.93	33.2	11.9
	Root	34.0	1.1	0.55	3.5	1.3	1.41	31.0	28.8
HERB	Shoot	40.2	2.1	1.95	17.3	16.3	4.58	19.3	0.70
	Root	41.1	0.9	1.41	14.3	3.8	1.92	45.9	15.5
ARB	Shoot	43.5	2.8	1.68	17.8	15.6	3.77	15.5	9.70
	Root	44.6	1.5	0.85	5.7	11.9	1.23	42.3	21.2
MIX1	Shoot	41.3	2.0	1.53	18.6	9.8	3.61	21.0	14.5
	Root	41.2	1.1	0.78	6.2	4.5	1.25	38.5	24.1
MIX2	Shoot	41.8	2.2	1.6	16.4	11.6	3.7	19.3	5.10
	Root	41.9	1.0	1.02	8.4	6.4	1.52	44.3	16.6

Herbaceous species: *Centrosema*, *S. obtusifolia*, *A. brasiliana* and *H. suaveolens*. Tree species: *M. tenuiflora*, *C. oncocalyx*, *C. pyramidale*, M. caesalpiniifolia and *C. sonderianus*. MIX1 = proportion of 45 % of grass + 27.5 % of herbaceous stratum species + 27.5 % of tree stratum species; MIX2: proportion of 25 % of grass + 37.5 % of herbaceous stratum species + 37.5 % of tree stratum species.

Table 3
Relative abundance (%) of precursor groups from the organic compounds determined by thermochemolysis TMAH-GC/MS from the shoot and roots of *M. maximum* (GRASS) and from groups of herbaceous plants (HERB), trees (ARB) and their combinations in different proportions (MIX1 and MIX2).

Plants groups	Plant components	Lipids	Carbohydrate derivatives	Cutin derivatives	Suberin derivatives	Compounds N	Lignin derivatives
GRASS	Shoot	3.24	0.00	6.53	2.48	0.00	14.4
	Root	2.21	7.14	10.6	4.75	1.48	32.8
HERB	Shoot	2.93	0.57	5.34	2.17	0.00	1.54
	Root	2.27	28.5	9.83	4.18	0.00	13.9
ARB	Shoot	2.04	1.73	13.1	8.20	0.00	27.3
	Root	5.58	21.1	7.12	2.41	0.00	22.4
MIX1	Shoot	2.06	0.00	11.0	5.30	0.00	28.6
	Root	2.16	4.59	12.5	5.27	1.38	25.8
MIX2	Shoot	1.37	0.00	10.5	5.03	0.00	11.1
	Root	1.21	2.74	16.0	6.10	0.00	15.7

Herbaceous species: *Centrosema*, *S. obtusifolia*, *A. brasiliana H. suaveolens* . Tree species: *M. tenuiflora*, *C. oncocalyx*, *C. pyramidale*, *M. caesalpiniifolia C. sonderianus* . MIX1 = proportion of 45 % of grass + 27.5 % of herbaceous stratum species + 27.5 % of tree stratum species; MIX2: proportion of 25 % of grass + 37.5 % of herbaceous stratum species + 37.5 % of tree stratum species.

2.5. Determination of the phospholipids fatty acid

The structure of the soil microbial community was characterized by the extraction and analysis of the phospholipids fatty acids (PLFA), following the protocol of (Fernandes and Chaer, 2010), adapted from the Bligh-Dyer method (Bligh and Dyer, 1959). Three grams of freeze-dried soil were extracted with a chloroform/methanol/phosphate buffer solution (pH ~ 7.0) (1:2:0.8 v/v/v) (White et al., 1979). Next, a PLFA fraction was purified by chromatography using a solid-phase extraction column (SPE). The dry phospholipids were then converted into fatty acid methyl esters (FAMEs) by mild alkaline methylation; during this stage 80 μL methyl nonadecanoate (19:0Me) internal standard was added to the vials. After the methylation of the FAMEs were measured by gas chromatography and mass spectrometry (GCMS-QP 2010 plus-Shimadzu).

From the chromatogram peaks and their respective retention times 17 biomarkers were identified (PLFAs): i15:0, a15:0, i16:0, 16:1ω7, 16:1ω5, 16:0, 10Me16:0, i17:0, a17:0, cy17:0, 10Me17:0, 18:2ω6, 18:1ω9, 18:1ω7, 18:0, 10Me18:0 and cy19:0. Then, the biomarkers from the following functional groups were selected: gram-positive bacteria - G+ (i15:0, a15:0, i16:0, i17:0, a17:0) (Zelles, 1999), gram-negative bacteria - G- (16:1ω7, cy17:0, 18:1ω7, 18:0, cy19:0) (Zelles, 1999), actinobacteria (10Me16:0, 10Me17:0, 10Me18:0) (Kaiser et al., 2010), fungi (18:1ω9, 18:2ω6) (Olsson et al., 2003; Zelles, 1999) and arbuscular mycorrhizal fungi - AMF (16:0, 16:1ω5) (Olsson et al., 2003). The concentration of all the identified PLFAs (nmol g^{-1} of soil) was determined using an internal standard (C19:0). The total PLFA biomass was determined by the sum of the peaks of all the PLFAs of each chromatogram and expressed in nmol g^{-1} of soil.

