

## Cover crops affect on soil organic matter fractions under no till system

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### Abstract

The purpose of this research was to identify the soil organic matter (SOM) fractions changes in a crop rotation system under no-till system (NTS). This research was carried out from October 2010 to February 2014 in a Rhodic Hapludox. The experiment was set up in completely randomized blocks in a factorial design with eight cover crops and three soil depths (0-5, 5-10 and 10-20 cm) with four repetitions. Cover crops: fall-winter corn, intercropping fall-winter corn with *Brachiaria ruziziensis*, intercropping fall-winter corn with *B. brizantha* cv. Marandu, intercropping fall-winter corn with *Crotalaria spectabilis*, *B. ruziziensis*, *B. brizantha* cv. Marandu, *Pennisetum glaucum* L. and set-aside area. The results of SOM granulometric fractionation showed that 6.5% of the total organic carbon (TOC) stocks were in the particulate organic carbon (POC) fraction. The low values of POC observed in this research are associated with the weather condition of experimental site, which shows high temperature and moisture. In relation to the labile carbon (LC), the highest LC stock was observed in 0-5 cm depth, which differed from the 10-20 cm depth. The POC, LC and labile nitrogen (LN) were the SOM fractions that showed to be more sensitive to detect the changes promoted by the cover crops and soil depths in NTS, as well as the carbon management index (CMI). The intercropping fall-winter corn with *B. brizantha* cv. Marandu and *B. ruziziensis* were efficient in increasing the CMI in deeper depth (10-20 cm).

**Keywords:** soil organic carbon; labile carbon; sustainability; conservation tillage; soybean.

**Abbreviations:** SOM\_Soil Organic Matter; CMI\_Carbon Management Index; TOC\_Total Organic Carbon; LC\_Labile Carbon; SOC\_Soil Organic Carbon; Mg ha<sup>-1</sup>\_ton ha<sup>-1</sup>; POC\_Part particulate Organic Carbon; TN\_Total Nitrogen; MOM\_Mineral-associated Organic Matter; NTS\_No Tillage System.

### Introduction

The carbon is found on the earth ecosystem in the geological formation, in atmosphere and oceans (Lal, 2005). On the earth ecosystem, the soil is the major carbon storage (2,500 Pg C), which represents four times the carbon storage in the vegetation and 3.3 times the atmosphere carbon (Lal, 2008). In soil, the capacity of maintaining carbon is associated with the soil tillage (Sá and Lal, 2009; Boddey et al., 2010). In general, the adoption of conservationist system may contribute to maintain or even increase the quantity of total organic carbon (TOC) and total nitrogen (TN) stocks in soil, thereby increasing the soil capacity in assimilate the CO<sub>2</sub> from atmosphere (Bayer et al., 2000; Cerri et al., 2004; Bayer et al., 2006). The no-till system (NTS) is a conservationist system that is promisor to decrease the emission of greenhouse gas (GHG). As reported by Abdalla et al. (2015), in absence of NTS the emissions of CO<sub>2</sub> increase 21%. The benefits of NTS in decreasing the emission of GHG and consequently the reduction of global warming is related to the capacity in increasing the TOC in soil (Amado et al., 2006),

due to higher input and maintenance of SOM, through the soil tillage just in the seeding row (Chivenge et al., 2007) and less microbial activity in NTS (Sainju et al., 2012).

The potential in assimilating CO<sub>2</sub> may be intensified when it is associated with adequate cover crops in a crop rotation system (Bayer et al., 2006). According to Wagger et al. (1998), the cover crops are the most important choice to determine the dynamics of carbon and nitrogen in agricultural systems. In relation to the species of cover crops, Lal (2004) considered that the association of crop rotation system with NTS may increase the SOM, and decrease the loss of carbon. The cover crops can promote increase in soil quality and water use efficiency, as well as decreasing the soil and nutrients loss, besides the increase of carbon through the biomass production (Sainju et al., 2002). As reported by Sisti et al. (2004), in NTS there are accumulations of total nitrogen. The insertion of cover crops in NTS also promotes recover of soil nitrogen stocks (Ryan et al., 2008). The accumulation of carbon can occur in labile fractions and in

stable SOM fraction, but this accumulation depends on the chemical composition of plant residues (C/N ratio), in association with the capacity of biomass production of the plants and soil tillage (Zhongkui et al., 2010). To assess the capacity of NTS and cover crops in increasing the SOM, nowadays it is used the evaluation of TOC and TN, but it is not enough to understand the changes caused by cover crops in SOM in short-time. This way, to improve the evaluation of the benefits that cover crops bring to soil, it is important to determine carbon stocks, labile nitrogen, granulometric fractionation of SOM and carbon management index.

The labile C and N are all the compounds that can be oxidable by  $\text{KMnO}_4$  as crop residues and polysaccharides (Yang et al., 2012). In relation to granulometric fractionation of SOM is possible to determine mineral-associated organic matter (MOM) and particulate organic carbon (POC) (Cambardella and Elliott, 1992). The MOM takes longer in soil due to the shelter of soil aggregate, it shows less sensible to alteration of management (Rossi et al., 2012). The POC represents a labile fraction with higher tax of organic compounds recycle, which can be changed in short-time (Feller and Beare, 1997; Bayer et al., 2002). The carbon management index (CMI) is very useful to obtain information about the effect of soil tillage and use impacts on SOM quality (Blair et al., 1995).

Researches focus to evaluate the capacity of cover crops in increasing and maintain the SOM ought to be necessary to improve this knowledge around the world. Based on these points of view above, the purpose of this research was to identify the SOM fractions changes in a crop rotation system under NTS in Brazilian Cerrado.

