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THE EFFECT OF DIVERSIFIED CROP ROTATIONS ON SOIL ORGANIC CARBON DYNAMICS IN A CLAYEY TROPICAL SOIL

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ABSTRACT. This study assessed the impact of crop diversification within no-till crop rotations on soil organic carbon and total nitrogen stocks, as well as on labile (Carbon and Nitrogen in the particulate organic matter) and persistent (Carbon and Nitrogen in the mineral-associated organic matter) Organic matter fractions. The objective was to identify practical indicators for monitoring public policies promoting low carbon emission agriculture. Field experiment was conducted in 2014/2015 cropping season using a complete random block design. Seven treatments were evaluated: soybean/cotton (CONTROL); maize/soybean (M/Sb); soybean/maize+brachiaria (Sb/M+Br); soybean/millet+brachiaria/crotalaria spectabiliscotton (Sb/Mt+Br/CrsCt); soybean/cotton/common beans/millet+ brachiaria (Sb/Ct/Cb/Mt+Br); millet-cotton/ soybean/maize/crotalaria spectabilis (Mt-Ct/Sb/M/Crs); crotalaria-cotton/soybean/ sorghum+brachiaria/crotalaria ochroleuca+ brachiaria (Cr-Ct/Sb/Sg+Br/Cro+Br). Sampling was done in May 2020 on an Oxisol in a neotropical savanna of the Central West region of Brazil (Capivara Experimental Research Farm of Embrapa Rice and Beans, Santo Antônio de Goiás, Goiás State, Brazil). Treatment comparisons were made after correction for equivalent soil mass per soil layer. The C-POM, N-POM, C-MAOM, and N-MAOM fractions were obtained through granulometric physical fractionation. Total SOC and SOC stocks were inadequate indicators of the impact of crop rotations on SOC. However, the distribution of C and N among the soil organic matter (SOM) fractions (C-POM, N-POM, C-MAOM, and N-MAOM) was influenced by crop rotations. Rotations with greater crop diversity, including gramineae, had higher concentration of C and N in the particulate SOC (C-POM and N-POM). Differences in rotation composition also affected the C to N ratio, particularly in the POM fraction, which was higher in rotations involving brachiaria grass and maize. Most diversified rotations contributed to maintaining higher C-POM stocks.

Keywords: carbon and nitrogen stocks; crop rotation; mineral-associated organic matter (MAOM); Oxisol; particulate organic matter (POM); zero-tillage.

INTRODUCTION

The need to reduce the level of carbon dioxide (CO_2) in the atmosphere by both reducing its sources (anthropogenic emissions) and increasing its sinks (sequestration of atmospheric CO_2 in terrestrial plants or soils) is on the increase worldwide. Scientists are generating knowledge on the best ways to manage croplands in order to meet the growing demand for food and at the same time increase carbon storage for extended periods in cropping systems (Teluguntla *et al*., 2015). Soil organic carbon (SOC) sequestration on agricultural lands contribute to enhance soil health, improving the resilience of agricultural systems, preventing soil degradation, decreasing the costs of climate change mitigation and promoting increased food security (Hoyle *et al*., 2011).

Soil organic matter (SOM) contributes to the formation of soil chemical, physical (Madari *et al*., 2005) and biological properties (Mendes *et al*., 2005) as well as acts as major store and source of plant nutrients. Soil organic carbon (SOC) is the carbon component of SOM that is present in organic compounds of different stability, depending on the structural properties and protection of SOM (Mandal *et al*., 2008*;* Simpson *et al*., 2007). The study of SOC dynamics of agricultural soils can provide important information on how to manage such soils to increase C stocks and promote C sequestration.

The SOC is vital to sustainable agricultural productivity in tropical regions, including in savanna ecosystems. It plays an important role in maintaining the productivity of tropical soils through promoting biological diversity by providing energy and substrates for soil organisms.

Hence, SOC helps to maintain soil quality and the critical functions of agro-ecosystems. SOC has a direct influence on soil quality, due to its effect on soil properties (Wendling *et al*., 2010). Long-term crop rotation, combined with no-tillage (NT) can restore SOC levels, which can lead to improved crop yields in tropical soils (Freixo *et al*., 2002*;* Sá *et al*., 2014; Sharma *et al*., 2013*)*. SOC plays key role in earth's carbon cycle since it accounts for the largest active terrestrial pool of carbon (Le Quere *et al*., 2018). Recently SOC has been associated with sustainable strategies to mitigate the emission of greenhouse gases (GHGs) (Arunrat and Pumijumnong, 2017; Ghimire *et al*., 2017*;*).

In the agro-ecosystem, SOC retention potential of soil depends on several factors, including C input level and type, soil use and management, and vegetation type (Wiesmeier *et al*., 2019). In cash crop production systems NT, crop rotations that include cover crops or green manure, application of manure or crop/biomass residue and residue of carbonized biomass or biochar are among soil and crop management practices that potentially improve SOC (Arunrat *et al*., 2020; Boddey *et al.,* 2010; Lal, 2011). Cover crops and green manure used in this study were *Crotalaria ochroleuca*, *Crotalaria spectabilis*, millet (*Pennisetum glaucum*), and brachiaria (*Urochloa ruziziensis*).

These crops are commonly used in rotations in Brazil due to their ability to fix nitrogen, improve soil structure, suppress weeds, and provide organic matter Boddey *et al.* (2004). They also help to enhance soil fertility and contribute to sustainable agricultural practices Sánchez *et al.* (2004).

The fractionation of SOM is a useful method for detecting quantitative and qualitative changes in SOC, as well as to characterize its vulnerability to decomposition (Janzen *et al*., 1992). Fractionation procedures based on the size or density of SOM and its position within the soil structure enable the assessment of labile pools of SOC that are more sensitive to differences in soil management, land use or cropping practices than total SOC (Barrios *et al*., 1996, 1997).

Tillage and notably NT, affects many soil characteristics that may influence nutrient availability, plant growth and yield (Ernani *et al*., 2002). Under NT, the SOM accumulates in the top few centimeters over time (Díaz - Zorita and Grove, 2002; Selles *et al*., 1997).

Tillage affects soil microorganism dynamics negatively by destroying soil structure, exposing the soil to changes in temperature and the moisture regime. Furthermore, anthropogenic climate warming can increase SOC losses due to increments in soil temperature, making tillage effects even more severe. Additionally, tillage reduces soil pH and changes SOC and nitrogen cycles (Behnke *et al*., 2020). Thus, feeding the human population will require innovative ideas that involve minimizing conventional agricultural practices in order to be able to use natural ecosystem functions as much as possible. No-tillage has a potential to increase microbial biomass (Helgason *et al*., 2010), improve soil carbon (Lal *et al*., 2003), increase mineralizable N (Spargo *et al*., 2011), soil moisture (Ma *et al*., 2008) and enzyme activities (Alvear *et al*., 2005). It is clear that NT and diversification of crop species in rotation can improve the soil's physical, chemical and biological properties. In fact, combining these two agricultural practices could have positive synergistic effects and improve soil function and services (Acharya *et al*., 2012, Veloso *et al*., 2018).

