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Chemical composition of cover crops and soil organic matter pools in no-tillage systems in the Cerrado

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Abstract

In no-tillage systems (NTS), cover crops are recommended to increase the productivity of agricultural systems. Furthermore, a greater diversity of cover crops in NTS favours an increase in soil carbon (C) stocks. However, there are scarce published data on the relationship between the chemical composition of cover crops and the accumulation of labile and stable fractions of SOM. We evaluated the relationship between the chemical composition of cover crops and SOM fractions, C stocks and maize yield. Hemicellulose, cellulose and lignin contents were determined for *Urochloa ruziziensis*, *Canavalia brasiliensis*, *Cajanus cajan* and *Sorghum bicolor*, cultivated in the off-season of maize. *Canavalia brasiliensis* had high N (20.96 g kg^{-1}) and hemicellulose (185.67 g kg^{-1}) contents, lower lignin content (39.50 g kg^{-1}) and high dry matter yield (3,251 kg ha^{-1}). All these characteristics resulted in a better SOM quality. *Urochloa ruziziensis*, with higher hemicellulose and lower lignin contents, and low lignin/N ratio, was associated with accumulation of TOC (19.95 and 18.33 g kg^{-1} in 0- to 10-cm and 10- to 20-cm layers, respectively) and mineral-associated organic C (on average, 16.68 g kg^{-1}) in the soil. Cover plants with N:lignin ratio lower than 2.0 are fundamental for soil C sequestration. In conclusion, it is recommended the adoption of *Urochloa ruziziensis* and *Canavalia brasiliensis* as cover plants improve maize production, soil organic matter quality and C sequestration in the Cerrado region.

KEYWORDS

cellulose, hemicelluloses, labile carbon, lignin, nitrogen, total organic carbon

1 | INTRODUCTION

Crop rotation and plant diversity in no-tillage systems (NTS) can provide more favourable conditions for accumulating carbon (C) and nitrogen (N) in the soil profile (Alhameid et al., 2017; Eberhardt et al., 2021; Sant'Anna et al., 2017; Schmatz et al., 2017) and for nutrient cycling, increasing the productivity of agricultural systems by improving soil quality (Soares et al., 2019). Inclusion of legumes in crop rotations provides N to microorganisms that synthesize stable fractions of soil organic matter (SOM), in addition to promoting protection and stability of C, consequently reducing greenhouse gas (GHG) emissions (Nishigaki et al., 2021; Yao et al., 2019). The adoption of cover crops in cropping systems is recommended as a management strategy to increase soil C stocks (Chahal et al., 2020; Rosolem et al., 2016).

In agricultural systems, crop residues are the main source of soil C. Their chemical composition affects soil C and N dynamics and strongly influences the decomposition rate and formation of microbial biomass and its by-products (De Bruijn & Butterbach-Bahl, 2010; Schmatz et al., 2017). According to Schmatz et al., (2017), in the short term, the residues of vetch decompose faster than those of wheat and peas because of their more favourable chemical composition with a higher soluble C fraction combined with the high N content in vetch tissues. These results align with studies that show faster decay rates for residues with a highly soluble fraction and are usually not sustained in the medium term because of exhaustion of this more labile fraction (Cotrufo et al., 2013).

The concentrations of N and organic compounds such as lignins, hemicelluloses and cellulose regulate the decomposition rate of crop residues (Carvalho et al., 2012) and consequently affect the dynamics and accumulation of soil organic matter fractions (Carvalho et al., 2014). Therefore, not only the total SOM contents are essential but also their fractions, which can express changes in soil quality more quickly (Duval et al., 2020; Fontana et al., 2011). In this sense, crop residues with higher proportions of labile constituents are more rapidly converted into microbial products and with greater use efficiency; they are also the major contributors to storing organic C in the soil (Cotrufo et al., 2013). Thus, sustainable intensification of farming systems with cover crops on Cerrado soils is central to cropping systems that promote SOM accumulation and stabilization (Sato et al., 2019).

In the Cerrado biome, an important factor for NTS implementation is the identification of alternative cover plants adaptable to the off-season droughts. Off-season or second harvest edaphoclimatic limitations in the Cerrado hinder preservation of large amounts of dry matter on the soil surface, leaving it unprotected. This is because of the limited production and accelerated decomposition of plant residues (Carvalho et al., 2009, 2012). When maize is cultivated as the main crop, cover crops in the off-season are recommended to protect and maintain soil quality (Soares et al., 2019). Cover crops can promote soil aggregation (Silva et al., 2016) and increase humic acid and particulate C fractions (Sato et al., 2019), which are the main components of SOM and indicators of soil quality (Duval et al., 2020). Soil organic matter is also a critical factor in global climate change because soils represent an important C reservoir on the Earth, accounting for approximately 2,500 Gt C, and also contribute to reduced GHG emissions to the atmosphere (Lal, 2004). In this context, it is essential to understand the relationships between the chemical composition of cover plants and the accumulation of SOM pools, which control mineralization and accumulation of C in the soil. These processes are decisive to mitigate GHG emissions and modulate C sequestration in the NTS.

Highlights

- The effect of cover crops on soil organic matter (SOM) pools was investigated
- The chemical composition of cover crops and stable and labile pools of SOM were measured
- Lignin-rich plant residues favour increase in stable SOM pools

While previous studies have successfully demonstrated the role of cover crops in increasing soil C stocks (Chowdhury et al., 2015; Rosolem et al., 2016), there is still a lack of understanding of the direct relationship between the chemical composition of cover crops and the accumulation of labile and stable fractions of SOM. This relationship can inform the selection of the best combination of cover crops for promoting soil C sequestration and GHG mitigation in the Cerrado region. In this sense, this is the first study focused on the direct relationship between the chemical composition of cover crops and SOM fractions, C stocks and maize yield in tropical regions. Furthermore, the results of the present study are relevant to the UN Sustainable Development Goals such as food security and climate mitigation (Bouma, 2019).