2.6. Gas chromatography coupled to mass spectrometry

The analysis was conducted in gas chromatography coupled to a Shimadzu GCMS-QP 2010 plus mass spectrometer equipped with capillary column RTX-5MS Crossbond 5 % diphenyl/95 % dimethyl polysiloxane (Restek, 30 m × 0.25 mm, film thickness of 0.25 μm) coupled by a heat transference line (200 °C) with the quadrupole mass spectrometer. The chromatographic separation followed the following temperature programming: 60 °C (1 min isothermal), elevating to 15 °C min^{-1} until 300 °C (10 min isothermal). Helium was the carrier gas (3.0 mL min^{-1}), the injector temperature was 290 °C and the split injection mode was 10 mL min^{-1}. The mass spectrum was obtained with electron impact ionization mode (70 eV). Integration of the peaks of the PLFA and TMAH compounds was based on comparison with the mass spectral library recorded in the NIST 2011 (Nacional Institute of Standards and Technology), using a similarity index of 85 % and the internal standard.

The TMAH data were expressed quantitatively as relative abundance in relation to the total peak intensity, according to the equation:

$$Relative\ abundance_{ij} = \left(\frac{x_{ij}}{\sum x_i}\right)100$$

where: x_{ij} is the integrated area of the compound j in the sample i and $\sum x_i$ is the sum of the area of all the integrated compounds.

The identification and classification of the TMAH compounds were done according to its probable origin through the searches in the literature (Chefetz et al., 2002; Otto et al., 2005; Spaccini and Piccolo, 2009; Stewart et al., 2015, 2011; Vinci et al., 2019). During the identification the compounds were grouped into six distinct categories, including

lipids, lignin, carbohydrates, N-bearing, cutin and suberin derivates and others.

The thermochemolysis analysis also allowed the separation of monomers derived from the main lignin precursor alcohols: p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, which provides information on the degree of oxidation of lignin. Once incorporated in the structure of the lignin macromolecule these monomers are referred to as p-hydroxyphenyl (P), guaiacyl (G) and syringyl (S) structures, respectively (Kačík et al., 2014).

2.7. Statistical analysis

Initially, the data were submitted to the normality test of Shapiro-Wilk and of homogeneity of variances of Levene at 5 % of probability. The analyses were done after the transformation of the data to *log* whenever necessary.

In order to test whether the microbial groups were influenced by the incubation of the different plant residues in the soil, a variance analysis was carried out (*two-way* ANOVA). The significative differences were tested with the *post-hoc* TukeyHSD test, at 5 % of probability. The analyses were done using the statistical software SISVAR (Ferreira, 2011). The Dunnett test, at 5 % of probability was also done in order to evaluate the statistical differences between the control treatment (soil without addition of residues) and the other treatments with the application of residues, and for that, the R software version 4.0.2 was used (R Core Team, 2020).

The biochemical composition data of the SOM fractions were evaluated by descriptive statistics using mean and standard error ($n = 4$). For the principal component analysis (PCA) the variables that contributed the most to the variation of the data set were selected, taking into consideration only the variables with *loadings* > 0.6. Two PCAs were done, the first one using as variables the PLFA biomarkers and the soil properties of each evaluated treatment. In the second one, the microbial groups were considered (Bacteria G+ and G-, actinobacteria, saprophytic fungi, and AMF) and the biochemical composition (aliphatic compounds, lignin derivatives, nitrogenous compounds, carbohydrates, cutin, and suberin) from POM and MAOM from the soil of each treatment. The analysis was done using the FactorMineR and Factorextra packages from the R software version 4.0.2 (R Core Team, 2020).

3. Results

3.1. Soil microbial community structure

The total PLFA biomass after the incubation for 120 days from the different residues of shoot and roots from plant species is presented in Fig. 2. The comparison by the Dunnett test has shown that the application of shoot and root residues increased in 92.2 % and 24.5 %, respectively the total PLFA biomass ($p < 0.01$) in the soil in relation to the treatment without the addition of residues, except for the treatments with GRASS and HERB roots (Fig. 2). The application of shoot residues increased total PLFA biomass by 54.4 % ($p < 0.05$) compared to root residue application.

A total of 17 biomarkers (PLFAs) were selected and, according to the C content and the quantified PLFAs structure, six microbial groups were identified (Fig. 2). The application of the shoot and roots residues in the soil increased in 48.7 % and 28.5 % the relative abundance of the fungi biomarkers, not only the saprophytic but also the AMF ($p < 0.01$). The shoot residues increased the abundance of bacteria G+ to 33.3 %, while the root residues decreased it to 2.6 %, when compared to the treatment