Long-term experiments have increased worldwide because they are the only means of identifying suitable early warning and long-term indicators for productivity decline and ecosystem damage (Bessam and Mrabet, 2003), such as SOC stocks. Thus the objective of this study was to evaluate effects of crop rotation intensification under no-tillage on soil organic carbon (SOC) stocks and the labile and persistent C pools, of an Oxisol in a long term field experiment under edaphic and climate conditions of a neotropical savanna, in the Central West region of Brazil, in order to identify practical indicators that can be used to monitor public policies implemented to promote low carbon emission agriculture.

The specific objectives are as follows: a) Assessing SOC stocks. b) Quantifying labile and recalcitrant SOC pools. C) Identifying optimal crop rotations that facilitate SOC accumulation.

MATERIALS AND METHODS

Study area and soil sampling

The study was conducted in 2020 as part of a long-term experiment initiated in 2014 at the Experimental Farm (Capivara Farm) of Embrapa Rice and Beans in Santo Antônio de Goiás, Goiás State, Brazil, in collaboration with Embrapa Cotton. The area, preceding the study for two years (2012/2013), was occupied by Brachiaria pasture, and no information was available on soil management and corrections during that period. The soil in the experimental area is classified as an Oxisol (580 g kg^{-1} clay). The climate at the experimental station is humid tropical with distinct dry and rainy seasons (Aw). The annual mean temperature is 23°C, and the average annual precipitation has been 1503 mm over the last 35 years.

The experimental design adopted a randomized block format with four repetitions. Each experimental plot measures 12 m in width and 14 m in length. All crops were cultivated and managed under rain-fed conditions without the use of irrigation. The experiment involved crop rotations featuring maize (*Zea mays*), aerobic rice (*Oryza sativa* L), common bean (*Phaseolus vulgaris*), cotton (*Gossypium herbaceum*), soybean (*Glycine max*), and various cover crops and green manure. Soil management for all treatments followed direct planting practices, with no soil disturbance using discs, harrows, or other mechanical equipment, except in the sowing line.

Seven treatments were examined, corresponding to rotation or succession schemes for soybean, common bean, corn (as the main crop and second crop), and cotton (as the main crop and second crop). The cover crops and green manure in the rotation included Crotalaria ochroleuca, Crotalaria spectabilis, millet (*Pennisetum glaucum*), and brachiaria (*Urochloa ruziziensis*). These crops are commonly used in crop rotation in Brazil due to their ability to fix nitrogen, improve soil structure, suppress weeds, and provide organic matter Boddey *et al.* (2004). They also help to enhance soil fertility and contribute to sustainable agricultural practices Sánchez *et al.* (2004). Annually, soybeans and common beans were sown in the spring between late October and early November, with a spacing of 45 cm between lines. Corn as the main crop was sown in November, while as the second crop, after soybean harvest, typically in February, depending on the sowing date, variety cycle, and soybean harvest date. Cotton as the main crop was sown between early December and early January, and as the second crop, after common bean or soybean harvest, usually between mid-January and late February.

Soil samples were collected in 2020 the 6th year of the experiment at six soil layers (0-10, 10-20, 20-30, 30-40, 40-50, 50-60 cm). These layers were chosen considering that carbon is typically concentrated in the top 0-30 cm layers, with stabilization occurring in the deeper layers from 40-60 cm. Additionally, cropping root systems can penetrate to 1m and beyond, impacting SOC. Composite samples, consisting of three sub-samples from each plot (in each repetition), were air-dried and ground to pass a 2-mm sieve to obtain the fine earth fraction (< 2mm) of the soil. Gravels (soil particles with a diameter larger than 2 mm) were not identified in this soil. The fine fraction of the soil underwent chemical and physical analysis. A portion of each composite sample was finely ground (> 180 μm) in preparation for total soil C and N analysis. Undisturbed soil samples were collected using cylinders and an auger to determine soil bulk density (BD).

Analyses and determinations

Total soil organic C and N were determined using the Dumas method with a Perkin Elmer CHNO/S 2400 II Analyzer. This analyzer utilizes high-purity gases such as Oxygen (for the combustion chamber) and Helium (transport gas). For analysis, 10-15 mg of finely ground (>180 μm) soil samples were weighed in a tin capsule using an ultra-microbalance. Quality control was maintained using Acetanilide and soil standards. Each sample underwent duplicate analysis, and the mean was considered the result. The furnace temperature was set at 971°C, and the reduction column temperature was maintained at 600°C.

Soil fertility analyses and soil texture were determined as follows: soil pH was measured using the electrode method (Thomas, 1996); phosphorus (P), calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^{+}) were extracted using diluted concentrations of strong acids (0.05 mol L^{-1} HCl +0.0125 mol L^{-1} H_2SO_4; Mehlich I) (Kuo, 1996). Phosphorus was determined colorimetrically, Ca^{2+} and Mg^{2+} by atomic spectroscopy, and K^{+} by flame emission spectrometry (Wright and Stuczynski, 1996). Aluminum (Al^{3+}) was extracted using a KCl solution and titrated with NaOH (Bertsch and Bloom, 1996). Soil texture was determined using a hydrometer method with a standard hydrometer Bouyoucos scale (Gee and Bauder, 1986).

For bulk density (BD) analysis, undisturbed soil samples were collected using a metal cylindrical tube with a diameter and height of 5 cm. The ratio of oven-dry weight of the soil (dried at 105°C for 24 h) core to its total volume yielded BD (kg dm^{-3}).

Soil carbon stock was calculated using the BD of the whole soil (FAO, 2019) (*Equation 1*):

$$SOCi\ stock\ (Mg ha^{-1}\ C) = \\ = OCi x BDi x (1 - gGi)\ x\ ti x\ 0.1 \quad (1)$$

where:

- *SOCi* (Mg ha^{-1} C) is the soil organic carbon stock of depth increment *i;*
- OC*i* (mg g^{-1} C) is the organic carbon content of the fine earth fraction (< 2 mm) of the depth increment *i;*
- BD*i* (g cm^{-3}) is the mass of soil per total volume of the soil sample of the depth increment*i;*
- gG*i* (g g^{-1}) is the mass fraction of coarse mineral fragment, thus (1-gG*i*) is the mass fraction fine earth (g fine earth g^{-1} soil) of the depth increment *i;*

- *ti* is the thickness (depth, in cm) of the depth increment *i;*
- and 0.1 is a factor for converting mg cm^{-2}C to Mg ha^{-1} C.

Soil organic carbon stocks were calculated after equivalent soil mass correction, widely used in the literature (Sisti *et al.*, 2004), for the 0-30 and 0-60 cm soil layers.

Soil organic carbon fractionation

Physical fractionation of soil carbon, was performed by adapting the method described by Cambardella and Elliot (1992) and Cotrufo *et al.* (2019), calibrated for the specific soil of the experiment. This method separates the total organic matter into two fractions, particulate organic matter (POM) and organic matter associated with minerals (MAOM), which intimately attaches to the clay and silt mineral fractions.