## Results and discussion

### *Mineral-associated organic matter and total organic carbon stocks measurements*

No significant effects ( $p>0.05$ ) was observed for mineral-associated organic matter (MOM). The total organic carbon stocks (TOC) showed significant difference ( $p\leq 0.01$ ) just for the soil depths (Table 1). The absence of cover crops and soil depths affects on MOM stocks may be due to the particles of this SOM fraction in soil that is linked to the clay fraction in soil which is more stable. This way, the alterations with cover crops used in a crop rotations system can show slow modification in soil physical-chemical properties, especially in soils with high amount of clay (Diekow et al., 2005). As reported by Chistensen (2001), the clay minerals shelter the SOM building the complex mineral-organic in soil resulting in decreasing the oxidation of SOM.

The weather condition of the experimental site may has influenced the absence of changes in MOM stocks, since the tropical region the process of SOM mineralization occur faster than in sub-tropical region, which implies in less time for SOM stabilization. The Brazilian Cerrado shows high air temperature ( $26^\circ\text{C}$  on average), high moisture (1500 mm on average) and 80% of the rainfall concentrated between November to April (Silva et al., 2004). Besides, the time of using for cover crops in fall-winter and spring-summer season could not be enough to observed statistic difference among the treatments due to the short-time of evaluation. As report by Geisseler and Horwath (2009), in five years of conservationist tillage little changes were observed in carbon content and its distribution in soil profile.

The highest TOC stocks were observed in 0-5 cm depth without changes in relation to 5-10 cm depths, but differed from 10-20 cm depth (Fig 1). These results can be explained

because of the soil movement in just seeding rows and the constant input of crop residues on soil surface in NTS. The stratification of TOC stocks have been observed in NTS, and it is higher than conventional tillage. Moreover, the stratification of carbon usually increases in the course of time under NTS (Franzluebbers, 2002; Moreno et al., 2006; Zhao et al., 2014). According to Sisti et al. (2004) and Alvarez et al. (2014), in NTS it is possible to observe higher amount of TOC stocks in soil surface, the majority because when the crop residues above-ground increases, it can increase the carbon content into the topsoil.

### *Cover crops affects on particulate organic carbon*

There was interaction between cover crops and soil depths ( $p\leq 0.01$ ), for particulate organic carbon (POC) (Table 1). The results of SOM granulometric fractionation showed that 6.5% of the TOC stocks were in the POC fraction. Awale et al. (2013) observed that the POC fraction showed on average 19.2% of the TOC stocks in no-till and Dou et al. (2008) found 14-31% of POC in the TOC stocks. For the weather condition of both researches, these values of POC are considered low, because in soil from cold region in the world, the reaches POC values surround 50% of TOC stocks (Cambardella and Elliot, 1992; Chan, 1997).

The low values of POC observed in this research are associated with faster mineralization of crop residues in tropical region, which shows high air temperature and rainfall. As reported by Lal and Logan (1995), the mineralization of crop residues in Brazilian Cerrado is faster than temperate region that can show ten times higher.

The POC fraction is considered the most labile of SOM, which can change easily in soil throughout the mineralization and tillage (Guimarães et al., 2012). On the other hand, the highest endurance of carbon in more recalcitrant fraction, as MOM may be explained by association with the complex mineral-organic that is linked to soils like Oxisol, where it is possible to obtain strong association of humidified organic matter with caulinic clays and Al/Fe oxy-hydroxides. Pinheiro et al. (2015) noticed predominance in the MOM in soil than the more labile fractions (POC), due to the highest decomposition in Oxisol. Besides, the highest content of clays in Oxisol increases the amount of microspores and decreases the chance of microorganism to mineralize the SOM (Hartman et al., 2014). As reported by Figueiredo et al. (2010), there is a negative correlation between POC and MOM, which indicates that the process of these fractions formation is opposite, this way; to increase the MOM it is necessary to decrease the POC by the process of mineralization. This pattern was observed in this experiment, which showed that independent of the cover crop used in the research, the MOM stocks and POC were negatively correlated ( $r^2=-0.55$ ). In comparison among the cover crops, higher POC stocks were verified in 0-5 cm depth with the intercropping fall-winter corn with *Brachiaria brizantha* cv. Marandu. In 5-10 cm depth, the single fall-winter corn and intercropping with *B. brizantha* cv. Marandu showed higher POC stocks, and in 10-20 cm depth the highest POC stocks were observed in *B. ruziziensis* (Fig 2).

The adoption of the intercropping fall-winter corn with *B. ruziziensis* or *C. spectabilis* did not differ among the soil depths, the same was observed in *B. ruziziensis* and millet in soil depths (Fig 2). The cultivation of single fall-winter corn and in intercropping with *B. brizantha* cv. Marandu showed higher POC stocks in 0-5 and 5-10 cm depth, which differed from 10-20 cm depth. On the other hand, the fall-winter corn

**Table 1.** Summary of analysis of variance (ANOVA).

Source of variation	DF	TOC	POC	MOM	LC	TN	LN	CMI
		F-value						
Block	3	3.344 <sup>NS</sup>	2.496 <sup>NS</sup>	2.071 <sup>NS</sup>	2.802 <sup>NS</sup>	2.901 <sup>NS</sup>	0.036 <sup>NS</sup>	2.284 <sup>NS</sup>
CC <sup>†</sup>	7	2.044 <sup>NS</sup>	5.225 <sup>**</sup>	1.599 <sup>NS</sup>	12.166 <sup>**</sup>	1.277 <sup>NS</sup>	14.751 <sup>**</sup>	35.827 <sup>**</sup>
D <sup>††</sup>	2	4.495 <sup>**</sup>	14.018 <sup>**</sup>	2.826 <sup>NS</sup>	7.345 <sup>**</sup>	20.994 <sup>**</sup>	4.422 <sup>**</sup>	10.05 <sup>**</sup>
CCxD	14	1.147 <sup>NS</sup>	3.175 <sup>**</sup>	1.205 <sup>NS</sup>	1.498 <sup>NS</sup>	1.004 <sup>NS</sup>	3.990 <sup>**</sup>	4.684 <sup>**</sup>
CV (%)		11.86	24.76	12.53	26.88	22.88	23.02	16.58

\*\* significant at 0.01 probability level by F-value. <sup>NS</sup> no significant at 0.05 probability level by F-value. DF=Degree of freedom; <sup>†</sup>CC= Cover crop; <sup>††</sup>D=depth. Total Organic Carbon (TOC); Particulate Organic Carbon (POC); Mineral-associated Organic Matter (MOM); Labile Carbon (LC); Total Nitrogen (TN); Labile Nitrogen (LN), Carbon Management Index (CMI).