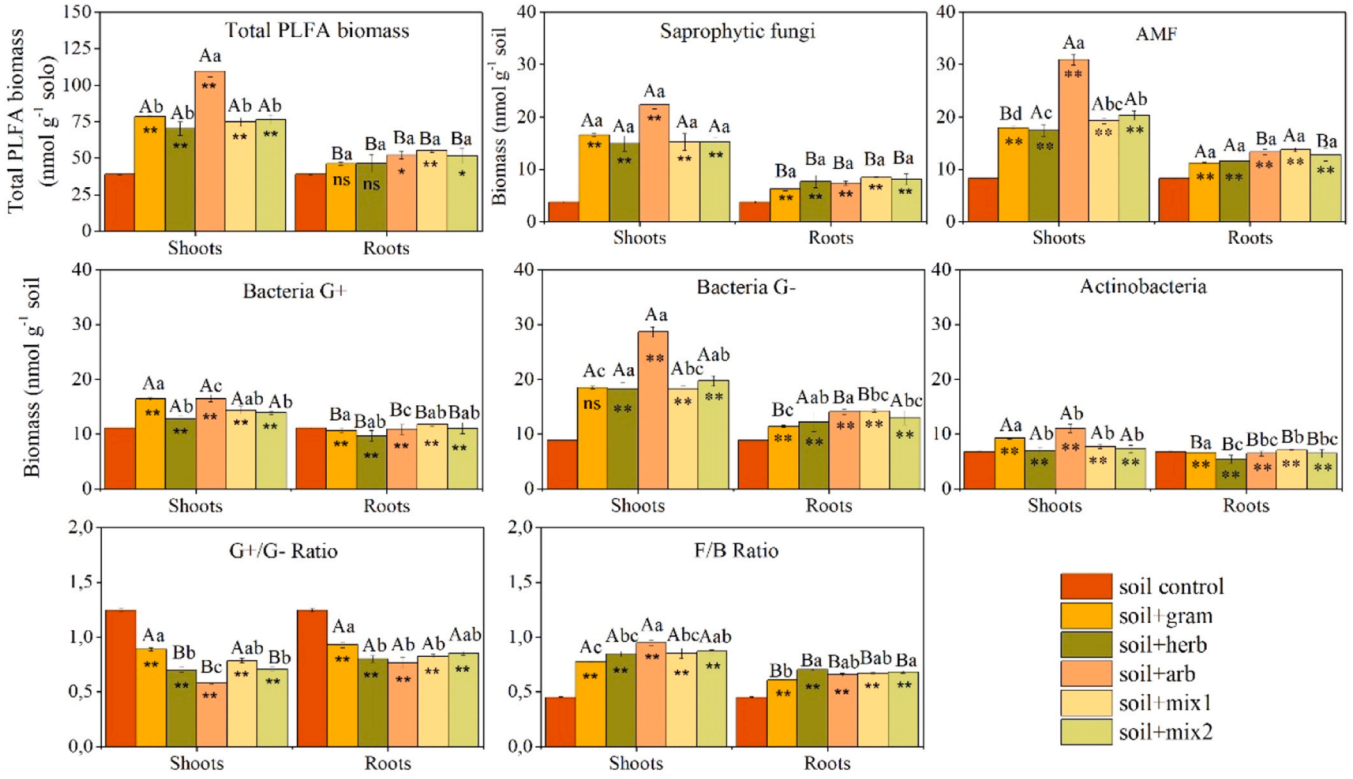

Fig. 2. Total PLFA and microbial groups biomass after 120 days of incubation in the soil with residues of shoot and roots of the Caatinga plant. HERB: *Centrosema*, *S. obtusifolia*, *A. brasiliana* and *H. suaveolens*. ARB: *M. tenuiflora*, *C. oncocalyx*, *C. pyramidale*, *M. caesalpiniifolia* and *C. sonderianus*. MIX1: proportion of 45 % of GRASS + 27.5 % of HERB + 27.5 % of ARB; MIX2: proportion of 25 % of GRASS + 37.5 % of HERB + 37.5 % of ARB. Similar lowercase letters do not differ significantly among the different compositions of species and similar uppercase letters do not differ statistically between shoot and root according to the Tukey test ($p < 0,05$). * $p < 0.05$, ** $p < 0.01$ or non-significant (*ns*) indicate the existence or not of differences between treatments in which there was application of residues and the control-treatment by the Dunnett test.

without addition of residues (control soil). The application of roots and shoot from the different groups of species implied distinct abundance in the several microbial groups.

When comparing treatments in which residues from each plant part were applied (shoot or roots), one noticed that for the bacteria G+, higher abundance in the soil ($p < 0.05$) was observed with the addition of the shoot of GRASS and MIX1. For the roots, the ARB treatment presented a lower abundance of G+ bacteria ($p < 0.05$), whereas the other treatments did not differ from each other ($p > 0.05$) (Fig. 2).

Regarding the saprophytic fungi, significant differences between the treatments were not observed either for the residues of the shoot or the roots. For the AMF, on the other hand, they were more abundant ($p < 0.05$) in the treatment with the addition of the shoot of tree species (ARB). When root residues were applied, there was no significant difference observed ($p > 0.05$) among the groups of evaluated species (Fig. 2).

In the principal component analysis (PCA), the two first components explained 82.5 % of the total variance observed. The results indicate that in a general way, the microbial communities, represented by the selected biomarkers, presented themselves distinct when residues of the shoot and roots were applied, forming two treatment groups: the ones of roots in quadrants 1 and 4 and the ones from the shoot in the quadrants 2 and 4 (except for HERB treatment) (Fig. 3).