Our hypothesis is that, in no-tillage systems, cover plants with a lower lignin/N ratio and higher concentration of hemicelluloses and N, grown in succession to corn in the Cerrado region, result in higher levels of labile carbon (LC) and MBC, providing better quality SOM. Thus, the objective of this work was to assess the effect of the chemical composition of cover crops on the SOM pools, soil C stocks and corn grain yield in no-tillage systems in the Cerrado.

2 | MATERIALS AND METHODS

2.1 | Location, experimental design and history of conducting the experiment

The experiment was carried out at Embrapa Cerrados, in Planaltina, Distrito Federal (15°35′30″S, 47º42′30″W), Brazil (Figure 1). The regional climate is Aw, according to the Köppen–Geiger classification, with an average annual rainfall of 1,345 mm. The rainiest quarter is concentrated in November, December and January, with average cumulative precipitation values close to 635 mm, representing

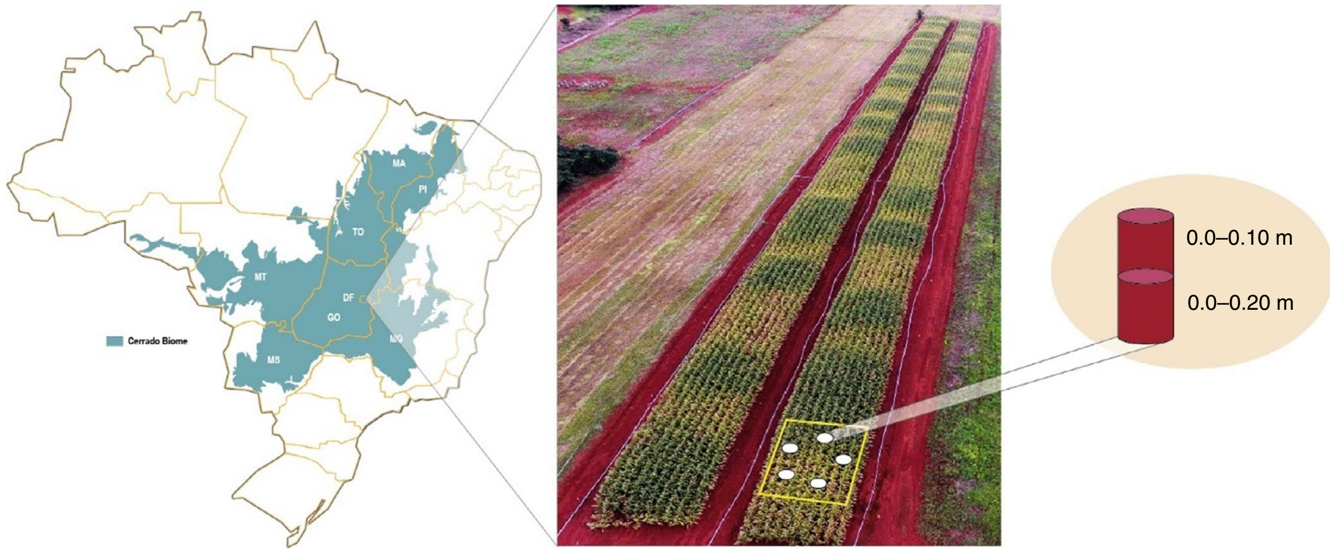

FIGURE 1 Location of the experimental area highlighting the soil sampling scheme

47% of the annual average, and an average annual temperature of 21.8°C (Silva et al., 2017).

The soil is classified as an Oxisol (Soil Survey Staff, 2014), and at the beginning of the experiment, the chemical and physical characteristics were as follows: pH (H_2O), 6.0; organic matter, 21.7 g kg^{-1}; $P_{Mehlich-1}$, 0.9 mg kg^{-1}; Al^{3+}, 0.1 cmol$_c$ kg^{-1}; Ca^{2+} + Mg^{2+}, 2.9 cmol$_c$ kg^{-1}; K^+, 0.1 cmol$_c$ kg^{-1}; fine sand, 258 g kg^{-1}; coarse sand, 76.7 g kg^{-1}; silt, 101.8 g kg^{-1}; and clay, 563.5 g kg^{-1}.

Before the experiment, from 1999 to 2004, soya bean and maize were grown in rotation. From the 2004/2005 to 2012/2013 crop seasons, maize and cover crops were grown in succession in a no-till system. Every crop season, maize was sown in November and harvested in March, and cover crops were sown in March and harvested at flowering (between May and August), depending on the plant species.

Aboveground inputs of carbon through different cover crops and maize over the years (2009–2012) were estimated by multiplying C content in shoots by the total dry matter of each plant (Table S1).

A randomized block experimental design with three replicates and a split-plot arrangement was used. Cover crops covered the entirety of the respective plots; subsequently, these plots were cultivated with maize and split between with nitrogen (WN) and no nitrogen (NN) topdressing treatments. Both WN and NN maize treatments received 20 kg N ha^{-1} at planting. For WN, two topdressings of N as urea (65 kg N ha^{-1}) were applied when plants had the fourth and the eighth pairs of leaves, respectively.

Cover crops were planted in April 2013 without fertilizer application, and the sowing density was 20 plants m^{-1} for *Cajanus cajan*, *Sorghum bicolor* and *Urochloa ruziziensis*, and 10 plants m^{-1} for *Canavalia brasiliensis*. The row spacing for all cover crops was 0.5m. At flowering, the *Cajanus cajan* and *Sorghum bicolor* were harvested in June. *Urochloa ruziziensis* and *Canavalia brasiliensis* were harvested in August. Crop residues were left on the soil surface.