**Table 2.** Some physical and chemical soil properties of the experimental site.

	Depths	
	0-20 cm	20-40 cm
pH (CaCl <sub>2</sub> )	5.10	4.66
SOM (g dm <sup>-3</sup> )	28.0	19.0
P (mg dm <sup>-3</sup> )	16.30	1.08
K <sup>+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0.41	0.10
Ca <sup>2+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	4.15	1.90
Mg <sup>2+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	1.30	0.75
H+Al (cmol <sub>c</sub> dm <sup>-3</sup> )	4.98	4.83
Al <sup>3+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0	0.34
SB (cmol <sub>c</sub> dm <sup>-3</sup> )	5.86	2.75
CEC (cmol <sub>c</sub> dm <sup>-3</sup> )	10.84	7.58
BS (%)	54.06	36.28
Clay (g kg <sup>-1</sup> )	390	
Sand (g kg <sup>-1</sup> )	310	
Silt (g kg <sup>-1</sup> )	300	

CEC: Cation Exchange Capacity; total acidity pH 7.0 (H<sup>+</sup> +Al<sup>3+</sup>); Exchangeable (KCl 1 mol L<sup>-1</sup>) Ca<sup>2+</sup>, Mg<sup>2+</sup> and Al<sup>3+</sup>; SB: Sum of Base=Σcations; BS: Base Saturation=(Σcations/CEC)x100.

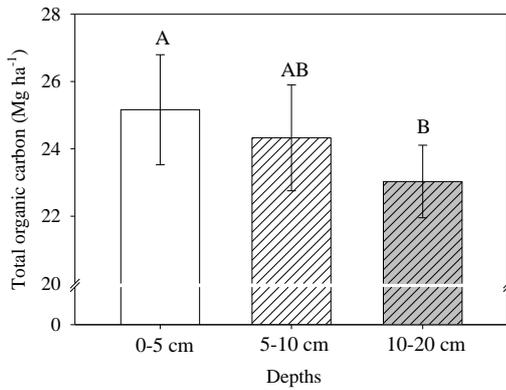
**Table 3.** The treatments carried from 2010 to 2014, it was cultivated cover crops in fall-winter and soybean in spring-summer.

Treatments (T)	Cover crops	Spring-summer season
T1	Fall-winter corn	Soybean
T2	Intercropping fall-winter corn with <i>B. ruziziensis</i>	Soybean
T3	Intercropping fall-winter corn with <i>B. brizantha</i> cv. Marandu	Soybean
T4	Intercropping fall-winter corn with <i>Crotalaria spectabilis</i>	Soybean
T5	<i>Brachiaria ruziziensis</i>	Soybean
T6	<i>Brachiaria brizantha</i> cv. Marandu	Soybean
T7	<i>Pennisetum glaucum</i> L.	Soybean
T8	Set-aside area	Soybean
T9	Reference area (native vegetation of Brazilian "Cerrado") to calculate the carbon management index (CMI).	Absence of crops

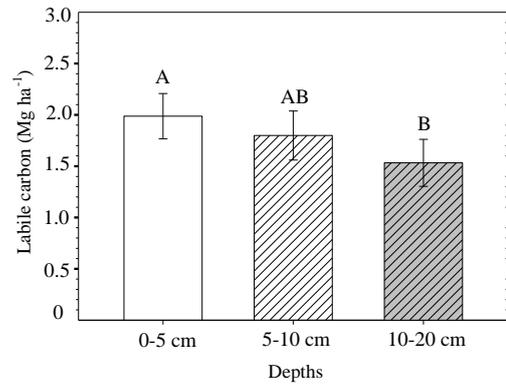
in intercropping with *B. brizantha* cv. Marandu and set-aside area showed higher POC stocks in 0-5 cm depth (Fig 2). The highest POC stocks with the implementation of these cover crops showed higher influence of crop residue in POC formation due to deposition above-ground and below-ground. As reported by Sá et al. (2001), in NTS more than 75% of POC are dependable of crop residues on soil in 0-2.5 cm depth and more than 50% for 2.5-5.0 cm depth. The results obtained in this research indicate that the use of intercropping fall-winter corn with *Brachiaria* showed great potential to contribute to increase the POC stocks. Preview researches indicate that in integrated crop-livestock system it is possible to increase the soil carbon due to the high amount of residues above-ground. Besides, the root system of the corn and *Brachiaria* spp. species increases the SOM and this effect is intensified with *Brachiaria* spp. due to high volume of roots that take place deeper in soil profile (Loss et al., 2013). As reported by Rossi et al. (2012), the positive effect of *Brachiaria* spp on POC may be associated with higher addition of organic residues and maintenance of vegetable residues in soil.