3.2. Biochemical characterization of SOM

The relative abundance of the six classes of compounds identified by the TMAH-GC/MS is shown in Fig. 4. The SOM fractions presented differences in the abundance among the classes of compounds and regarding the plant compounds (shoot vs. roots). Overall, no great variation in the abundance of the compounds was observed (Fig. 4).

Among all the identified compounds, the aliphatic ones were the most abundant, with a general average of 20.0 ± 0.3 % in the POM and 31.6 ± 5.0 % in the MAOM, with the application of shoot residues (56.6 ± 4.0 %) as well as root residues (43.4 ± 4.0 %). In the POM, the shoot residues provided a higher abundance of aliphatic compounds in the GRASS, ARB, and MIX1 treatments in relation to the control treatment. With the application of the root residues, these compounds were more

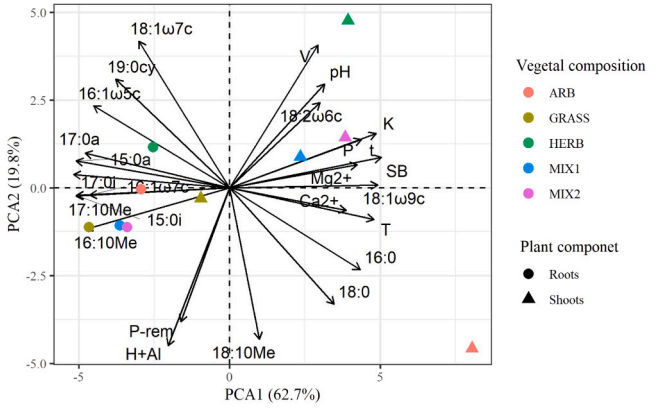

Fig. 3. Graphic dispersion in two principal components (PCA) from the treatments and from the 17 biomarkers (PLFAs): i15:0, a15:0, i16:0, i17:0, a17:0 (Gram+ bacteria); 16:1ω7, cy17:0, 18:1ω7, 18:0, cy19:0 (Gram- bacteria); 10Me16:0, 10Me17:0, 10Me18:0 (actinomycetes); 18:1ω9, 18:2ω6 (fungi) and 16:0, 16:1ω5 (arbuscular mycorrhizal fungi – AMF) and the soil chemical characteristics (pH, P, K^+, Ca^{2+}, Mg^{2+}, effective CTC (t), potential CTC (T), sum of bases (SB), saturation by bases (V), saturation by Al (H + Al) e P-rem) after 120 days of incubation of shoot and roots residues of Caatinga plant species in the soil. HERB: *Centrosema*, *S. obtusifolia*, *A. brasiliana* and *H. suaveolens*. ARB: *M. tenuiflora*, *C. oncocalyx*, *C. pyramidale*, *M. caesalpiniifolia* and *C. sonderianus*. MIX1: proportion of 45 % of GRASS + 27.5 % of HERB + 27.5 % of ARB; MIX2: proportion of 25 % of GRASS + 37.5 % of HERB + 37.5 % of ARB.

abundant in HERB, ARB, and MIX1. In the MAOM, there was a higher abundance of aliphatic ones with the incubation of shoot residues of MIX1 when compared to the control treatment. The incubation with the roots in the soil, on the other hand, provided a higher abundance in the control treatment and similar abundances among the other treatments (Fig. 4).

Lignin was the second class of more abundant compounds, now with the predominance in the POM (18.0 ± 0.6 %) in relation to the MAOM (9.8 ± 0.9 %) in all the treatments. In the POM, the shoot residues of GRASS and ARB provided a higher abundance of lignin compounds. With the root residues, the lignin was more abundant in MIX2, ARB, and GRASS. In the MAOM, the highest abundance of lignin was with the incubation of the shoot residues of GRASS (Fig. 4).

The N-bearing compounds were less abundant in the POM (3.2 ± 0.9 %) in all the treatments, in the shoot as well as the roots, whereas in the MAOM they were 21 ± 2.0 %. In the POM the N-bearing compounds were more abundant with the application of the ARB residues, in the shoot (4.4 ± 0.7 %) as well as in the roots (8.9 ± 4.4 %). In the MAOM, there was the highest abundance of these compounds in the control treatment (25.1 ± 5.5 %) (Fig. 4).

The carbohydrate class presented similar abundance for both fractions (MAOM 1.6 ± 0.6 % and POM 1.4 ± 0.4 %). In the POM fraction, a higher abundance was observed with the application of shoot residues of GRASS, HERB, and the MIXs, whereas the ARB treatment provided a higher abundance of carbohydrates in the MAOM (Fig. 4).

The cutin derivates were found in higher abundance in the POM (11.3 ± 0.2 %) of both plant compounds (shoot and roots), in relation to the MAOM (4.1 ± 1.4 %). In the POM, the residues of the shoot of GRASS and ARB provided a higher abundance of cutin (Fig. 4). The compounds derived from suberin were found in higher quantity in the POM (7.7 ± 0.2 %) in relation to the MAOM (4.3 ± 1.6 %) (Fig. 4).