In the November 2013, the maize hybrid 30F53VYHR was planted at a row spacing of 0.75 m with five seeds m^{-1}, giving a population of 65,000 plants ha^{-1}. At sowing, maintenance fertilizer application was applied to the planting furrow, consisting of 500 kg ha^{-1} NPK 4–30–16, 2 kg ha^{-1} Zn ($ZnSO_4.7H_2O$) and 10 kg ha^{-1} FTE BR 12 as the micronutrient source (3.2% S; 1.8% B; 0.8% Cu; 2.0% Mn; 0.1% Mo; 9.0% Zn; and 1.8% Ca). As mentioned above, upon growth of the fourth and eighth leaves, respectively, two topdressings of 65 kg N ha^{-1} were applied as urea (WN). The 130 total N ha^{-1} from the topdressings and the 20 kg N ha^{-1} applied at planting resulted in a total of 150 kg N ha^{-1}, as recommended by Sousa and Lobato (2004).

2.2 | Plant analysis and maize yield

Urochloa ruziziensis, *Canavalia brasiliensis*, *Cajanus cajan* and *Sorghum bicolor* were sampled at flowering using two rectangular iron sampling frames per subplot (38 × 58 cm). Plant biomass samples were dried at 65°C, and a subsample was weighed to quantify plant dry matter, converted to kilograms per hectare. One portion of the dried samples was ground, and a 3 g sample was heated in a porcelain crucible in an oven at 105°C for 8 h. Dry matter was calculated as the difference between sample weight before and after this procedure. The total N content (TN) of the cover crop was analysed by colorimetry with a Lachat 228 Quikchem flow injection analyzer (Lachat Instruments, 5600 Lindbergh Drive, Loveland, CO 80539, USA).

The dry matter, acid detergent fibre (ADF), neutral detergent fibre (NDF) and lignin contents of the cover crop straw were analysed at 105°C (Robertson & van Soest, 1981). Hemicellulose and cellulose contents were computed as the differences between NDF and ADF and between ADF and lignin, respectively.

In March 2014, 4-m rows of each split plot were harvested to determine the maize grain yield (moisture corrected to 13%).

2.3 | Soil analysis

For analysis of the SOM fractions, soil samples were collected after corn harvesting at the depths of 0–10 cm and 10–20 cm. Five subsamples from each subplot, randomly collected from between the rows, were composited to produce one sample (Figure 1). These two soil layers were chosen because of the nature of the management system adopted. The no-tillage system promotes SOM stratification with greater accumulation of C on the soil surface, mainly in the 0-to 10-cm layer. Therefore, it is widely recommended to evaluate these layers separately. After collection, soil samples were air-dried and passed through a 2-mm mesh sieve. After the harvest of the maize, soil was sampled in March to determine C stocks at depths of 0–5, 5–10, 10–20, 20–40, 40–60, 60–80 and 80–100 cm. For each depth, samples were composited by homogenization of eight subsamples per plot. The same protocols of soil sampling were performed in a native Cerrado area localized around 50 m from the experimental area, used as a reference. For the bulk density (BD) assessment, undisturbed samples were collected at the same depth intervals, from 120-cm-deep trenches; samples were collected in duplicate at each depth, using two alternating walls of the trench, with steel stainless rings (Embrapa, 1997). For C analysis, samples were ground in an agate mortar, passed through a 0.150-mm sieve and subjected to the total C content determination elementary analysis with dry combustion using a Macro Vario Cube analyzer. For each layer of the soil profiles, carbon stocks were calculated using the equivalent soil mass method, adopting the soil mass of the natural vegetation system (Cerrado) as a reference in the calculation (Sisti et al., 2004).

Total organic carbon (TOC) was determined by wet oxidation with potassium dichromate in the presence of sulphuric acid, followed by titration with ferrous ammonium sulphate, without an external heat source (Walkley & Black, 1934).

The physical granulometric fractionation of the SOM was carried out according to the procedures of Cambardella and Elliott (1992), with adjustments for the weight of the sample used (Bayer et al., 2004; Bongiovanni & Lobartini, 2006). Twenty grams of the soil samples (<2.00 mm) was stirred with 70 ml of a sodium hexametaphosphate solution (5 g L^{-1}) for 15 hr. Afterwards, the suspension was passed through a 53-µm mesh sieve. The material retained on the sieve, which consists of particulate organic matter (>53 µm), was dried in an oven at 45°C, ground and analysed for its particulate organic C (POC) content by wet oxidation, without an external heat source (Walkley & Black, 1934). The mineral-associated organic carbon (MOC) was estimated at the difference between TOC and POC.

The carbon of fulvic acids, humic acids and humin was determined according to the methodology described by Sato et al., (2019). For this, soil samples (1 g) were extracted with 20 ml of 0.1 mol L^{-1} NaOH. The fulvic acid fraction was soluble in the alkaline extract, and the humic acid fraction corresponded to the precipitated portion after decreasing the extract pH to values between 1 and 1.5. The humin fraction was considered as all the residue insoluble in acid and alkaline medium, retained in the centrifuge tube after centrifugation of the alkaline extractant.

After extraction, the humic substance C was determined by oxidation with a 1N $K_2Cr_2O_7$ solution with external heat source under reflux and titration with a solution of [(NH4)2 Fe(SO4)2.6H2O)] (Nelson & Sommers, 1996). The humic acid/fulvic acid (AH/AF) ratio was also estimated as an indicator of SOM quality.

The labile C (LC) content was determined by oxidation of 1g soil sample with 0.033 mol L^{-1} of a $KMnO_4$ solution and subsequent absorbance reading in a spectrophotometer (Biospectro, model SP220; São Paulo, Brazil) at a wavelength of 565 nm (Sato et al., 2019).