Five grams of fine earth were placed in 250 mL plastic bottles, and 30 mL of sodium hexametaphosphate at a concentration of 5.0 g L^{-1} was added. The mixture was supplemented with twelve glass beads to enhance dispersion and subjected to ultrasonic dispersion in a horizontal shaker at 130 oscillations min^{-1}. This process allowed optimal soil disaggregation due to the very high clay content of 580 g kg-1. The resulting soil was then digested in a 53 µm sieve and washed with a weak jet of distilled water. The total particulate organic matter retained on the sieve (> 53 µm) was dried at 60°C, then ground in a porcelain mortar and passed through a 0.149 mm sieve, and analyzed for C and N concentration (%) using an elemental analyzer (LECO TruSpec CN). Stocks (Mg ha^{-1}) of particulate (POC) and mineral (MAOC) fractions of C and N were calculated as described above.

The soil C/N ratio was calculated according with Cotrufo *et al.* (2019) that applied mass balance approach (*Equation 2*):

$$C/N_{SOM}=C/N_{MAOM} \times f_{MAOM}+ \\ +C/N_{POM} \times (1-f_{MAOM}) \qquad (2)$$

where C/N_{SOM}, C/N_{POM} and C/N_{MAOM} are the C/N ratios of SOM, POM and MAOM, respectively, and f_{MAOM} is the MAOM proportion of SOM.

Statistical analysis

The experiment was designed as a Randomized Complete Block with 4 blocks. The PROC MIXED model (linear mixed model) was employed for the analysis of variance, with blocks considered as a random effect in the model. Treatment means were compared to the CONTROL using the Dunnett test, and differences were considered significant at $p \leq 0.10$.

RESULTS AND DISCUSSION

Soil texture and bulk density

The retention of Soil Organic Matter (SOM) and, consequently, Soil Organic Carbon (SOC) is influenced by various soil and environmental factors. In the agro-ecosystem, SOC retention potential depends on factors such as C input level and type, soil use and management, and vegetation type (Wiesmeier *et al*., 2019). Soil texture is a crucial factor affecting carbon retention due to differences in the specific surface area of soil fractions. The specific surface area of clay is higher than that of sand or silt, impacting the soil's carbon sequestration potential (Zinn *et al*., 2005).

In this study, we assessed the comparability of soil treatments by examining soil clay levels, given that clay dominates the soil texture. Statistical analysis revealed differences between the CONTROL and other treatments in four out of 42 cases, considering treatment and soil depth combinations. In these instances, the treatments exhibited higher clay content (6-10% higher) compared to the CONTROL. Notably, variations in clay content were observed at 0-10 cm, 20-30 cm, and 40-50 cm depths. Treatments including Mt-Ct/Sb/M/Crs and Crs-Ct/Sb/Sr+Br/Crs+Br showed higher clay content compared to the CONTROL at 0-10 cm and 40-50 cm depths. At 20-30 cm depth, Sb/M+Br exhibited the lowest clay content among treatments, while Crs-Ct/Sb/Sr+Br/Crs +Br showed the highest (*Table 1*).

Although literature suggests that increased diversity in crop rotation may raise soil clay content by enhancing soil organic matter return, this study did not consistently observe such changes. Considering the low occurrence and small magnitude of changes in clay content, we considered the treatments comparable to the CONTROL.

Soil bulk density (BD) is influenced by soil properties, including mineralogy, chemical interactions, biological activity, and composition. Soil management and plant cover, however, have a more immediate impact on BD. Differences in BD between the CONTROL and treatments were expected, particularly in soil layers directly affected by practices and plant root systems. BD is also a crucial factor affecting SOC stocks. In this study, BD varied among treatments compared to the CONTROL at 40-50 cm and 50-60 cm soil depths. At 40-50 cm, Sb/M+Br/Cro-Ct and Mt-Ct/Sb/M/Crs differed significantly from the CONTROL, exhibiting lower BD values. At 50-60 cm depth, all treatments varied significantly, with the CONTROL showing the highest BD and M/Sb and Crs-Ct/Sb/Sr+Br/Crs+Br exhibiting the lowest values (*Table 2*).

Diversified rotations resulted in lower BD values, a trend observed in other studies. More diversified rotations tend to enhance SOC content and reduce soil compactness. Liu *et al*. (2011) demonstrated that a wheat-sweet clover rotation increased SOC content and decreased soil bulk density. Similarly, Ibrahim *et al*. (2015) reported that a 4-year rotation system and no-tillage significantly increased SOM compared to a 2-year rotation and conventional tillage. These results suggest that diversified rotations contribute to improved soil structure and reduced soil compaction, ultimately enhancing SOC stocks. The observed decrease in BD under more diverse rotations aligns with the positive impact of crop diversity on microbial activity, SOM content, and aggregate stability in the soil profile.

Soil carbon and nitrogen concentrations

Table 3 and *Table 4* report the concentrations of total Soil Organic Carbon (SOC) and total Nitrogen (TN), respectively. The concentration of carbon showed no significant variation among the treatments at various depths, except at 0-10 cm where the Sb/M+Br treatment varied from the CONTROL, exhibiting the highest carbon concentration (3.08%).

Table 1 – Clay content in six soil layers of an Oxisol cultivated with a succession of soybean/cotton (CONTROL) and with biodiversity Intensification for the soy/cotton system at six years (2020) after implementation of the field experiment in Santo Antônio de Goiás, GO, Brazil

Treatments	Soil layers (cm)											
	0-10		10-20		20-30		30-40		40-50		50-60	
	Estimate	*p-value*	Estimate	*p-value*	Estimate	*p-value*	Estimate	*p-value*	Estimate	*p-value*	Estimate	*p-value*
	CLAY CONTENT (g kg^{-1})											
CONTROL	513	*NA*	583	*NA*	620	*NA*	620	*NA*	630	*NA*	665	*NA*
M/Sb	538	*0.35*	603	*0.464*	600	*0.301*	670	*0.196*	680	*0.191*	670	*0.814*
Sb/M+Br	508	*0.859*	578	*0.854*	**580***	*0.047*	620	*1.000*	675	*0.237*	680	*0.483*
Sb/M+Br/Cro-Ct	538	*0.35*	593	*0.713*	610	*0.601*	630	*0.791*	670	*0.291*	640	*0.248*
Sb/Ct/Cb/Mt+Br	543	*0.269*	593	*0.713*	605	*0.435*	620	*1.000*	675	*0.237*	650	*0.483*
Mt-Ct/Sb/M/Crs	**563***	*0.073*	613	*0.277*	620	*1.000*	615	*0.895*	680	*0.191*	675	*0.639*
Crs-Ct/Sb/Sr+Br/Crs+Br	**558***	*0.104*	628	*0.11*	630	*0.601*	660	*0.297*	**705***	*0.056*	670	*0.814*
Std. Error	*26*		*27*		*19*		*37*		*37*		*21*	

Table 2 – Soil bulk density in six soil layers of an Oxisol cultivated with a succession of soy/cotton (CONTROL) and with biodiversity intensification for the soy/cotton system at six years (2020) after implementation of the field experiment in Santo Antônio de Goiás, GO, Brazil