#### Labile carbon in soil within the top 20 cm depth

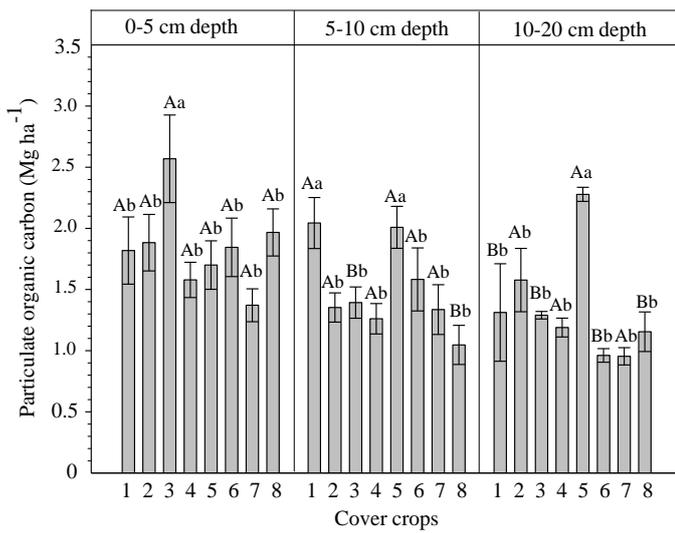
In relation to the labile carbon (LC), the highest LC stock was observed in 0-5 cm depth, which differed from the 10-20 cm depth (Fig 3). In NTS, the absence of soil movement in association with crop rotation and above-ground dry matter may contribute to increase the LC in the first layer (0-5 cm depth) of soil depth. As reported by Saraiva et al. (2014), the highest carbon stocks in the soil surface (0-5 cm depth) is due to the deposition of cover crops in association with the root system concentrated into the first layers of soil. Nevertheless, it is possible to observe that in NTS, the fertilization in the seeding row with deposition of fertilizer in 8 cm depth promotes higher concentration of roots in this depth due to the nutrients placed, this fact increased the SOM in 5-10 cm depth as it was possible to observe in this research, this way, no significant difference was observed between 0-5 cm and 5-10 cm depth (Fig 3). In comparison among the cover crops,



**Fig 1.** Total organic carbon (TOC) in soil. Different uppercase letters indicate significant difference ( $p \leq 0.05$ ) among different soil depths by Tukey test of means.

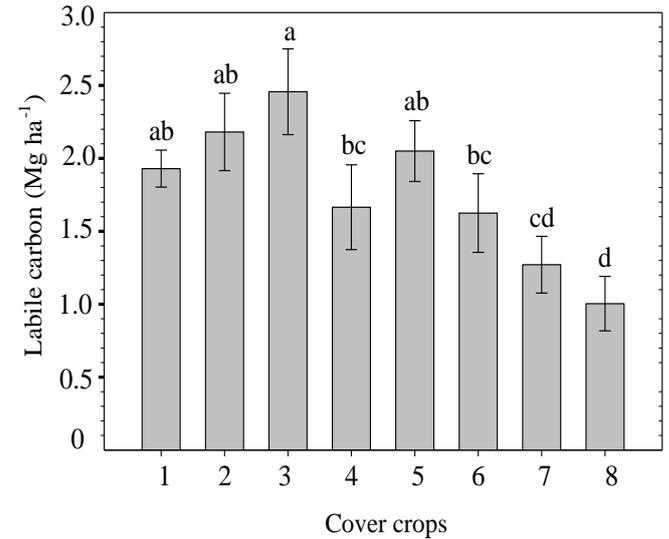


**Fig 3.** Labile carbon stocks in soil profile. Different uppercase letters indicate significant difference ( $p \leq 0.05$ ) among different soil depths by Tukey test of means. The error bars are the standard errors.

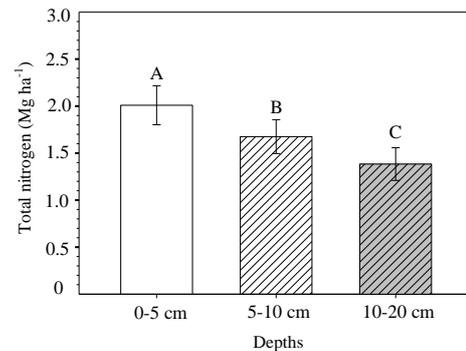


**Fig 2.** Particulate organic carbon (POC) affected by cover crops and soil depths. Different uppercase letters indicate significant difference ( $p \leq 0.05$ ) among different soil depths within same cover crop while the different lowercase letters indicate significant difference ( $p \leq 0.05$ ) among different cover crops within same soil depth by Tukey test of means. The error bars are the standard errors. (1) Fall-winter corn; (2) Intercropping fall-winter corn with *B. ruziziensis*; (3) Intercropping fall-winter corn with *B. brizantha* cv. Marandu; (4) Intercropping fall-winter corn with *C. spectabilis*; (5) *B. ruziziensis*; (6) *B. brizantha* cv. Marandu; (7) *Pennisetum glaucum* L; (8) Set-aside area.