Microbial biomass C (MBC) was estimated by the irradiation–extraction method described by Islam and Weil (1998), using a correction factor of 0.33.

2.4 | Statistical analysis

Data were analysed using a two-way ANOVA, followed by Tukey's HSD test (considering $p < .10$ for carbon stocks and $p < .05$ for other variables) as a post hoc method to detect statistically significant differences among all treatments. Pearson's linear correlation analysis was performed between the chemical composition of cover crops and soil carbon stocks. These analyses were performed using XLSTAT software (Addinsoft, 2013). Additionally, a principal component analysis (PCA) was performed using data from the soil surface layer (0–10 cm) in a matrix composed of 24 rows (four cover crops, two nitrogen levels and three replications) and 17 columns with soil and agronomic variables (hemicelluloses, HEM; cellulose, CEL; lignin, LIG; lignin:N ratio, LIGN; nitrogen content, NC; nitrogen uptake, NU; dry matter, DM; grain yield, GY; total organic carbon, TOC; microbial biomass carbon, MBC; labile carbon, LC; particulate organic carbon, POC; mineral-associated organic carbon, MOC, humic acid, HA; fulvic acid, FA; humic:fulvic ratio, HA:FA; and humin, HUM). This was used to identify, among the variables corresponding to the edaphic and agronomic data, which variables had the greatest weight in the linear combination of the first two principal components. In addition to the correlation diagram from the PCA, an ordering diagram for cover crops was constructed to test for the significance of differences between cover crops in relation to the two first components. A discriminant analysis based on the Mahalanobis distance was used to compare the mathematical distances between the samples. This step was performed in the ADE4 software (Thioulouse et al., 1997).

3 | RESULTS AND DISCUSSION

3.1 | Chemical composition of cover crops and maize yield

The chemical composition of the cover crops determined at flowering is shown in Table 1. There were significant differences in the N content in the aboveground biomass of cover plants; *U. ruziziensis* (24.40 g kg^{-1}), *C. brasiliensis* (20.96 g kg^{-1}) and *C. cajan* (20.91 g kg^{-1}) had higher N concentrations ($p < .05$) than *S. bicolor* (14.87 g kg^{-1}). The highest concentration of hemicelluloses was measured in the aboveground biomass of *U. ruziziensis* (263.16 g kg^{-1}). *S. bicolor* (132.49 g kg^{-1}) and *C. cajan* (124.98 g kg^{-1}) had the least hemicellulose concentrations ($p < .05$). *Sorghum bicolor* (240.8 g kg^{-1}) had a higher concentration of cellulose ($p < .05$) than *C. brasiliensis* (202.81 g kg^{-1}) and *C. cajan* (202.28 g kg^{-1}), but not distinct from *U. ruziziensis*.

The highest lignin content was measured in the aboveground biomass of *C. cajan* (85.49 g kg^{-1}), while the lowest ($p < .05$) was measured for *U. ruziziensis* (19.77 g kg^{-1}). The lowest lignin:N ratio in the aboveground biomass was found for *U. ruziziensis* (0.81 g kg^{-1}) and *C. brasiliensis* (1.90 g kg^{-1}); *C. cajan* had the highest lignin:N ratio (4.10) (Table 1).

Urochloa ruziziensis is rich in hemicelluloses and cellulose and has little lignin (Carvalho et al., 2012). This cover plant produces more readily decomposable organic matter, with an accumulation of MOC at the expense of more recalcitrant aromatic compounds, whose structural precursor component is lignin. In turn, *C. cajan* favoured the accumulation of POC in the soil. According to Carvalho et al., (2009), this may be a consequence of the increased lignin content, hindering the rapid decomposition of residues and favouring the accumulation of POC. The organic fraction of soils located in tropical regions is dominated by humin. Inclusion of legumes as green manure provides organic N, which is preferentially used by microorganisms that synthesize stable SOM fractions, such as humin (Ribeiro et al., 2011).

The present study showed that in NTS, *C. cajan* is a suitable cover crop when plant residues with higher proportion of aromatic C and lower decomposition rates are desired. On the other hand, for faster nutrient recycling, as is the case of green manures, *C. brasiliensis* should be used because of the faster decomposition rates of its biomass, contributing to the formation of HA and LC.

The lignin/N ratio is also a key indicator of the cover crop ability to protect the soil surface and promote efficient nutrient cycling. Cover plants with a lower lignin/N ratio such as *U ruziziensis* and *C. brasiliensis* are preferentially recommended for nutrient cycling because of their faster decomposition. In contrast, plants with higher lignin/N, such as *C. cajan*, are more efficient for soil protection, in

TABLE 1 Nitrogen (N), hemicellulose, cellulose and lignin contents and lignin:N ratio in the aboveground biomass of cover crops.

Cover crops	N	Hemicellulose	Cellulose	Lignin	Lignin/N
	(g kg^{-1})				-
Urochloa ruziziensis	24.40a	263.16a	222.92ab	19.77c	0.81d
Sorghum bicolor	14.87b	132.49c	240.85a	45.42b	3.11b
Canavalia brasiliensis	20.96a	185.67b	202.81b	39.50b	1.90c
Cajanus cajan	20.91a	124.98c	202.28b	85.49a	4.10a
CV%	8.97	11.84	6.76	13.07	15.13

Note: Equal letters for each variable indicate no statistical differences between treatments according to Tukey's test ($p < .05$).

CV, coefficient of variation.