Treatments	Soil layers (cm)											
	0-10		10-20		20-30		30-40		40-50		50-60	
	Estimate	*p-value*	Estimate	*p-value*	Estimate	*p-value*	Estimate	*p-value*	Estimate	*p-value*	Estimate	*p-value*
	BULK DENSITY (g cm^{-3})											
CONTROL	1.233	*NA*	1.310	*NA*	1.388	*NA*	1.478	*NA*	1.493	*NA*	1.538.	*NA*
M/Sb	1.248	*0.876*	1.290	*0.759*	1.360	*0.597*	1.448	*0.580*	1.478	*0.782*	**1.340***	*0.000*
Sb/M+Br	1.220	*0.897*	1.323	*0.848*	1.428	*0.444*	1.438	*0.462*	1.480	*0.817*	**1.403***	*0.008*
Sb/M+Br/Cro-Ct	1.230	*0.979*	1.308	*0.969*	1.320	*0.203*	1.453	*0.644*	**1.398***	*0.092*	**1.388***	*0.004*
Sb/Ct/Cb/Mt+Br	1.255	*0.815*	1.250	*0.362*	1.345	*0.416*	1.460	*0.746*	1.418	*0.177*	**1.408***	*0.010*
Mt-Ct/Sb/M/Crs	1.323	*0.356*	1.355	*0.492*	1.418	*0.564*	1.425	*0.337*	**1.378***	*0.045*	**1.438***	*0.039*
Crs-Ct/Sb/Sr+Br/Crs+Br	1.298	*0.502*	1.225	*0.202*	1.310	*0.147*	1.435	*0.435*	1.403	*0.109*	**1.365***	*0.001*
Std. Error	*0.095*		*0.064*		*0.051*		*0.053*		*0.053*		*0.045*	

Table 3 – Carbon concentrations, in six soil layers of an Oxisol cultivated with a succession of soy/cotton (CONTROL) and with biodiversity intensification for the soy/cotton system at six years (2020) after implementation of the field experiment, in Santo Antônio de Goiás, GO, Brazil

Treatments	Soil layers (cm)											
	0-10		10-20		20-30		30-40		40-50		50-60	
	Estimate	*p-value*	Estimate	*p-value*	Estimate	*p-value*	Estimate	*p-value*	Estimate	*p-value*	Estimate	*p-value*
	C CONCENTRATION (%)											
CONTROL	2.313	*NA*	1.723	*NA*	1.270	*NA*	1.035	*NA*	0.805	*NA*	0.713	*NA*
M/Sb	2.805	*0.274*	1.615	*0.667*	1.378	*0.559*	1.093	*0.803*	0.753	*0.794*	0.585	*0.285*
Sb/M+Br	**3.080***	*0.096*	1.535	*0.455*	1.223	*0.796*	1.165	*0.574*	0.900	*0.638*	0.600	*0.344*
Sb/M+Br/Cro-Ct	2.495	*0.681*	1.680	*0.865*	1.020	*0.183*	1.140	*0.649*	0.900	*0.638*	0.748	*0.766*
Sb/Ct/Cb/Mt+Br	3.000	*0.132*	1.635	*0.726*	0.233	*0.838*	1.108	*0.753*	0.675	*0.521*	0.563	*0.211*
Mt-Ct/Sb/M/Crs	2.540	*0.608*	1.783	*0.810*	1.280	*0.956*	1.113	*0.737*	0.923	*0.561*	0.693	*0.865*
Crs-Ct/Sb/Sr+Br/Crs+Br	2.295	*0.968*	1.543	*0.473*	0.015	*0.175*	1.045	*0.965*	0.980	*0.389*	0.565	*0.219*
Std. Error	*0.436*		*0.246*		*0.181*		*0.227*		*0.198*		*0.116*	

Table 4 – Nitrogen concentrations, in six soil layers of an Oxisol cultivated with a succession of soy/cotton (CONTROL) and with biodiversity intensification for the soy/cotton system at six years (2020) after implementation of the field experiment, in Santo Antônio de Goiás, GO, Brazil

Treatments	Soil layers (cm)											
	0-10		10-20		20-30		30-40		40-50		50-60	
	Estimate	*p-value*	Estimate	*p-value*	Estimate	*p-value*	Estimate	*p-value*	Estimate	*p-value*	Estimate	*p-value*
	N CONCENTRATION (%)											
CONTROL	0.170	*NA*	0.125	*NA*	0.085	*NA*	0.085	*NA*	0.055	*NA*	0.055	*NA*
M/Sb	0.213	*0.238*	0.120	*0.800*	0.098	*0.385*	0.073	*0.912*	0.050	*0.723*	0.038	*0.752*
Sb/M+Br	0.228	*0.116*	0.115	*0.613*	0.098	*0.385*	0.083	*0.982*	0.060	*0.723*	0.038	*0.752*
Sb/M+Br/Cro-Ct	0.188	*0.621*	0.125	*1.000*	0.075	*0.486*	0.078	*0.947*	0.065	*0.480*	0.050	*0.928*
Sb/Ct/Cb/Mt+Br	0.220	*0.168*	0.123	*0.899*	0.093	*0.600*	0.073	*0.912*	0.045	*0.480*	0.140	*0.137*
Mt-Ct/Sb/M/Crs	0.188	*0.621*	0.128	*0.899*	0.090	*0.726*	**0.293***	*0.078*	0.065	*0.480*	0.075	*0.719*
Crs-Ct/Sb/Sr+Br/Crs+Br	0.175	*0.887*	0.113	*0.528*	0.070	*0.300*	0.070	*0.894*	0.068	*0.379*	0.058	*0.964*
Std. Error	*0.035*		*0.019*		*0.014*		*0.111*		*0.014*		*0.055*	

This was followed by Sb/Ct/Cb/Mt+Br (3.00%), while the lowest value was observed in Crs-Ct/Sb/Sr+Br/Crs+Br (2.29%). Literature suggests that more diverse crop rotations tend to have higher soil carbon and microbial biomass, especially with the inclusion of cover crops (McDaniel *et al*., 2014).

However, the study did not observe significant effects of diversified crop rotations on carbon concentration, except for the Sb/M+Br treatment at 0-10 cm, which included Brachiaria grass known for its ability to sequester and accumulate substantial amounts of SOC.

Nitrogen concentration exhibited significant variation only at the 30-40 cm depth, where the Mt-Ct/Sb/M/Crs treatment differed from the CONTROL, showing the highest nitrogen value. In other layers, treatments did not affect soil TN concentrations. In diversified rotations involving a mix of grasses, leguminous, and non-leguminous cover crops, it would be expected that nitrogen concentration increases due to the influence of crop type on residue mass and quality. Both residue quality and mass contribute to nitrogen immobilization in no-tillage systems, influencing SOC stock changes. The incorporation of legumes into pasture has been shown to stimulate nitrogen accumulation, emphasizing the importance of crop type in nitrogen supply and biomass production.

McDaniel *et al*., (2014) observed that cover crop incorporation within diversified crop rotations can increase SOC and TN, particularly when leguminous cover crops are present for longer periods. Soil quality improvement and conservation benefits, including nitrogen supply and carbon sequestration, are associated with a mixture of grain crops, leguminous, and non-leguminous cover crops.