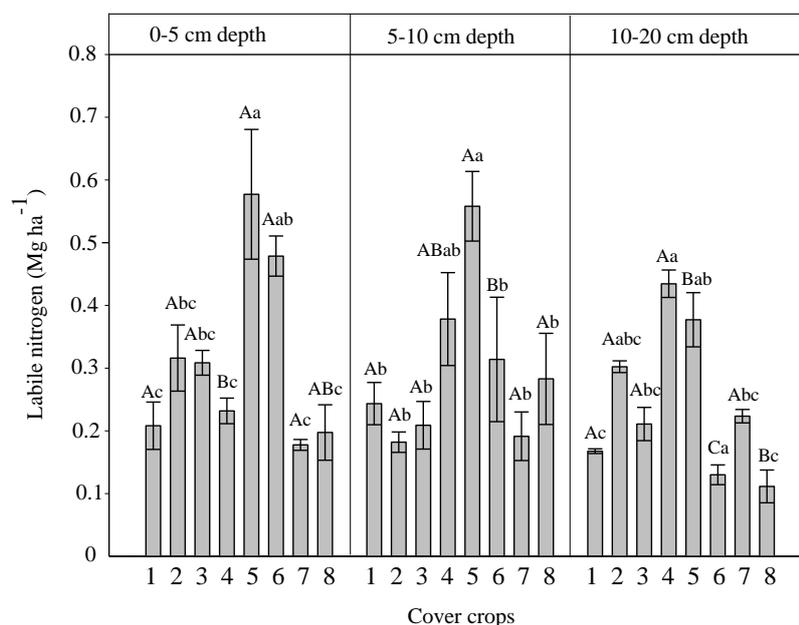
the LC stocks were higher with the adoption of these crops in rotation than in set-aside area. The LC stocks increases follow this order: intercropping fall-winter corn with *B. brizantha* cv. Marandu = fall-winter corn with *B. ruziziensis* = *B. ruziziensis* = fall-winter corn > intercropping fall-winter corn with *C. spectabilis* = *B. brizantha* > millet > set-aside area (Fig 4). These results are associated with the above-ground and below-ground dry matter production. Besides, the implementation of cover crops maintains the above-ground dry matter production constantly and in higher amount than areas with absence of cover crops. As reported by Chan et al. (2001), the *Brachiaria* spp. can improve the LC stocks in soil profile.



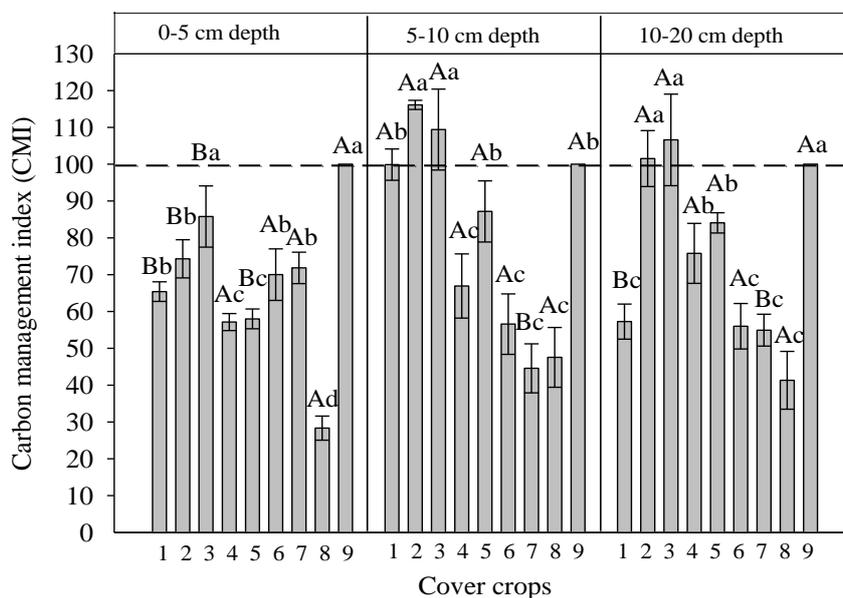
**Fig 4.** Labile carbon stocks in soil profile. Different lowercase letters indicate significant difference ( $p \leq 0.05$ ) among different cover crops. The error bars are the standard errors. (1) Fall-winter corn; (2) Intercropping fall-winter corn with *B. ruziziensis*; (3) Intercropping fall-winter corn with *B. brizantha* cv. Marandu; (4) Intercropping fall-winter corn with *C. spectabilis*; (5) *B. ruziziensis*; (6) *B. brizantha* cv. Marandu; (7) *Pennisetum glaucum* L; (8) Set-aside area.



**Fig 5.** Total nitrogen stocks in soil profile. Different uppercase letters indicate significant difference ( $p \leq 0.05$ ) among different soil depths by Tukey test of means.



**Fig 6.** Cover crops and soil profile effects on labile nitrogen. Different uppercase letters indicate significant difference ( $p \leq 0.05$ ) among different soil depths within same cover crop while the different lowercase letters indicate significant difference ( $p \leq 0.05$ ) among different cover crops within same soil depth by Tukey test of means. The error bars are the standard errors. (1) Fall-winter corn; (2) Intercropping fall-winter corn with *B. ruziziensis*; (3) Intercropping fall-winter corn with *B. brizantha* cv. Marandu; (4) Intercropping fall-winter corn with *C. spectabilis*; (5) *B. ruziziensis*; (6) *B. brizantha* cv. Marandu; (7) *Pennisetum glaucum* L; (8) Set-aside area.



**Fig 7.** Cover crops and soil profile effects on carbon management index. Different uppercase letters indicate significant difference ( $p \leq 0.05$ ) among different soil depths within same cover crop while the different lowercase letters indicate significant difference ( $p \leq 0.05$ ) among different cover crops within same soil depth. The error bars are the standard errors. Dash line means the CMI of the native vegetation (Brazilian “Cerrado”). (1) Fall-winter corn; (2) Intercropping fall-winter corn with *B. ruziziensis*; (3) Intercropping fall-winter corn with *B. brizantha* cv. Marandu; (4) Intercropping fall-winter corn with *C. spectabilis*; (5) *B. ruziziensis*; (6) *B. brizantha* cv. Marandu; (7) *Pennisetum glaucum* L; (8) Set-aside area; (9) native vegetation.