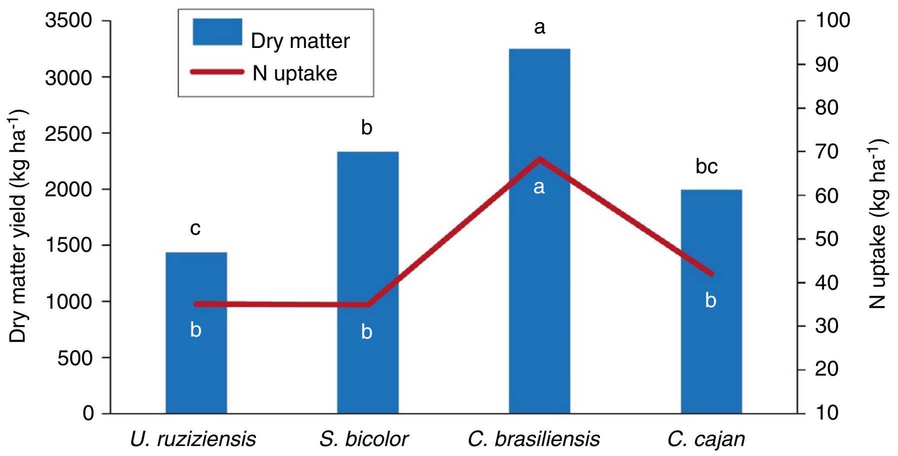

FIGURE 2 Dry matter yield and N uptake in the aboveground biomass of cover crops. Equal letters for each variable indicate no statistical differences between treatments according to Tukey's test ($p < .05$)

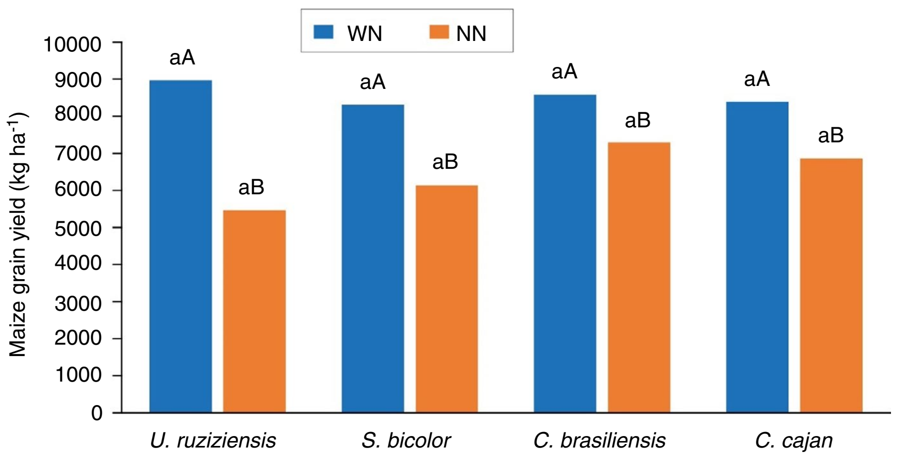

FIGURE 3 Maize grain yield in the 2013/2014 cropping season in succession to cover crops. Equal letters for each variable indicate no statistical differences between treatments according to Tukey's test ($p < .05$). Comparisons between cover crops and N fertilizer application are indicated by lowercase and uppercase letters, respectively

the short term, because of the higher proportion of aromatic C (Carvalho et al., 2009), which favours the accumulation of POC in soils (Ramos et al., 2020).

Canavalia brasiliensis had the highest dry matter yield (3251.1 g kg^{-1}) (Figure 2) corroborating previous reports (Carvalho et al., 2012). Larger amounts of biomass with higher C:N ratio, as reported for *S. bicolor* by Carvalho et al., (2012), result in slower decomposition rates. Consequently, competition for available N from the soil can reduce the yield of maize planted in succession to cover crops.

Canavalia brasiliensis had the highest N uptake (68.27 kg ha^{-1}), statistically significant. Total N uptake was not different among the other cover crops ($p > .05$). This confirms previous reports of large biomass yields and higher N levels for this plant (Carvalho et al., 2012).

Recalde et al., (2015) reported that the dry matter of cover plants, both in quantity and in quality, can effectively contribute to the increase in SOM with gains in C stocks over the years (Ramos et al., 2020). This is desirable for the management of soils, since SOM is linked to key processes such as the cycling and accumulation of nutrients and the stability of soil aggregates (Nascimento et al., 2019; Silva et al., 2016).

Despite the differences in chemical composition of the aboveground biomass, in the present study cover crops did not affect the yield of the subsequently planted maize ($p > .05$) (Figure 3). *C. brasiliensis* led to an increase in maize yield ($p < .05$) of 1284 kg ha^{-1} for the treatment receiving fertilizer. In comparison, *U. ruziziensis* increased maize yield by 3501 kg ha^{-1}, a 40% yield increase when N was added as a topdressing application compared with the unfertilized treatment (Figure 3).

Even without significant differences in maize yields between treatments, it should be noted that cover plants with a lower lignin/N ratio, such as *U. ruziziensis* and *C. brasiliensis*, might facilitate better N use efficiency because of its release synchronized with the needs of the following maize crop (Carvalho et al., 2012).