Soil organic carbon and total nitrogen stocks

Table 5 and *Table 6* present SOC and TN stocks, with no significant difference observed between the treatments and CONTROL at the three soil layers (0-30 cm, 30-60 cm and 0-60 cm). The experiment, initiated in the 2014/2016 planting season, was in its sixth year at the time of sampling in May 2020. Slow SOC changes, even under moderate conditions, can be challenging to detect statistically, as observed in other long-term experiments. The results align with data from Denmark and England, revealing slow changes in SOC levels under temperate conditions in response to alterations in land use.

For nitrogen stocks, all treatments showed lower N stock compared to the CONTROL at the 0-30 cm layer, except for Mt-Ct/Sb/M/Crs, which exhibited the highest value. At the 30-60 cm layer, all treatments significantly differed from the CONTROL, with lower nitrogen values. The Soy/Cotton (CONTROL) treatment had the highest nitrogen value, followed by Mt-Ct/Sb/M/Crs, and the lowest was in Soy/Corn.

Similarly, at the 0-60 cm layer, all treatments showed significant variation from the CONTROL, with the highest value in the Soy/Cotton (CONTROL) treatment, followed by Mt-Ct/Sb/M/Crs, and the lowest in M/Sb. The lower nitrogen stocks in the treatments compared to the CONTROL may explain the lack of explicit carbon accumulation relative to the CONTROL. Nitrogen controls soil carbon accumulation through their C:N ratio, which typically falls between 8 and 16. Although a mostly positive difference in SOC accumulation between the CONTROL and the treatments was observed, it was not statistically significant at the chosen probability level (10%).

The M/Sb treatment exhibited the highest positive difference compared to the CONTROL in both the 0-30cm and 0-60cm layers, while the Crs-Ct/Sb/Sr+Br/Crs+Br treatment recorded the highest negative difference to the CONTROL. At the 30-60cm layer, Sb/M+Br/Cro-Ct presented the highest value, whereas Sb/Ct/Cb/Mt+Br had the lowest (negative) value, followed by Crs-Ct/Sb/Sr+Br/Crs+Br.

Numerous studies have illustrated that no-till practices can enhance soil carbon, particularly at the soil surface (West and Post, 2002). Moreover, research indicates that this rise in carbon is linked to increased soil aggregation (Cambardella and Elliott, 1992; Madari *et al*., 2005; Six *et al*., 2000). Melero *et al.* (2011) and Onunwa *et al.* (2020) observed that management practices influence the balance between input and output in a system, impacting the rate of organic matter decomposition. Wright and Hons (2005) concluded that employing appropriate land use and management practices, such as no-till (NT) and crop rotations, is crucial for enhancing carbon sequestration and accumulation potential in croplands.

Conversely, practices involving no soil disturbance, coupled with high input of crop residues from cover crops like brachiaria and green manure (*C. ochroleuca*), are effective management approaches for promoting soil carbon sequestration (Bayer and Dieckow, 2020).

In general, treatments involving both leguminous and non-leguminous cover crops, especially soy and brachiaria, exhibited the highest carbon stock across various depths (0-30cm, 30-60cm, and 0-60cm). The elevated carbon stock in the upper 30cm signifies substantial carbon storage in this soil layer, particularly in NT systems combined with crop rotation.

However, this stored carbon is susceptible to loss if the upper 30cm soil layer is disturbed. Additionally, the presence of a high carbon stock in the 30-60cm layer emphasizes the importance of this depth in carbon storage.

Conversely, the existence of soil organic carbon (SOC) in the lower (0-60 cm) soil layers indicates the significance of deeper layers in preserving soil organic carbon over extended periods (persistence of SOC).

Several studies have reported the presence of relatively more SOC in upper soil layers compared to lower or deeper layers (Grüneberg *et al*., 2010; Ehrenbergerová *et al*., 2016; Zádorová *et al*., 2015). Crop rooting depth and root mass likely influenced cumulative SOC stocks at depth (Fan *et al.*, 2016). Soil organic carbon at deeper soil depths is likely derived more from root inputs than from aboveground biomass, although C allocation from aboveground biomass through bioturbation and possibly preferential flow also plays a role in soil C dynamics (Doran *et al*., 1984; Kätterer *et al*., 2011; Wilts *et al*., 2004).

Table 5 – Carbon(COS) stocks and COS accumulation in three soil layers of an Oxisol cultivated with a succession of soy/cotton (CONTROL) and with biodiversity intensification for the soy/cotton system at six years (2020) after implementation of the field experiment, in Santo Antônio de Goiás, GO, Brazil

Treatments	Soil layers (cm)											
	0-30			30-60			0-60			0-30	30-60	0-60
	Estimate	*Std. Error*	*p-value*	Estimate	*Std. Error*	*p-value*	Estimate	*Std. Error*	*p-value*			
	COS Stocks (Mg ha^{-1})									COS (Mg ha^{-1}) accumulation relative to control**		
CONTROL	68.683	*6.633*	*NA*	38.203	*4.187*	*NA*	106.890	*9.333*	*NA*	*NA*	*NA*	*NA*
M/Sb	81.573	*7.660*	*0.222*	38.547	*4.835*	*0.958*	120.120	*10.777*	*0.367*	12.9	0.3	13.2
Sb/M+Br	74.408	*6.633*	*0.550*	39.970	*4.187*	*0.769*	114.380	*9.333*	*0.578*	5.7	1.8	7.5
Sb/M+Br/Cro-Ct	67.223	*6.633*	*0.878*	41.585	*4.187*	*0.576*	108.810	*9.333*	*0.886*	-1.5	3.4	1.9
Sb/Ct/Cb/Mt+Br	75.620	*6.633*	*0.470*	34.300	*4.187*	*0.519*	109.920	*9.333*	*0.821*	6.9	-3.9	3.0
Mt-Ct/Sb/M/Crs	74.173	*6.633*	*0.567*	40.923	*4.187*	*0.652*	115.100	*9.333*	*0.543*	5.5	2.7	8.2
Crs-Ct/Sb/Sr+Br/Crs+Br	59.660	*7.660*	*0.386*	34.833	*4.835*	*0.606*	94.490	*10.777*	*0.397*	-9.0	-3.4	-12.4

Table 6 – Nitrogen(TN) stocks and TN accumulation in three soil layers of an Oxisol cultivated with a succession of soy/cotton (CONTROL) and with biodiversity intensification for the soy/cotton system at six years (2020) after implementation of the field experiment, in Santo Antônio de Goiás, GO, Brazil