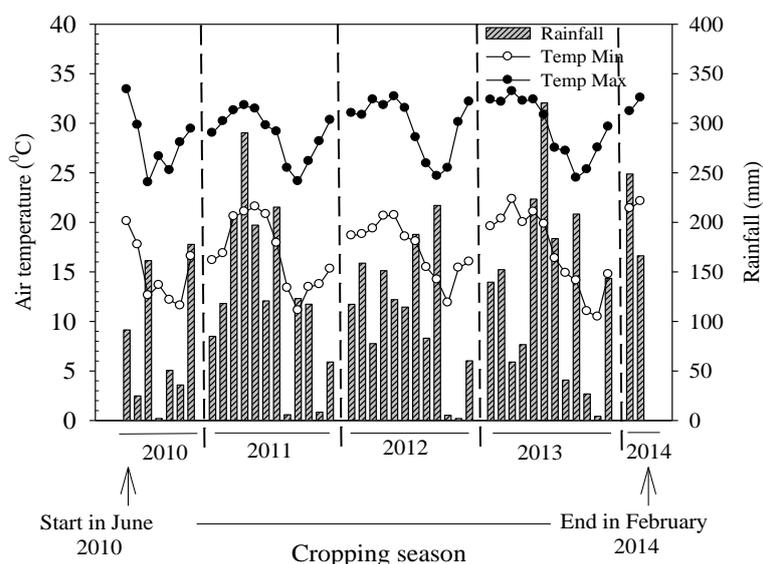


Fig 8. Rainfall and temperature monthly in the period from June 2010 to February 2014.

### Total nitrogen within the top 20 cm depth

The TN showed significant difference ( $p \leq 0.01$ ) for soil depths (Table 1). In this investigation, it was observed higher TN stocks ( $2.0 \text{ Mg ha}^{-1}$ ) in 0-5 cm depth, which differed 5-10 cm and 10-20 cm depth (Fig 5). As reported by Mishra et al. (2010), in NTS they found higher TN stocks and this concentration decreased in deeper layers, which was attributed to the higher amount of dry matter on soil surface. The SOM is the highest pool of nitrogen in soil, and in NTS due to the amount of SOM in soil surface that tends to increase this quantity (Liu et al., 2014). Higher accumulation of TN in 0-5 cm depth ( $2 \text{ Mg ha}^{-1}$ ) is very important for plant nutrition, because in soil surface due to the higher activity of microorganisms, the SOM is under a faster process of mineralization. This source of nitrogen can decrease the need of nitrogen fertilizer for plant uptakes, which ought to be more study to be possible the use of this information in management of fertilizer in agricultural systems.

### Cover crops effects on labile nitrogen

The labile nitrogen (LN) stocks showed significant difference ( $p \leq 0.01$ ) for all depths evaluated (Table 1), which indicated that this SOM pool is more sensitive to identify the changes in soil tillage (Fig 6). In 0-5 cm depth, the adoption of *B. ruziziensis* and *B. brizantha* cv. Marandu promoted higher LN stocks ( $0.57$  and  $0.47 \text{ Mg ha}^{-1}$ ), respectively. In 5-10 cm and 10-20 cm depth, higher LN stocks were obtained in intercropping fall-winter corn with *C. spectabilis* and *B. ruziziensis*. The benefits of *Brachiaria* spp. in increasing the nitrogen stocks in more labile fractions of SOM is associated with the highest input of plant residues above-ground and below-ground left in soil. The capacity of increasing the nitrogen in soil, leguminosae as *C. spectabilis*, in crop rotation system is probably due to the capacity of nitrogen assimilation from the atmosphere air (Giller, 2001), this fact increases the capacity of providing nitrogen from plant residue to soil solution.

### Cover crops affects on carbon management index

The calculation of carbon management index (CMI) was defined to obtain information about the carbon dynamic in

this NTS and to provide quality and quantity measurement of SOM, as suggested by Blair et al. (1995). So that, the soils are considered better management when the CMI is higher (Diekow et al., 2005) and above 100 as it is possible to observe in some cases in Fig 7. The results of CMI showed interactive effect ( $p \leq 0.01$ ) between cover crops and soil depths (Table 1). In comparison among cover crops in 0-5 cm depth, higher CMI was observed in intercropping fall-winter corn with *B. brizantha* cv. Marandu and native vegetation (Brazilian "Cerrado"). In 5-10 cm and 10-20 cm, the intercropping fall-winter corn with *Brachiaria* spp. showed higher values of CMI, nevertheless, there was no significant difference ( $p > 0.05$ ) in comparison to native vegetation in 10-20 cm (Fig 7). The millet showed the highest CMI in 0-5 cm depth. On the other hand, the intercropping fall-winter corn with *Brachiaria* spp. and single *B. ruziziensis* promoted the highest CMI in deeper layers (5-10 cm and 10-20 cm). The intercropping fall-winter corn with *C. spectabilis*, single *B. brizantha* cv. Marandu, set-aside area and native vegetation did not differ statistically ( $p > 0.05$ ) among the depths evaluated (Fig 7). The variability of CMI in soil profile indicates that this index is adequate to evaluate the soil quality and changes in carbon pools in soil surface and deeper layers, as well as reported by Zhao et al. (2014). In comparison to the treatment without cover crops (set-aside area) in 5-10 cm depth, the intercropping fall-winter corn with *B. ruziziensis* and *B. brizantha* cv. Marandu increased the CMI in 144.2 and 130.2%, respectively. In 10-20 cm depth, it was observed equal trend, the intercropping fall-winter corn with *B. ruziziensis* and *B. brizantha* cv. Marandu increased the CMI in 145.6 and 158%, respectively. With the finds obtained in this experiment, it was verified that the intercropping with *Brachiaria* sp. promoted positive effects on CMI especially for deeper layer of soil, because in this layer (10-20 cm) the CMI achieved values above 100, which indicates that this cover crops in NTS are efficient in maintaining and increasing the SOM stocks. The *Brachiaria* sp. can increase the labile pools of SOM because of the higher amount of SOM from its roots system (Salton et al., 2014). Their roots show a continuity exudation of labile substance in the soil (Carvalho et al., 2009). These features combined might increase the CMI, because a higher value of CMI is associated with higher LC in soil. Based on the results obtained for CMI, it is possible to assure that the

intercropping fall-winter corn with *Brachiaria* sp is a valuable measurement to indicate changes in SOM and it is possible to achieve CMI above the native vegetation of Brazilian Cerrado. The CMI close to or above the reference area was very important to observe how valuable these intercropping and crop rotations are to improve better in SOC management.