3.2 | Relationship between cover crops, soil organic matter pools and carbon stocks

The soil organic C contents were affected by the cover crops studied. *U. ruziziensis* and *C. cajan* were associated with the highest TOC contents in both soil layers (0–10 cm and 10–20 cm). *C. cajan* and *S. bicolor* led to similar TOC

TABLE 2 Total organic carbon and soil organic matter pools in response to cover crops and N fertilizer application

	TOC	FA	HA	HUM	HA/FA
	(g kg^{-1})				
Cover crops	**0–10 cm**				
Urochloa ruziziensis	19.95a	7.07a	1.52b	9.09b	0.23a
Sorghum bicolor	17.27b	6.42a	1.46b	9.92b	0.22a
Canavalia brasiliensis	15.49c	7.01a	2.18a	12.09a	0.31a
Cajanus cajan	18.71ab	6.91a	1.48b	8.93b	0.21a
Fertilizer application					
WN	17.91a	7.05a	1.51a	9.89a	0.22a
NN	17.79a	6.66a	1.81a	10.12a	0.27a
CV%[1]	4.73	13.41	17.24	7.48	21.18
CV%[2]	3.66	29.03	32.42	12.19	29.52
Cover crops	**10–20 cm**				
Urochloa ruziziensis	18.33a	6.24a	1.87a	8.89b	0.31a
Sorghum bicolor	15.91bc	6.39a	1.18a	9.72b	0.18a
Canavalia brasiliensis	13.79c	6.21a	1.76a	11.65a	0.28a
Cajanus cajan	17.42ab	5.33a	1.40a	8.90b	0.27a
Fertilizer application					
WN	16.44a	5.87a	1.52a	9.72a	0.26a
NN	16.28a	6.22a	1.59a	9.86a	0.26a
CV%[1]	6.57	13.21	31.49	5.24	33.58
CV%[2]	4.72	19.73	28.4	10.33	23.27

Note: Equal letters for each variable indicate no statistical differences between treatments according to Tukey's test ($p < .05$).

[1]Coefficient of variation related to cover crops.

[2]Coefficient of variation related to the fertilizer application effect.

contents in both soil layers ($p < .05$) (Table 2). The results of the PCA (Figure S1) revealed a trend of association between *U. ruziziensis* with MOC, and *C. brasiliensis* with the MBC and LC fractions in the 0- to 10-cm layer (Figure S1). The extensive root system of *U. ruziziensis* may have favoured TOC accumulation below 10 cm (Ramos et al., 2020; Rossi et al., 2012; Santos et al., 2014), confirming the hypothesis that tropical grasses may be recommended as cover crops to improve some soil attributes, through the action of their roots, can be just as important as the production of soil cover (Rosolem et al., 2016). A deeper root system makes these cover crops more resistant to drought, which is favourable in the edaphoclimatic conditions of the Cerrado, and also contributes to deep C accumulation and the formation of soil aggregates (Loss et al., 2012; Silva et al., 2016). These results indicate the potential of these plants to favour the soil C stocks and consequently collaborate in reducing GHG emissions in tropical agricultural systems.

The fulvic acid (FA) contents were not affected by cover crops for the studied soil depths ($p > .05$). On the other hand, for *C. brasiliensis* HA and HUM were increased in the 0- to 10-cm layer, with no difference between the other cover crops (Table 2). Higher dry matter yield associated with a higher N content and lower lignin/N in the aboveground biomass of *C. brasiliensis*, and consequently the higher decomposition rate (Carvalho et al., 2011), may explain the increase in HA formation at the 0- to 10-cm layer. According to Rosa et al., (2017), even when they do not alter the TOC levels, legumes promote the accumulation of HA in the soil surface layer. The PCA also revealed a trend of association between *C. cajan* and *S. bicolor*, which are associated with high lignin levels ($p > .01$; Figure S1a,c).

In general, short-term crop residues whose chemical composition is more favourable to biodegradation, with a higher soluble fraction combined with high N content, favour the formation of humic substances (Schmatz et al., 2017). Canellas et al., (2007) suggested that the higher proportions of HA indicate improvement in the SOM quality. In Oxisols, there are usually a decrease in the HA content and a predominance of FA (Rosa et al., 2017).

The use of *C. brasiliensis* also favoured the accumulation of HUM in the soil ($p < .05$), regardless of the depth studied (Table 2). As highlighted for HA, *C. brasiliensis* has high dry matter yield, N content, lignin content and lignin:N ratio in the aboveground biomass (Table 1 and Figure 2), which favour rapid decomposition (Carvalho et al., 2011) and subsequent SOM humification processes. Interaction between the mineral fraction of an oxidic nature and the dehydration reactions favoured by the alternation of dry and wet periods favours humin formation (Canellas et al., 2002).

The HA/FA ratio varied between 0.21 and 0.31 in the 0- to 10-cm layer and 0.18 to 0.31 in the 10- to 20-cm layer (Table 2). However, there were no differences ($p < .05$) between cover plants. The HA/FA ratio can be considered an indicator of humus quality (Sousa et al., 2015) as it expresses the degree of the SOM humification and the potential for C mobility in the soil. In highly weathered soils, such as in the tropics, HA/FA ratio is less than 1.0 indicating a rapid and intense mineralization of plant residues, and consequently a faster humification (Canellas et al., 2002; Rosa et al., 2013). This also indicates a limited likelihood for the development of SOM because of edaphic or management reasons and recent inputs of SOM (Canellas et al., 2007).

Table 3 shows the C contents of granulometric and labile SOM fractions, in addition to MBC. There was a significant interaction between cover crops and N application for the following SOM pools (Table 3): particulate organic carbon (POC), mineral-associated organic carbon (MOC), labile carbon (LC) and MBC. The cover crop treatments differed ($p < .05$) with regard to POC levels in the soil (Table 3). In the 0- to 10-cm layer, the soil cultivated with *C. cajan*, both with and without N fertilizer application, had a higher POC ($p < .05$) in relation to the other cover plants. In the 10- to 20-cm layer, *C. brasiliensis*, *U. ruziziensis* and *S. bicolor* led to similar levels of POC, regardless of N fertilizer application. It should be highlighted that as the adoption of conservation systems strengthens the soil aggregation, the POC contents must be increased in the occluded form in stable aggregates (Conceição et al., 2013).