Treatments	Soil layers (cm)											
	0-30			30-60			0-60			0-30	30-60	0-60
	Estimate	*Std. Error*	*p-value*	Estimate	*Std. Error*	*p-value*	Estimate	*Std. Error*	*p-value*			
	TN Stocks (Mg ha^{-1})									TN (Mg ha^{-1}) accumulation relative to control**		
CONTROL	7.208	*0.424*	*NA*	9.485	*0.392*	*NA*	16.690	*0.619*	*NA*	*NA*	*NA*	*NA*
M/Sb	**1.560***	*0.489*	*<.0001*	**1.437***	*0.452*	*<.0001*	**2.997***	*0.714*	*<.0001*	-5.6	-8.0	-13.7
Sb/M+Br	**2.355***	*0.424*	*<.0001*	**1.623***	*0.392*	*<.0001*	**3.975***	*0.619*	*<.0001*	-4.9	-7.9	-12.7
Sb/M+Br/Cro-Ct	**2.513***	*0.424*	*<.0001*	**2.280***	*0.392*	*<.0001*	**4.795***	*0.619*	*<.0001*	-4.7	-7.2	-11.9
Sb/Ct/Cb/Mt+Br	**4.728***	*0.424*	*0.001*	**5.043***	*0.392*	*<.0001*	**9.765***	*0.619*	*<.0001*	-2.5	-4.4	-6.9
Mt-Ct/Sb/M/Crs	7.920	*0.424*	*0.252*	**5.550***	*0.392*	*<.0001*	**13.470***	*0.619*	*0.002*	0.7	-3.9	-3.2
Crs-Ct/Sb/Sr+Br/Crs+Br	**3.823***	*0.489*	*<.0001*	**3.473***	*0.452*	*<.0001*	**7.300***	*0.714*	*<.0001*	-3.4	-6.0	-9.4

Experimental design: Completely Randomized Block with 4 blocks; block are random effect in linear mixed model; *Std. Error* = standard error of the differences of least squaresmeans(n = 4); *p-value* = nominal significance level is given by contrasts between treatments and CONTROL by the Dunnett test; *NA*= not applicable. *Estimate is significantly different from CONTROL ($p \leq 0.1$). **Statistical analysis not applied. Sb: Soybean (*Glycine max* L.); Cb: Common-bean (*Phaseolus vulgaris* L.); M: Maize (*Zea mays* L.); Br: Brachiaria (*Urochloa ruziziensis*); Cro: *Crotalaria ochroleuca;* Crs: *Crotalaria spectabilis;* Mt: Millet (*Pennisetum glaucum*); Ct: Cotton (*Gossypium herbaceum*); Sr: Sorghum (*Sorghum bicolor*).

Particulate and mineral-associated soil organic carbon and nitrogen

In the 0-10 cm layer, both particulate carbon (C-POM) and nitrogen (N-POM) content were higher in two treatments compared to the CONTROL (Soy/Cotton), namely Sb/Ct/Cb/Mt+Br (1.67 g kg^{-1} and 0.104 g kg^{-1}) and Crs-Ct/Sb/Sr+Br/Crs+Br (1.498 g kg^{-1} and 0.093 g kg-1), as opposed to the CONTROL (0.812 g kg^{-1} and 0.051 g kg^{-1}) (*Table 9*). For both C and N of mineral-associated organic matter (C-MAOM and N-MAOM), only one treatment varied from the CONTROL, exhibiting the highest values (29.927 g kg^{-1} and 2.213 g kg-1, respectively), while Crs-Ct/Sb/Sr+Br/Crs+Br showed the lowest values for both C-MAOM and N-MAOM (21.403 g kg^{-1} and 1.632 g kg^{-1}). Regarding the C to N ratio of POM (CN-POM), Sb/M+Br/Cro-Ct (24.875) varied from the CONTROL (16.186), having the highest value, while the CONTROL itself exhibited the lowest value.

No variation was observed, compared to the CONTROL, in the C to N ratio of the whole soil or of the MAOM. Similar to the C-POM content, the C-POM stock was higher in the Sb/Ct/Cb/Mt+Br and Crs-Ct/Sb/Sr+Br/Crs+Br treatments than in the CONTROL. There was no effect of the treatments on the C-MAOM stocks in this layer (*Table 7*).

In the 10-20cm layer, only one treatment, Sb/Ct/Cb/Mt+Br, showed variation from the CONTROL for both C-POM and N-POM, exhibiting the highest values (1.162 g kg^{-1} and 0.069 g kg^{-1}). For CN-POM, Sb/M+Br/Cro-Ct (24.750) and Sb/M+Br (22.875) showed variation with the highest values, while the CONTROL had the lowest value (15.572). Similar to the C-POM concentration, the C-POM stock was also higher in the Sb/Ct/Cb/Mt+Br treatment than in the CONTROL, and similarly to the previous layer, there was no effect of the treatments on the C-MAOM stocks (*Table 7*).

In the 20-30cm layer, Sb/Ct/Cb/Mt+Br (0.613 g kg^{-1}), Mt-Ct/Sb/M/Crs (0.612 g kg^{-1}), and Crs-Ct/Sb/Sr+Br/Crs+Br (0.567 g kg^{-1}) showed significant variation from the CONTROL (0.394 g kg^{-1}) for C-POM, with higher values. The highest value was observed in Sb/Ct/Cb/Mt+Br, while the CONTROL exhibited the lowest value. For N-POM, only Sb/Ct/Cb/Mt+Br (0.036 g kg^{-1}) differed from the CONTROL (0.019 g kg^{-1}), exhibiting the highest value, while the CONTROL showed the lowest. Only Sb/M+Br differed from the CONTROL for CN-POM (25.438) and CN-MAOM (12.664), representing the highest value for CN-POM while the lowest for CN-MAOM. The C stock in the POM fraction of the more diversified rotations (Sb/Ct/Cb/Mt+Br, Mt-Ct/Sb/M/Crs, and Crs-Ct/Sb/Sr+Br/Crs+Br) increased (0.822, 0.859, and 0.740 Mg ha^{-1}, respectively) compared to the CONTROL (0.547 Mg ha^{-1}). However, the C stock in the C-MAOM fraction of one of the diversified rotations (Crs-Ct/Sb/Sr+Br/Crs+Br, 12.491 Mg ha^{-1}) decreased compared to the CONTROL (17.023 Mg ha^{-1}) (*Table 7*).

At 30-40cm, there were no significant differences across treatments for C-POM, C-MAOM, and CN-POM. The N-POM, only in the case of Sb/M+Br, was higher than the

CONTROL. The N-MAOM was higher than the CONTROL only in the Mt-Ct/Sb/M/Crs treatment. Several treatments had higher C to N ratios in the whole soil and the MAOM fraction compared to the CONTROL, mainly those with corn or brachiária in the rotation. There was no difference in C-POM and C-MAOM stocks between the CONTROL and the treatments (*Table 7*).

At 40-50cm, the difference between the CONTROL and the other treatments for the C to N ratio in the whole soil and the MAOM fraction was no longer present.

At 50-60cm, almost all the treatments differed from the CONTROL, except Sb/Ct/Cb/Mt+Br and Mt-Ct/Sb/M/Crs, with low values for C-POM and N-POM. The highest value was observed in Sb/M+Br/Cro-Ct (1.808 g kg^{-1}C-POM), while the CONTROL exhibited the lowest value (0.184 g kg^{-1}C-POM). Simultaneously, C-MAOM decreased in the M/Sb, Sb/M+Br, and Crs-Ct/Sb/Sr+Br/Crs+Br treatments. The C to N ratio of the POM increased in all treatments (from 13.063 to 16.844) compared to the CONTROL (10.333); however, there was no effect on CN-MAOM or CN-Soil. The C-POM stock increased, and the C-MAOM stock decreased in various treatments relative to the CONTROL (*Table 7*).