The principal components analysis (PCA), presented in Fig. 5, was applied taking into consideration the variables associated with the different groups of microbial community and the biochemical composition of the SOM fractions. From the total variance, 77.8 % was explained by the principal components 1 and 2. The separation of the treatments into two distinct groups was observed, and they were correlated with the parts of the plant used in the incubation, that is, the shoot (quadrants 2 and 3) and roots (quadrants 1 and 4) (Fig. 5).

3.3. Biochemical characterization of lignin

In the POM fraction, there was a higher abundance of guaiacyl subunits, in the incubation of shoot residues (47.2 ± 3.0 %), as well as in the roots (52.6 ± 2.0 %). In general, the incorporation of the shoot and roots residues increased the abundance of the p-hydroxyphenyl subunits in the POM in relation to the control treatment. The p-hydroxyphenyl subunits were the most abundant with the incubation of GRASS residues not only in the shoot but also in the roots (Fig. 6).

In all the treatments incorporating shoot residues a higher abundance of siringyl subunits was found in relation to the control, with a highlight for the GRASS which provided a higher abundance (38.2 ± 3.0 %). With the incorporation of the root residues, the ARB was responsible for the highest abundance of these compounds (Fig. 6).

In the MAOM, a higher abundance of guaiacyl subunits was observed, not only in the incubation of shoot residues (41.8 ± 7.0 %) but also of roots (44.8 ± 7.0 %). In general, the incorporation of shoot residues and roots promoted the reduction in the abundance of the guaiacyl units and the increase in abundance of syringyl and p- hydroxyphenyl units and, comparatively to the treatment without residue addition (control treatment) (Fig. 6).

The guaiacyl subunits were more abundant in the control soil (74.6 %) in relation to the treatments with the application of shoot residues. Among the treatments with residues, ARB provided the highest guaiacyl abundance. The same behavior was observed in the treatments with root residues (Fig. 6).