For both tested soil layers, both in the presence and in the absence of N, *U. ruziziensis* was associated with a higher MOC content than *C. brasiliensis*, with no differences between the other cover plants. The higher dry matter yield of *C. brasiliensis* did not result in a greater accumulation of C in the granulometric fractions of SOM. *C. brasiliensis* led to lower TOC content and greater

TABLE 3 Particulate organic carbon (POC) and mineral-associated organic carbon (MOC) in g kg^{-1}, microbial biomass carbon (MBC) in mg kg^{-1} and labile carbon (LC) in g kg^{-1} in Latosol with cover crops cultivated in succession to maize with (WN) and without N (NN) fertilizer application

	POC		MOC		MBC		LC	
	WN	NN	WN	NN	WN	NN	WN	NN
Cover crops	**0–10 cm**							
Urochloa ruziziensis	2.28bA	2.58bA	16.46aA	16.91aA	195.85aA	159.63aA	1.55aA	1.50aA
Sorghum bicolor	1.75cA	1.09cB	14.46abA	15.58abA	272.94aA	117.64aB	1.40aA	1.38aA
Canavalia brasiliensis	2.01bcA	2.16bcA	13.15bA	13.14bA	220.51aA	148.71aB	1.95aA	2.16aA
Cajanus cajan	2.94aB	3.64aA	15.44abA	14.51abA	174.97aA	139.18aA	1.31 aA	1.56aA
CV%[1]	8.6		8.1		36.1		30.1	
CV%[2]	9.6		5.7		18.9		20.7	
	10–20 cm							
Urochloa ruziziensis	2.28abA	2.58abA	16.43aA	16.91aA	211.30abA	115.76aB	1.14bA	1.22bA
Sorghum bicolor	1.70bA	1.91bA	14.46abA	15.58abA	166.30abA	121.01aA	1.02bA	0.98bA
Canavalia brasiliensis	2.01abA	2.16abA	13.12bA	13.14bA	242.70aA	196.64aA	2.14aA	2.31aA
Cajanus cajan	2.93aB	3.64aA	15.44abA	14.51abA	130.58bA	172.20aA	1.13bA	0.92bA
CV%[1]	25.9		11.9		26.3		37.7	
CV%[2]	23.3		5.2		15.9		17.6	

Note: Equal letters for each variable, uppercase in the column and lowercase in the row, indicate no statistical differences between treatments according to Tukey's test ($p < .05$).

[1]Coefficient of variation related to cover crops.

[2]Coefficient of variation related to the fertilizer application effect.

accumulation of humified SOM fractions in the form of HA and HUM (Table 2). The higher concentration of N in *C. brasiliensis* biomass might have intensified the SOM humification reactions (Liu et al., 2018), reducing the C content in granulometric pools of SOM, and consequently the TOC.

The relationship between MOC and POC (Table 3) suggests that supplying labile organic matter to soil can promote the degradation of already-stabilized SOM (Fontaine et al., 2003). This phenomenon of degradation of stable organic matter after adding fresh organic matter has been previously reported (Bell et al., 2003; Fontaine et al., 2007). A common explanation is that microorganisms use N from SOM (relatively low C/N), which accelerates its decomposition and consequent CO_2 emission (Kuzyakov, 2010). It should be noted that the addition of substrates with a higher C/N ratio can be understood as a supply of fresh organic material.

In general, MBC was the SOM fraction most affected by the N application to maize. In the 0- to 10-cm layer, *S. bicolor* and *C. brasiliensis* when grown succeeding maize fertilized with N, promoted greater MBC. Likewise, in the 10- to 20-cm layer MBC was reduced for non-fertilized maize following *U. ruziziensis*. These results reinforce the importance of improving N to increase the soil microbial biomass. Despite this, irrespective of application of N, the cover crops did not yield differences in the MBC contents in the 0- to 10-cm layer. In the 10- to 20-cm layer, there was a difference between cover crop treatments when maize received N; *C. brasiliensis* resulted in higher levels of MBC in the soil ($p < .05$) compared with *C. cajan*. Soil cultivated with *C. brasiliensis* favoured the accumulation of about 53% more MBC than the soil with *C. cajan*, possibly because of the N content and the low lignin/N ratio of its biomass, which favours the mineralization of the SOM. According to Cotrufo et al., (2013), high-quality residues with a low lignin/N ratio, such as *C. brasiliensis*, in which more labile constituents predominate, are quickly and more efficiently converted into microbial products.

According to Santos et al., (2004), the greater immobilization of C by microbial biomass after cover crops is because of the increase in TOC via the decomposition of residues. The combination of increased dry matter yield and higher N content resulted in higher N uptake, in addition to the lower lignin/N ratio ($p < .05$) in the aboveground biomass of *C. brasiliensis* (Table 1). This may have favoured the greater accumulation of MBC in the soil cultivated with this plant. Figure 2 also shows that *C. brasiliensis* with the highest production of dry matter ($p < .05$) favoured the accumulation of MBC in the 10- to 20-cm layer, especially when N was applied to maize (Table 3).

Regarding labile carbon (LC), differences between the cover crop treatments ($p < .05$) can be seen only in the 10- to 20-cm layer (Table 3). In this layer, *C. brasiliensis* was associated with more LC ($p < .05$). The higher N content and the lower lignin/N ratio (Table 1 and Figure 2) in the aboveground biomass of this legume could have favoured the formation of easily decomposable organic matter such as labile carbon (LC) (Cotrufo et al., 2013). The higher LC content combined with the higher MBC after *C. brasiliensis* indicates that this cover plant has the potential to improve the short-term SOM quality, as well as the cycling of nutrients, with emphasis on N in addition to C stocks.