In the soil, although the proportion of Particulate Organic Matter (POM) associated with the sand fraction was higher in the bulk soil, the majority of Soil Organic Carbon (SOC) stock was situated in the Mineral-Associated Organic Matter (MAOM) fraction due to higher MAOM-C concentration than POM-C concentration. This phenomenon may be attributed to the soil texture; clayey soils rich in Fe and Al oxides, as well as kaolinite, contribute to enhancing organic carbon stability through the formation of organo-mineral complexes (Roscoe and Buurman, 2003). This aligns with previous studies (Bol *et al*., 2009; Flessa *et al*., 2008) reporting that over 88% of SOC is found in the silt and clay fraction. Guo et al. (2019) noted that in a Vertisol, 74%–92% of SOC was associated with MAOM. From a climate change perspective, a larger carbon pool in MAOM is more intriguing, as it represents the soil's most stable and long-term carbon reservoir compared to POM (Lavelle *et al*., 2020).

SOC is a complex mixture of heterogeneous organic materials that can be separated into particulate or light fraction and heavy fraction, free and occluded, based on chemical-physical characteristics and localization within the soil structure (Gregorich and Ellert, 1993; Janzen *et al*., 1992; Mendonça and Matos, 2017; Sohi *et al*., 2001). Agricultural practices affect the light or free fraction more rapidly and sensitively than the heavy, mineral-associated fraction, as observed in this study. The light fraction mainly comprises plant residues, small animals, and microorganisms, providing substrates for microbial activity and acting as cohesion material to bind soil aggregates (Mueller *et al*., 1998; Sohi *et al*., 2005). Diversified rotations, particularly those including gramineous cover crops like brachiaria grass and millet, demonstrated improved Particulate Organic Matter (C-POM and N-POM) in the upper soil layers (0-10, 10-20, 20-30 cm).

Table 7 – C and N concentrations and C and N stocks of the particulate and mineral associated SOM fractions in an Oxisol cultivated with a succession of soybean/cotton (CONTROL) and with biodiversity intensification for the soybean/cotton system at six years (2020) after implementation of the field experiment, in Santo Antônio de Goiás, GO, Brazil

Treatments	SOM fractions									
	C-POM	N-POM	C-MAOM	N-MAOM	CN-POM	CN-MAOM	f(MAOM/CT)	CN-Soil	C-POM-Stock	C-MAOM-Stock
	g kg^{-1}								Mg ha^{-1}	
					0-10 cm					
CONTROL	0.812	0.051	22.275	1.637	16.186	13.543	0.964	13.637	1.001	27.563
M/Sb	0.717	0.040	27.308	2.072	18.375	13.169	0.975	13.300	0.899	34.319
Sb/M+Br	0.861	0.050	**29.927***	**2.213***	18.486	13.608	0.973	13.741	1.045	36.381
Sb/M+Br/Cro-Ct	1.120	0.049	24.493	1.876	**24.875***	13.162	0.953	13.708	1.429	31.037
Sb/Ct/Cb/Mt+Br	**1.670***	**0.104***	28.330	2.071	16.072	13.893	**0.942***	14.015	**2.086***	35.703
Mt-Ct/Sb/M/Crs	1.092	0.071	24.296	1.804	16.517	13.510	0.958	13.613	1.416	32.282
Crs-Ct/Sb/Sr+Br/ Crs+Br	**1.498***	**0.093***	21.403	1.632	16.544	13.082	**0.935***	13.289	**1.989***	28.055
Std.Error	*0.221*	*0.018*	*4.220*	*0.331*	*1.999*	*0.502*	*0.009*	*0.458*	*0.309*	*6.493*
					10-20 cm					
CONTROL	0.516	0.036	16.660	1.189	15.572	14.050	0.970	14.082	0.682	21.867
M/Sb	0.722	0.034	15.403	1.142	18.375	13.668	0.954	14.605	0.936	19.915
Sb/M+Br	0.558	0.026	14.755	1.112	**22.875***	13.133	0.961	13.492	0.736	19.472
Sb/M+Br/Cro-Ct	0.484	0.020	16.291	1.205	**24.750***	13.548	0.968	13.910	0.623	21.317
Sb/Ct/Cb/Mt+Br	**1.162***	**0.069***	15.164	1.132	17.308	13.383	**0.931***	13.645	**1.458***	18.933
Mt-Ct/Sb/M/Crs	0.597	0.036	17.229	1.227	17.063	14.126	0.966	14.227	0.808	23.417
Crs-Ct/Sb/Sr+Br/Crs+Br	0.682	0.042	14.706	1.071	16.406	13.806	0.956	13.944	0.854	18.092
Std.Error	*0.185*	*0.014*	*2.399*	*0.192*	*2.749*	*0.544*	*0.010*	*0.704*	*0.245*	*3.536*

Table 7 (continued)

Treatments	SOM fractions									
	C-POM	N-POM	C-MAOM	N-MAOM	CN-POM	CN-MAOM	f(MAOM/CT)	CN-Soil	C-POM-Stock	C-MAOM-Stock
	g kg^{-1}								Mg ha^{-1}	
	20-30 cm									
CONTROL	0.394	0.019	12.257	0.832	19.542	14.774	0.969	15.050	0.547	17.023
M/Sb	0.409	0.024	13.342	0.927	17.583	14.649	0.967	14.756	0.558	18.016
Sb/M+Br	0.489	0.021	11.711	0.942	**25.438***	**12.664***	0.955	13.538	0.696	16.716
Sb/M+Br/Cro-Ct	0.484	0.024	9.678	0.714	17.875	13.957	**0.949***	14.370	0.647	12.782
Sb/Ct/Cb/Mt+Br	**0.613***	**0.036***	11.688	0.852	16.875	13.669	**0.949***	13.863	**0.822***	15.719
Mt-Ct/Sb/M/Crs	**0.612***	0.027	12.175	0.848	22.833	14.573	**0.951***	14.983	**0.859***	17.344
Crs-Ct/Sb/Sr+Br/Crs+Br	**0.567***	0.026	9.546	0.649	22.396	14.696	**0.944***	15.115	**0.740***	**12.491***
Std.Error	*0.077*	*0.006*	*1.808*	*0.143*	*2.431*	*0.825*	*0.009*	*0.910*	*0.104*	*2.570*
	30-40 cm									
CONTROL	0.401	0.021	9.937	0.817	19.000	12.574	0.957	12.857	0.594	14.580
M/Sb	0.276	0.019	10.625	0.694	15.333	**15.588***	0.972	**15.573***	0.399	15.494
Sb/M+Br	0.515	**0.041***	11.123	0.759	16.500	14.717	0.955	14.571	0.743	15.891
Sb/M+Br/Cro-Ct	0.400	0.020	10.963	0.743	19.833	**15.228***	0.963	**15.432***	0.592	15.755
Sb/Ct/Cb/Mt+Br	0.489	0.305	10.561	0.682	16.146	**16.292***	0.954	**16.240***	0.715	15.385
Mt-Ct/Sb/M/Crs	0.371	0.019	10.742	**2.894***	20.542	14.812	0.964	14.985	0.525	15.128
Crs-Ct/Sb/Sr+Br/Crs+Br	0.508	0.025	9.917	0.651	19.600	**15.545***	0.949	**15.810***	0.724	14.308
Std.Error	*0.103*	*0.011*	*2.261*	*1.114*	*2.252*	*1.421*	*0.012*	*1.375*	*0.150*	*3.156*