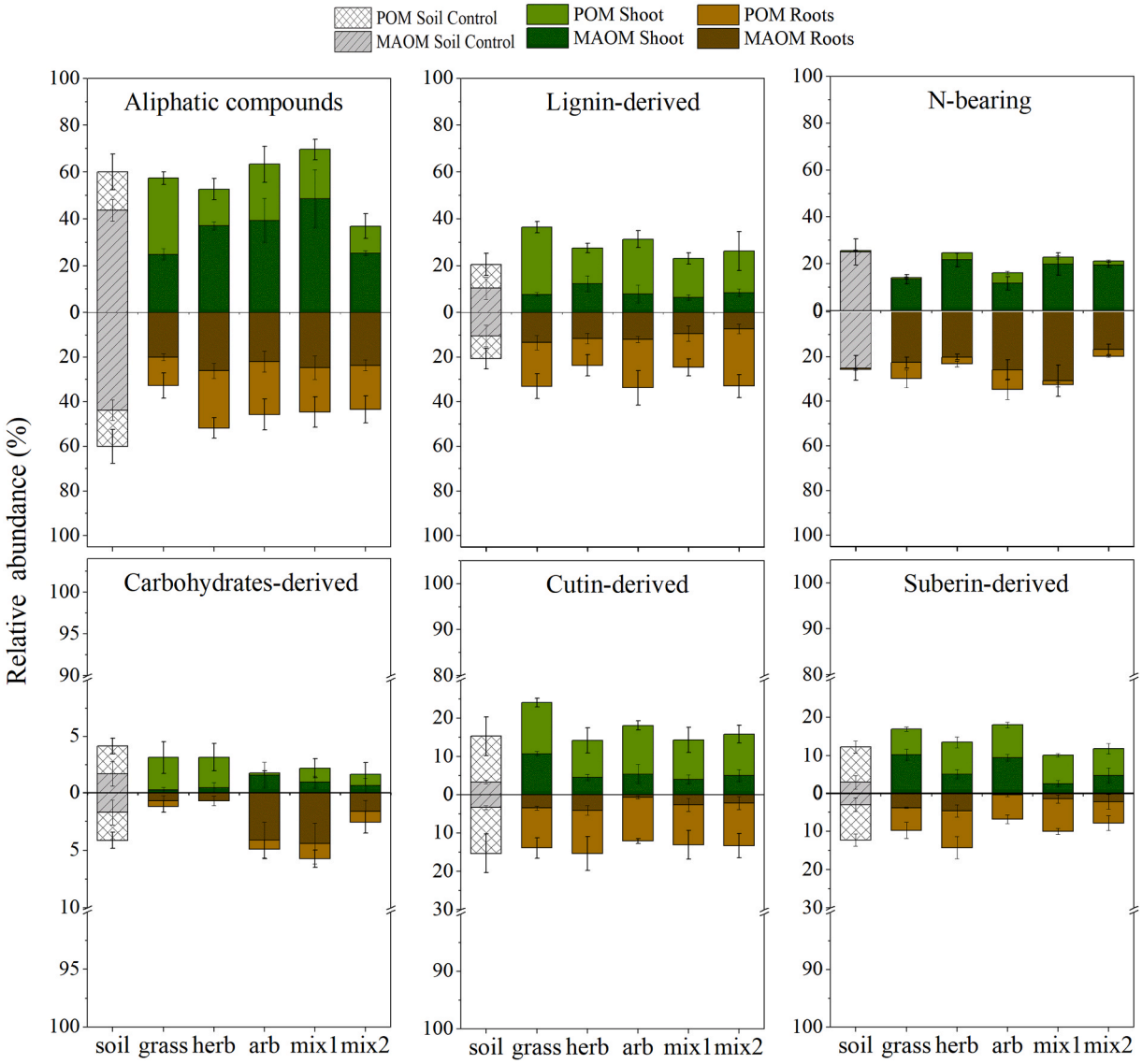

Fig. 4. Relative abundance of the classes of compounds present in the particulate fractions (POM) and associated with the minerals (MAOM): aliphatic compounds, derivated from lignin, nitrogen compounds, derivated from carbohydrates, cutin, and suberin identified by TMAH-GC/MS after 120 days of incubation of shoot and roots residues of Caatinga plant species in the soil. Error bars represent the standard error of the mean, $n = 4$. HERB: *Centrosema*, *S. obtusifolia*, *A. brasiliana* and *H suaveolens*. ARB: *M. tenuiflora*, *C. oncocalyx*, *C. pyramidale*, *M. caesalpiniifolia* and *C. sonderianus*. MIX1: proportion of 45 % of GRASS + 27.5 % of HERB + 27.5 % of ARB; MIX2: proportion of 45 % of GRASS + 27.5 % of HERB + 27.5 % of ARB.

4. Discussion

4.1. Soil microbial community structure

In general, the results showed that the structure of the soil microbial community was altered by adding different plant residues to the soil, increasing the total PLFA biomass. Changes in plant species composition alter the soil C and N input, impacting its microbial community structure and biomass (Prommer et al., 2020). It is important to understand the contribution that different vegetation covers have on the structure of the soil microbial community to plan management strategies aimed at preserving C in the soil.

Shoot residues seem to favor fungal biomarkers compared to root residues, probably due to differences in their biochemical compounds, as evidenced by a lower abundance of AMF when shoot residues of grass, with an elevated C:N ratio (Table 2), were added to the soil (Fig. 2). In contrast, Bossuyt et al. (2001) observed that changes in substrate quality alter the fungi:bacteria ratio, with residues having a low C:N ratio favoring bacteria, while fungi are favored by plant residues with a high C:N ratio.

Chen et al. (2019) observed an increase in the biomass of fungi and bacteria with an increase in the diversity of plant species and a higher fungi:bacteria ratio in a mixture of plants than in a monoculture. A higher fungi:bacteria ratio is usually associated with a higher storage of C in the soil (Dickens et al., 2015) and may be used as an indicator of sustainable soil management (Si et al., 2017). Fungi play an important role in litter decomposition, implying greater C storage (Table 2 – Supplementary material). Furthermore, Gram-negative (G-) bacteria preferentially use fresh plant inputs as C sources, whereas Gram-positive (G+) bacteria prefer recalcitrant soil organic matter (SOM) that is more processed by microorganisms (Kramer and Gleixner, 2008).

We believe that a shift towards fungal dominance in the microbial community increases soil C accumulation by decreasing its turnover rate. This aspect is particularly significant for soil C sequestration, greenhouse gas emissions, and the implementation of land use restoration management practices, underscoring the importance of vegetation

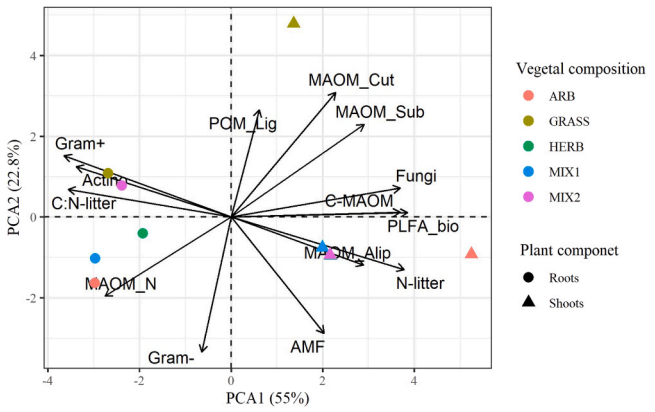

Fig. 5. Graphic dispersion of two principal components (PCA) from the treatments and the microbial groups identified in the PLFA analysis: G+ bacteria (Gram+); G- bacteria (Gram-); actinobacteria (Actin); fungi and arbuscular mycorrhizal fungi (AMF) and from the biochemical composition: aliphatic compounds (Alif), lignin (lig), nitrogen compounds (N), cutin (Cut) and suberin (Sub) identified by the TMAH/GC-MS analysis in the litter and in the particulate fractions (POM) and associated to the minerals (MAOM) from SOM, after 120 days of incubation of shoot and roots residues of Caatinga plant species in the soil. HERB: *Centrosema*, *S. obtusifolia*, *A. brasiliana* and *H. suaveolens*. ARB: *M. tenuiflora*, *C. oncocalyx*, *C. pyramidale*, *M. caesalpiniifolia* and *C sonderianus*. MIX1: proportion of 45 % of GRASS + 27.5 % of HERB + 27.5 % of ARB; MIX2: proportion of 25 % of GRASS + 37.5 % of HERB + 37.5 % of ARB.