3.3 | Carbon stocks in the 0- to 100-cm depth

Carbon stocks up to 100 cm deep differ significantly between the cover crop species (Figure 3). *Uroclhoa ruziziensis* followed by *C. brasiliensis* were associated with the largest carbon stocks ($p < .10$) of 118.86 and 118.70 Mg ha^{-1}, respectively. *Cajanus cajan*, a leguminous species, had slightly lower C stocks at 114.31 Mg ha^{-1}. In general, *U. ruziziensis* and *C. brasiliensis*, which showed the highest quality plant tissues properties (i.e. high contents of hemicelluloses and lower contents of lignin) resulted in higher C soil stocks.

Based on the data of C stocks in the adjacent native vegetation area (198.84 Mg C ha^{-1}), a decrease in soil C stocks was observed after deforestation and land use.

The following C inputs, including cover crops and maize aboveground dry matter biomass, were calculated (accumulated from 2009 to 2012) (Table S1): *U. ruziziensis*, 19.26 Mg ha^{-1}; *S. bicolor*, 23.62 Mg ha^{-1}; *C. brasiliensis*, 20.62 Mg ha^{-1}; and *C. cajan*, 18.90 Mg ha^{-1}. These results show that other characteristics such as a large root system, low lignin and higher N contents in the aboveground biomass explain the higher soil C stocks after the cultivation of *U. ruziziensis*.

The high biomass production of *C. brasiliensis* and elevated N accumulation of *U. ruziziensis* and its accelerated decomposition may have contributed to the higher C stocks in the plots with these cover crops (Carvalho et al., 2014). Furthermore, the lower proportions of recalcitrant aromatic C in these two species contributed to the faster decomposition of their residues (Carvalho et al., 2009). Soil C stocks are a function of C and N inputs and the chemical composition of plant tissues and decomposition. The role of N input for increasing the potential C sequestration in tropical soils has been investigated recently. Leguminous cover crops play an important role as a N source, with positive effects on C sequestration (Bayer et al., 2006).

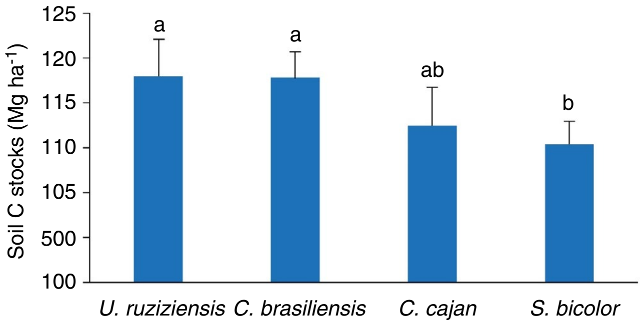

FIGURE 4 Soil carbon stocks under cover crops cultivated in succession to maize, 0- to 100-cm depth, Planaltina-Brazil

According to Cotrufo et al., (2013), *U. ruziziensis* and *C. brasiliensis* stood out among several cover crops with their high content of more labile constituents, such as hemicelluloses and lignin/N, respectively, that can be quickly converted into microbial products. In the present study, these are represented by the MBC and LC fractions, with a greater C use efficiency, which thus releases less CO_2 into the atmosphere. Therefore, these SOM fractions are the major contributors to the soil C stocks.

Schmatz et al., (2017) assessed how the crop residue quality and soil type influence the priming effect and highlighted the need to set up cropping systems with crop rotations (based on different species and botanical families) that allow for optimization of the trade-off between several ecosystem services.

In this context, the results reveal the importance of diversification of soil cover rotation with cover crops in intense crop cultivation systems to maintain soil C stocks (Chahal et al., 2020; Rosolem et al., 2016). The chemical composition of different cover crops with an appropriate N:lignin ratio is fundamental for C sequestration in the soil under systems using cover crop rotations in the Cerrado region. *Canavalia brasiliensis* and *U. ruziziensis* in succession with maize allow for increasing or maintaining soil C stocks. These results illustrate the importance of cover crop diversification (ecological intensification) in intense crop cultivation systems to maintain soil fertility and sustainability in agroecosystems.

As shown earlier, cover crops affected soil C stocks differently (Figure 4). Despite that, the correlation coefficients between the chemical composition of cover crops and soil C stocks were not significant ($p > .05$; Table S2). In the present study, carbon stocks were determined up to 100 cm deep, showing that other characteristics such as the amount of dry matter produced and the morphology of the root system are driving factors of carbon stocks at that depth.

4 | CONCLUSIONS

The sustainable development of crop productivity depends mainly on the continuous development of soil fertility renewal. The present study showed the importance of carrying out qualitative–quantitative assessments of the chemical composition of different cover plants and the soil carbon content to more accurately assess the contribution of each management system (or succession) on C dynamics in the environment. The results confirm the hypothesis that in tropical Cerrado conditions cover plants with a higher concentration of soluble fractions such as hemicelluloses and a lower concentration of lignin and lignin:N ratio promote the greater accumulation of SOM labile fractions and soluble humic substances. For *Canavalia brasiliensis*, a high N content associated with high dry matter yield resulted in higher SOM quality, expressed by the accumulation of HA and LC, and a higher HA/FA ratio. As a result of this greater accumulation of SOM fractions, *Urochloa ruziziensis* and *Canavalia brasiliensis* promoted higher soil C stocks than *S. bicolor*. The highest lignin concentrations and the highest lignin:N ratio in *Cajanus cajan* were associated with a more significant accumulation of POC, but not related to the indicators of SOM quality. Nitrogen fertilizer application increased maize grain yields in all cover crop treatments. Additionally, microbial biomass carbon and particulate organic carbon were sensitive and increased with N fertilizer application. In conclusion, it is recommended the adoption of *Urochloa ruziziensis* and *Canavalia brasiliensis* as cover plants improve maize production, soil organic matter quality and C sequestration in the Cerrado region.

DATA AVAILABILITY STATEMENT

Data available on request from the authors

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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