## Materials and Methods

### Site description

This research was carried out from October 2010 to February 2014 in a Rhodic Hapludox, clayey texture, clay mineralogy is constituted mainly by Al/Fe oxy-hydroxides, according to taxonomy of Santos et al. (2013), located in the municipality of Maracaju, State of Mato Grosso do Sul, Brazil (approximately 21°36'52''S, 55°10'06''W, average altitude 384 m above sea level). According to Köppen (1948), the region is classified as tropical climate of type Am, with rainy summer and dry winter. The rainfall and temperature in the region of the experimental site is showed in Fig. 8.

### Historic of the area

During ten years, the experimental area was cultivated with soybean in spring-summer and maize in fall-winter season. Before the implementation of the experiment in June 20<sup>th</sup> 2010, it was collected soil to analysis the chemical and physical properties, in 0-20 and 20-40 cm depth (Table 2). The dolomitic lime (2.4 Mg ha<sup>-1</sup>) and gypsum (CaSO<sub>4</sub>·2H<sub>2</sub>O) (0.6 Mg ha<sup>-1</sup>) were applied in 2010, 30 days before soybeans sowed. The lime showed calcium carbonate equivalent (CCE) to 64%. The lime and gypsum were accomplished by spreading on the soil without incorporation. In the end of October 2010, it was sowed soybean and after the soybean harvesting in February 2011 the cover crops were sowed in fall-winter season. The experiment was carried out from 2011 to 2014, in crop rotation with soybean and cover crops (Table 3). The soil measurements were taken after soybean harvesting on March 15<sup>th</sup> 2014.

### Experimental design and treatments

The experiment was carried out in completely randomized blocks, in a factorial design with eight cover crops cultivated in fall-winter season (Table 3) and three soil depth (0-5, 5-10 and 10-20 cm), with four repetitions. The experimental plot had dimension of 12 m length and 2.5 m width (30 m<sup>2</sup>). The cover crops were cultivated during four year at the same spot, but the evaluations were taken just in 2014.

### Plant material and steps of treatments implementation

The major species in the set-aside area were basically for the weeds *Bidens pilosa*, *Eleusine indica*, *Euphorbia heterophylla*, *Brachiaria* spp. and *Ipomoea grandifolia*. The sowing of the fall-winter corn in the growing seasons was accomplished in no-till system right after the soybean harvest. It was used the fall-winter corn hybrid DKB 390 VTPRO. The sowing of fall-winter corn was executed depositing three seeds per meter in the spacing between rows of 50 cm. At the moment of sowing in February of each year, the fertilizer was accomplished with 256 kg ha<sup>-1</sup> of the formulation 12-15-15 (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O), this fertilizer recommendation was based on the results of soil chemical and adequate nutrient range according to Cantarutti et al.

(1999). The intercropping fall-winter corn with forages grass (*B. ruziziensis* and *B. brizantha* cv. Marandu) was sowed at the same moment through the use of automatized machine. The space between rows for forages grass was 21 cm. The seed cultural value for *B. ruziziensis* and *B. brizantha* cv. Marandu were 50% Both species of forages grass showed seeds with 80% of germination and 62.5% of purity. To establish the intercropping fall-winter corn with *B. ruziziensis* and *B. brizantha* cv. Marandu, it was used 3.0 and 3.5 kg ha<sup>-1</sup> of pure life seeds, respectively. The plots sowed with only *B. ruziziensis* and *B. brizantha* cv. Marandu were established with 4.0 and 5.0 kg ha<sup>-1</sup> of pure life seeds, respectively. The *P. glaucum* L. sowing was accomplished in the space between rows of 50 cm. The *P. glaucum* L. cultivar sowed was BRS 1501, which was used 11 kg ha<sup>-1</sup> of seeds. The intercropping fall-winter corn with *C. spectabilis* was accomplished with 6 kg ha<sup>-1</sup> of seeds in the space between rows of 45 cm. The sowing of *C. spectabilis* was done manually 15 days after the winter corn sowed. In the soybean sowing (2011/2012, 2012/2013 and 2013/2014 growing seasons), it was used an automatized machine. In the occasion of sowing in October of each year, it was accomplished the fertilizer with 380 kg ha<sup>-1</sup> of the formulation 02-20-20 (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) in sowing furrow, this fertilizer recommendation was based on soil chemical results and soybean needs according to Cantarutti et al. (1999). The soybean cultivar sowed was BMX Potência Roundup Ready (99% of purity and 95% of germination). It was sowed 15 seeds per meter. The soybean seeds were treated with fungicide [Carboxin + Thiran (48 g L<sup>-1</sup>)], insecticide [Fipronil (40 g L<sup>-1</sup>)], micronutrients [cobalt (2.32 g L<sup>-1</sup>) and molybdenum (40.6 g L<sup>-1</sup>)], and these doses were in gram of active ingredient per 80 kg of seeds. Besides, the seeds were inoculated before the sowing with inoculate in turf, which contented the bacteria *Bradyrhizobium elkani* (Race Semia 5019) and *Bradyrhizobium japonicum* (Race Semia 5079) in the concentration of 5x10<sup>9</sup> viable cells per gram of inoculate. It was used 100 mL of inoculate in each 80 kg of soybean seed. The desiccation of cover crops was accomplished 20 days before the soybean sowing, through the use of glyphosate-salt-isopropylamine (1,440 g ha<sup>-1</sup> of acid equivalent) plus 1,209 g ha<sup>-1</sup> of active ingredient of 2,4-D, dimethylamine salt.