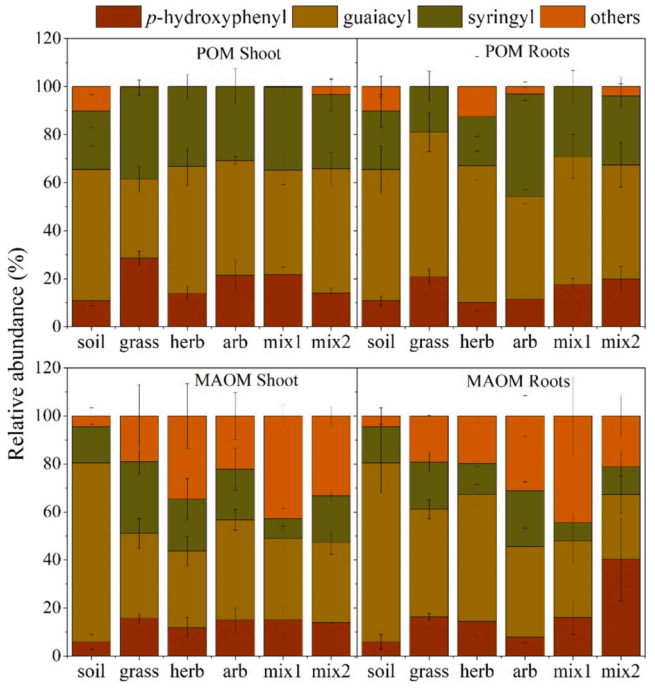

Fig. 6. Relative abundance of the lignin subunits released after the thermochemolysis of the organic matter particulate fraction (POM) and associated with the minerals (MAOM) induced by tetramethylammonium hydroxide (TMAH), after 120 days of incubation of shoot and roots residues of Caatinga plant species in the soil. HERB: *Centrosema*, *S. obtusifolia*, *A. brasiliana* and *H. suaveolens*. ARB: *M. tenuiflora*, *C. oncocalyx*, *C. pyramidale*, *M. caesalpiniifolia* and *C. sonderianus*. MIX1: proportion of 45 % of GRASS + 27.5 % of HERB + 27.5 % of ARB; MIX2: proportion of 25 % of GRASS + 37.5 % of HERB + 37.5 % of ARB. Error bars represent the standard error of the mean, $n = 4$.

cover in contributing to residue accumulation in the soil.

4.2. Biochemical characterization of SOM after incubation of residues

Knowledge about the mechanisms responsible for stabilizing C in the soil has recently gained significant interest due to its relevance in understanding the global C cycle (Lützow et al., 2006). Detecting the diversity of biochemical compounds in SOM fractions is important for identifying the complexity and longer persistence of SOM (Jones et al., 2023). In the context of Brazilian semi-arid soils, this information assumes heightened importance due to the absence of available data.

The quality of the litter plays a crucial role in determining the retention of C, the rate of conversion into SOM, and its residence time within the soil (Shahbaz et al., 2017). The elevated abundance of N-bearing compounds found in the MAOM with the incorporation of grass, herbaceous, and tree species and their combinations (MIXs) (Fig. 4) demonstrates that labile compounds also stabilize themselves in the MAOM and not only the recalcitrant ones (Lavallee et al., 2018; Yao et al., 2019). These findings suggest that the N-bearing compounds present in the MAOM may originate from microbial activities that require N, thus linking the capacity of soils to store C to nitrogen availability (Cotrufo et al., 2019). It is important to promote soil management strategies that ensure high microbial activity, supported by the sustained input of organic plant residues, as a precursor to the formation of the MAOM fraction (Wu et al., 2024). The MAOM fraction has high stability, making it crucial for soil C sequestration (Lavallee et al., 2020). Organic residues that contribute to the C sequestration in the MAOM fraction, when incorporated into the soil, are very important, especially in semiarid regions due to the extensive degradation that reduces soil C in these areas (Silva et al., 2023). Meanwhile, POM is a fraction of SOM characterized by a low degree of decomposition, making it more vulnerable to disturbances and highly sensitive to changes in land use (Cotrufo et al., 2019). The POM fraction is primarily derived from plants (Lavallee et al., 2020); this explains the high abundance of lignin, cutin, and suberin found in POM (Fig. 4). On the other hand, the decomposition of the POM fraction, when regulated by microorganisms, drives the formation of the MAOM fraction (Witzgall et al., 2021). Thus, the higher the quality of the substrate, the more efficiently it is utilized by microorganisms, resulting in more stable SOM. Therefore, understanding how plant residues with different degrees of lability control the formation and composition of SOM, and how they signify the quality of resources available to decomposer microorganisms, will help to elucidate the mechanisms of C formation and accumulation in SOM fractions. Cotrufo et al. (2022) found that the physical nature of the plant input (structural vs. soluble) influenced both the pathways and efficiencies of SOM formation, confirming the importance of the direct sorption of soluble inputs onto silt-and-clay-sized minerals for the formation of MAOM in bulk soil. The increase in labile and stable forms of organic matter in soils is desirable for improving the sequestration and stabilization of C and N through physical and biochemical pathways (Cotrufo et al., 2015).