Table 7 (continued)

Treatments	SOM fractions									
	C-POM	N-POM	C-MAOM	N-MAOM	CN-POM	CN-MAOM	f(MAOM/CT)	CN-Soil	C-POM-Stock	C-MAOM-Stock
	g kg^{-1}								Mg ha^{-1}	
	40-50 cm									
CONTROL	0.293	0.018	7.745	0.519	16.833	15.081	0.961	15.117	0.440	11.690
M/Sb	0.249	0.020	7.252	0.468	12.708	15.612	0.967	15.517	0.368	10.720
Sb/M+Br	0.236	**0.037***	8.740	0.562	**12.516***	15.309	0.970	14.998	0.348	12.760
Sb/M+Br/Cro-Ct	0.283	0.015	8.692	0.598	**21.250***	14.354	0.965	14.728	0.396	12.204
Sb/Ct/Cb/Mt+Br	0.359	0.023	6.378	0.415	15.938	15.683	**0.945***	15.664	0.509	8.992
Mt-Ct/Sb/M/Crs	0.263	0.016	8.950	0.609	16.875	14.757	0.970	14.838	0.363	12.251
Crs-Ct/Sb/Sr+Br/Crs+Br	0.286	0.020	9.489	0.655	17.917	14.629	0.971	14.697	0.406	13.413
Std.Error	*0.043*	*0.007*	*1.963*	*0.130*	*2.468*	*0.791*	*0.008*	*0.735*	*0.066*	*2.838*
	50-60 cm									
CONTROL	0.184	0.021	6.916	0.517	10.333	15.825	0.974	16.165	0.284	10.617
M/Sb	**1.379***	**0.082***	**4.447***	0.281	**16.844***	16.432	**0.720***	16.419	**1.860***	**5.866***
Sb/M+Br	**1.472***	**0.101***	**4.503***	0.262	**15.987***	16.801	**0.714***	16.677	**2.087***	**6.118***
Sb/M+Br/Cro-Ct	**1.808***	**0.100***	5.879	0.401	**15.917***	16.820	**0.804***	16.992	**2.085***	8.144
Sb/Ct/Cb/Mt+Br	0.323	0.024	5.303	1.376	**13.063***	17.377	0.942	17.120	0.457	**7.442***
Mt-Ct/Sb/M/Crs	0.267	0.016	6.633	0.709	**16.625***	16.759	0.961	16.895	0.385	9.540
Crs-Ct/Sb/Sr+Br/Crs+Br	**1.560***	**0.103***	**4.065***	0.447	**14.337***	**19.002***	**0.696***	16.952	**2.135***	**5.443***
Std.Error	*0.362*	*0.026*	*1.232*	*0.548*	*1.302*	*1.591*	*0.092*	*1.482*	*0.554*	*1.606*

Experimental design: Completely Randomized Block with 4 blocks; block are random effect in linear mixed model; *Std. Error* = standard error of the differences of least squares means (n = 4); *p-value* = nominal significance level is given by contrasts between treatments and CONTROL (soybean/cotton) by the Dunnett test; *NA*= not applicable. *Estimate is significantly different from CONTROL ($p \leq 0.1$).C-POM: C concentration of particulate organic matter; N-POM: N concentration of particulate organic matter; C-MAOM: C associated to the mineral fraction of the soil (clay and silt); N-MAOM: N associated to the mineral fraction of the soil (clay and silt); CN-POM: C to N ratio of the particulate organic matter; CN-MAOM: C to N ratio of the mineral associated organic matter; CN-soil: C to N ratio of the soil; C-POM-stock: C stock of the particulate organic matter; C-MAOM-Stock: C stock of the mineral fraction (clay and silt); Sb: Soybean (*Glycine max* L.); Cb: Common-bean (*Phaseolus vulgaris* L.); M: Maize (*Zea mays* L.); Br: Brachiaria (*Urochloa ruziziensis*); Cro: *Crotalaria ochroleuca;* Crs: *Crotalaria spectabilis;* Mt: Millet (*Pennisetum glaucum*); Ct: Cotton (*Gossypium herbaceum*); Sr: Sorghum (*Sorghum bicolor*).

This suggests that these cover crops efficiently contribute to fresh organic matter input into the soil, given their high C/N ratio (Carvalho *et al*., 2022) and influence on maintaining POM values (Cotrufo *et al*., 2013). The higher concentration of C and N in the particulate organic matter fraction is likely due to the extensive root volume of gramineae. The presence of these cover crops, especially in rotations following pasture, may have minimized POM loss compared to the CONTROL.

Management systems such as no-tillage and crop-livestock integration, utilizing crop rotation along with cover crops and pastures, can increase POM levels (Komatsuzaki *et al*., Ohta, 2007; Zilverberg, 2012). It is recommended to use cover crops like brachiaria and millet in diversified systems, as they provide a more recalcitrant input of organic material with higher POM contents.

The study observed that less diversified rotations increased Mineral-Associated Organic Carbon (C-MAOM) and nitrogen (N-MAOM) at lower soil depths, likely due to the physical and chemical stability of mineral-associated carbon. Crop rotations, especially those with corn and brachiaria grass, demonstrated a higher C to N ratio in the POM fraction through the soil profile. This higher C/N ratio, sustained by continued input of high C/N ratio corn and brachiaria tissues, is beneficial for increasing the longer-term N stock of the soil. Cover crops with more recalcitrant characteristics, such as brachiaria grass, help sequester carbon and balance the mineralization process of soil organic matter.

CONCLUSIONS

The labile SOC fraction, the particulate organic matter (C-POM), isolated by physical fractionation based on granulametric separation, was more sensitive and efficient in describing the effect of crop rotations of varying diversity on SOC.

Because of the management history of the area where the experiment was installed, and which had pasture on the experimental area before the implementation of the long-term experiment, our conclusions could be drawn more on the effect of the crop rotations on SOC after implementation of rotation of annual cropping systems after pasture, and less on the effect of the cropping systems compared among themselves.

Diversified crop rotations, particularly those including leguminous and non-leguminous cover crops, had a significant impact on soil C and N distribution among different fractions. Gramineous cover crops, like brachiaria grass and millet, contributed to improve Particulate Organic Matter in upper soil layers. The study recommends using cover crops in diversified systems to enhance soil organic matter and carbon sequestration. Moreover, maintaining a higher C/N ratio, especially with cover crops like brachiaria, is crucial for achieving long-term soil health and sustainability.

As a final remark, it is important to mention that pastures usually have positive impact on SOC accumulation when compared to annual crops. The success of crop-livestock integration in sequestering SOC is one of the practical advantages of including pasture in intensified agriculture systems (Oliveira *et al.*, 2022). This study confirmed the negative impact on SOC of cropping systems that exclude cover crops.

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