### Soil measurement

The soil sampling was collected after the soybean harvesting in 0-5, 5-10 and 10-20 cm depth, through the opening of trenches perpendicular to the seed rows. It was collected undisturbed soil sample in each experimental plot to determine soil bulk density (Claessen, 1997). To measure the quantitative analysis of organic matter (OM), the soil samples were crushed, ground and sifted in sieve with mesh of 0.210 mm. The total organic carbon (TOC) was determined following the methodology of oxidation via dampening, with external warming, as described by Yeomans and Bremner (1988). It was followed the methodology described by Cambardella and Elliott (1992), to obtain the granulometric fractionation of SOM [particulate organic carbon (POC) and mineral-associated organic matter (MOM)]. The POC was quantified per oxidation via dampening (Yeomans and Bremner, 1988), and the MOM was obtained by the difference between TOC and POC. To determine the content of oxidized carbon by KMnO<sub>4</sub> [labile carbon (LC)], it was weighted 1 gram of crushed soil and sifted in sieve of 0.210 mm mesh, right after the solution was stored in tube of 50 mL

mixed with 25 mL of  $\text{KMnO}_4$  ( $0.033 \text{ mol L}^{-1}$ ) solution (Shang and Tiessen, 1997).

This solution was shook in horizontal shaker to 130 rpm (rotation per minute) for one hour, and centrifuged to 2500 rpm. Right after the centrifugation, pipette 100  $\mu\text{L}$  of supernatant in test tube and the volume was completed to 10 mL of distilled water. The readings were taken in spectrophotometer of wavelength of 565 nm, and the labile carbon (LC) was measured from the standard curve equation. The standard curve was obtained from the concentration of 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of  $\text{KMnO}_4$  ( $0.033 \text{ mol L}^{-1}$ ) solution that was stored in volumetric flask of 100 mL, which was completed the rest volume with distilled water. The nitrogen was measured by Kjeldahl methodology as described by Tedesco et al. (1985). The total amounts of total nitrogen ( $\text{dag kg}^{-1}$ ) on soil were calculated using the following equation:

$$N(\text{dag kg}^{-1}) = \frac{(V_{ts} - V_{wt})(H^+)(1.4)}{\text{Soil weight (g)}}$$

Where:  $V_{ts}$  = volume of HCl spending to the titration of the sample;  $V_{wt}$  = volume of HCl in white titration;  $[H^+]$  = molarity of the hydrochloric acid ( $\text{mol L}^{-1}$ ); 1.4 = equivalent weight of N (14) divided by 10 (conversion unit -  $\text{g kg}^{-1}$  for  $\text{dag kg}^{-1}$ ).

The first step to calculate the CMI was the survey of the soil samples (0-5, 5-10 and 10-20 cm) in a forestry area (native vegetation without the anthropic influence) close to the experimental site. This area was located 100 meters from the experimental site, and it was called as reference area (RA). Based on the total organic carbon (TOC) changes, between the reference area (RA) and cultivated area (experimental site), it was calculated the carbon compartment index (CCI), as the equation below.

$$CCI = \frac{\text{TOC in cultivated area}}{\text{TOC in no cultivated area (reference area)}}$$

vegetation of Brazilian "Cerrado"). These two indices were used to calculate the carbon management index (CMI), obtained by the following equation ( $\text{CMI} = \text{CCI} \times \text{LI} \times 100$ ), according to Blair et al. (1995).

The total organic carbon (TOC) stocks, labile carbon (LC) and oxidized carbon were obtained by the multiplication of the carbon content ( $\text{g kg}^{-1}$ ) and soil mass, in each depth studied ( $\text{kg ha}^{-1}$ ) (0-5, 5-10 and 10-20 cm). The soil mass was obtained by the multiplication of width of each depth (cm), for its soil bulk density ( $\text{kg dm}^{-3}$ ), and the soil volume ( $\text{dm}^3$ ). The carbon and nitrogen stocks in each depth were calculated according to the following equation (Cardoso et al., 2010):

$$\text{Stock} = \frac{\text{content} \times \text{Soil bulk density} \times W}{10}$$

Where: Stock of carbon or nitrogen in  $\text{Mg ha}^{-1}$ ; Content = the content of TOC or total nitrogen in  $\text{g kg}^{-1}$ ; soil bulk density in each depth in  $\text{kg dm}^{-3}$ ; W, means the width of the depth in cm.

### Statistical analysis

The variables assessed in this experiment were submitted to the analysis of variance (ANOVA) by the *F*-test, which was through a joint analysis between the cover crops and soil depths. The means were compared through the Tukey test of means ( $p \leq 0.05$ ). These tests were carried out with the use of SISVAR software (Ferreira, 2010).

### Conclusion

The particulate organic carbon, carbon labile, nitrogen labile and CMI were the SOM fractions that showed to be more sensitive to identify the changes promoted by the cover crops within the top 20 cm depth of soil in a crop rotation system under NTS. The NTS associated with the cover crops promoted the stratification of TOC, LC and TN. The intercropping fall-winter corn with *B. brizantha* cv. Marandu, *B. ruziziensis* and *Crotalaria spectabilis* promoted the increase of LN stocks in soil. The intercropping fall-winter corn with *B. brizantha* cv. Marandu and *B. ruziziensis* were efficient in increase the CMI in deeper layer (10-20 cm). The intercropping corn with *Brachiaria* sp indicated that in short-time of crop rotation implementation is possible to achieve CMI values above the native vegetation of Cerrado, thus this management and land use can be applied with high possibility to improve CMI.

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