4.3. Composition of lignin in MOS fractions after incubation of different residues

The S/G ratio is usually used as an indicator of lignin degradation in woods (Vane et al., 2001a), agricultural cultures (Vane et al., 2001b), and organic compounds (Spaccini et al., 2009; Spaccini and Piccolo, 2009; Vane et al., 2003). As observed in the present study, it can also be used as a bioindicator of the degree of lignin degradation in the SOM fractions, indicating its degree of stabilization and persistence in the SOM.

The predominance of guaiacyl subunits in relation to the syringyl subunits in the soil (Fig. 6), indicating a higher oxidative alteration of the lignin molecule (Vane et al., 2006). This proportion tends to decrease when the lignin is decomposed by specific fungi groups, such as

white rot fungi (Vane et al., 2005). Lignin compounds enriched in guaiacyl units are more resistant to decomposition, due to the greater number of carbon-carbon bonds (C—C), whereas syringyl units are more easily oxidized by the enzymes of the ascomycete fungi (Vane et al., 2005). Chefetz et al. (2000) have observed a reduction in the S/G ratio, and they attribute this reduction to a degradation preferential to the S units by microorganisms during the SOM decomposition process. The preferential decomposition of S units can also be explained by the fact that these units contain fewer aryl-aryl bonds and lower redox potential than G (Nilsson and Daniel, 1989).

The S/G ratio increased in all the treatments, especially in the POM (Fig. 6), where a higher abundance of lignin-derived compounds was found (Fig. 4). These results indicate that this tendency of lignin decomposition can be attributed to several factors, including the different proportions of structural polymers present in plants, environmental factors, and varying microbial composition. (Vane et al., 2006) have observed that the white rot fungi and some species of soft-rot fungi are responsible for a great part of the lignin degradation. One observed that the incorporation of leaves of woody species in the soil is associated with a higher abundance of fungi (Fig. 5), which may indicate that these species are more likely to contribute S-type lignin subunits, making the lignin present in organic matter more susceptible to degradation. Unit S is more susceptible to lignin degradation, whereas unit G has a slower turnover (Bahri et al., 2006).

In an incubation experiment, root-derived compounds were observed to be a source of C with higher relative stability, while litter was found to be the most active C source in soil (Pisani et al., 2016). However, the results of this study showed that the biochemical abundance of the shoot and root compounds were quite similar (Fig. 4). Possibly these results can be attributed to the fact that this is an ex-situ study, with an evaluation of dead roots, not being possible to quantify the release of exudates during the incubation period since they are released directly to the soil in situ (Angst et al., 2016). In comparison to the litter, the decomposition of the roots and the influence factors are less understood and involve more complex processes due to the microorganism-plant-soil interactions (Zhang and Wang, 2015).

5. Conclusions

The results obtained in this study partially confirm the hypothesis that mixing grasses with residues from different plant covers is a management practice that alters the structure of the microbial community and the biochemical composition of the SOM. The application of the different plant residues to the soil, in the shoot as well as in the root increases the total PLFA biomass and fungi biomarkers (saprophytic and AMF). However, that reduces the abundance of G+ bacteria and actinobacteria. Notably, the input of C derived from the shoot increases the total PLFA biomass when compared to the roots, and it is related to lower concentrations of recalcitrant compounds and low C:N ratios in the aboveground residues. The incorporation to the soil of shoot residues, that is, of higher quality (low C:N ratios and lignin:N) favors the fungi biomarkers, whereas the ones from the roots (high C:N ratios) are related to the bacteria biomarkers. The biochemical composition of SOM was altered by the application of the different residues, resulting in a higher abundance of aliphatic and nitrogen compounds in the MAOM fraction and compounds derived from lignin in the POM. The SOM lignin composition was also evaluated, and it was dominated by syringyl compounds with the incorporation of residues in the soil, indicating that the lignin present in the SOM is more susceptible to degradation. The results of this study highlight the important role of the biodiversity of plants and the biochemical composition of these species in the regulation and formation of the C originating from the microbial biomass, POM and MAOM, mediating the impacts of the climatic changes and promoting C storage through improved management of Caatinga vegetation in the Brazilian semi-arid region.

The knowledge gap between what has been observed of the chemical structures detected in the SOM fractions motivates further studies. Unraveling the effects of Caatinga species litter integrated with microbial communities is essential for the development of management systems aimed at the conservation of organic matter and the sustainability of this important Biome.

CRediT authorship contribution statement

José Ferreira Lustosa Filho: Visualization, Supervision, Resources. **ANACLÁUDIA ALVES PRIMO:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Teogenes Senna de Oliveira:** Supervision, Project administration, Funding acquisition, Data curation, Conceptualization. **Ivo Ribeiro da Silva:** Resources, Investigation, Conceptualization. **Rafael Gonçalves Tonucci:** Visualization, Supervision, Resources. **Helen Botelho Marota:** Methodology, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.pedobi.2024.150985.

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