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Mineral-associated and particulate organic matter in aggregates as a proxy for soil C changes in pasture-sugarcane land use transitions

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ABSTRACT: To meet the growing demand for bioenergy such as ethanol, the area cultivated with sugarcane has expanded, especially in areas currently occupied by extensive pastures with low productivity. However, land-use change (LUC) from pasture to sugarcane implies changes in soil structure and variations in organic matter (SOM) stored in the soil. This study aimed to quantify the impact of LUC on organic matter fractions – particulate organic matter (POM) and mineral-associated organic matter (MAOM) – in soils with contrasting textures, and to explore the correlations between possible alterations in soil aggregation and the effects on carbon (C) stocks and SOM fractions. The study was conducted in two areas in Central-Southern Brazil, one with clayey soil and the other with sandy soil. In each area, a LUC chronosequence was evaluated: native vegetation (NV), pasture (PA), short-term sugarcane (SC1), the area analyzed during the sugarcane plant cycle; long-term sugarcane (SC2), area analyzed during the ratoon sugarcane cycle. In each use, undisturbed and disturbed samples were collected and macroaggregates and microaggregates were obtained by wet sieving and soil samples. In these samples, the physical fractionation of the SOM and the calculation of the C contained in each fraction of the SOM and C total stock of each use were performed. The conversion of NV to PA increased C stocks by more than 50 %, mainly in the MAOM fraction, and maintenance of macroaggregates (more than 80 %) in sandy site; and reduction of C stocks by more than 30 %, mainly MAOM in the clayey area. These benefits acquired from grazing on sandy area were lost with the expansion of sugarcane, a reduction of more than 20 % in macroagregation and C stocks. The sugarcane expansion into pasture with clayey site resulted in C accumulation (more than 2 Mg C ha^{-1} yr^{-1}) and recovery of stocks on a SC2 basis. Expanding sugarcane areas into low-productivity pasture areas can be considered a sustainable strategy, especially in clayey soils, in which multiple gains occur through C sequestration and improved soil quality, as well as the reduction of CO$_2$ emissions through the diversification of the energy matrix with the production of ethanol.

Keywords: soil quality, biofuels, macroaggregates, microaggregates.

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INTRODUCTION

Global warming and climate change have affected all regions of the planet (IPCC, 2021). Therefore, strategies that help to decarbonize the atmosphere (i.e., reduce greenhouse gas (GHG) emissions and remove CO_2 in the atmosphere) have been proposed and intensely debated in different technical-scientific and political forums around the world. A major alternative to climate change mitigation is to replace fossil fuels with the insertion of renewable energies in the energy matrix. Brazil is one of the major players in the bioenergy sector, especially regarding sugarcane derives. The country accounts for about 38 % of global sugarcane production (FAO, 2020), and it is estimated that it will produce more than 24 billion L of ethanol in the 2022/23 harvest (Conab, 2022).

To meet these growing demands, the area cultivated with sugarcane has expanded by 40 % in the last 15 years (from 5.8 to 8.2 thousand ha^{-1}) (Conab, 2022) and continues to expand (despite having reduced in the last five years), especially in areas currently occupied by extensive pastures that have low productivity and some degree of degradation (Dias et al., 2016; Oliveira et al., 2019; Cherubin et al., 2021). Recently, a study proposing a new agricultural zoning for sugarcane cultivation in Brazil found that 37 million hectares were suitable for sugarcane cultivation, of which 20 million hectares are currently occupied by pasture (Hernandes et al., 2021). Although this is considered a promising strategy, the conversion of pasture areas into areas cultivated with sugarcane changes the vegetation cover as well as the management practices, which directly and indirectly affects soil quality (Cherubin et al., 2016a) and the provision of multiple ecosystem services, including soil C sequestration (Oliveira et al., 2019; Cherubin et al., 2021).

Soil is the largest terrestrial C pool (approximately 1500 Pg of C in the 1 m layer) (Lal, 2018; Lal et al., 2021) and plays a vital role in the regulation of C fluxes and, thus, in climate change mitigation. In soil, C is stored in the form of soil organic matter (SOM) (Lehmann and Kleber, 2015; Lal, 2016). Soil organic matter is not homogeneous, containing components with different formation, behavior, and persistence (Lavallee et al., 2020; Cotrufo and Lavallee, 2022). Thus, knowing the characteristics of SOM is essential to determine the stability and persistence of the C stored in the soil.

The use of the physical fractionation of SOM has been widely applied to understand and predict the soil C dynamic (Cotrufo and Lavallee, 2022). Based on particle size, SOM is divided into two main components: particulate organic matter (POM) and soil mineral-associated organic matter (MAOM) (Lavallee et al., 2020). Particulate organic matter contains fragments from plants and organisms, partially decomposed through the fragmentation process by soil organisms (Lavallee et al., 2020). Thus, POM has a particle size greater than 53 µm; can be occluded, protected by aggregates, or free in the soil; and is more available for decomposition processes (Cotrufo and Lavallee, 2022). On the other hand, MAOM has a particle size of less than 53 µm and is composed of low molecular weight microscopic molecules and rainfall leachate from the plant, root exudates, and microbial decomposition products (Cotrufo and Lavallee, 2022). It is in an advanced stage of decomposition and forms associations with soil minerals (silt and clay fraction). As it forms organo-mineral complexes, MAOM is protected against decomposition in the micropores of soil aggregates (Totsche et al., 2018), which makes it less accessible to the action of decomposing microorganisms and their enzymes (Lavallee et al., 2020; Cotrufo et al., 2021).

The conversion of land to expand sugarcane cultivation causes changes in soil structure, which can intensify degradation due to excessive machinery traffic and periodic soil tillage (Cherubin et al., 2016b; Barbosa et al., 2019; Cavalcanti et al., 2020). Mechanical tillage disrupts the soil, breaking up and reducing the proportion of macroaggregates (Six et al., 2000), accelerating organic matter losses (La Scala Jr et al., 2006; Silva-Olaya et al., 2013; Tenelli et al., 2019). Bordonal et al. (2017) observed, as an undesirable outcome of this process, high oxidation of organic compounds and the formation of large proportions of aromatic compounds in SOM, which were exposed to microbial attack by the aggregate breakdown due to soil disturbance. For sandy soils, the effects of sugarcane expansion worth even more attention (Bordonal et al., 2017; Tenelli et al., 2021).

The large amount of C inputs, via straw and roots, are associated to soil C accretion in sugarcane areas without burning or straw removal in Brazil (Mello et al., 2014; Oliveira et al., 2017a; Tenelli et al., 2021). Usually, increases in POM may be associated with C input by above-ground residues (Mitchell et al., 2021), while alterations in MAOM are associated with the entry of C through roots and its exudates (Cotrufo and Lavallee, 2022). However, any source of C may be associated with MAOM accretion. Root residues and exudates are more prone to transformation and MAOM formation because microbial abundance in the rhizosphere is 2–20 times higher than in the bulk soil (Cotrufo et al., 2013; Lange et al., 2015; Kuzyakov and Blagodatskaya, 2015).

Land-use change and soil management can affect carbon storage in the soil through changes in aggregate formation and stability (Franco et al., 2020). Studies are needed to understand the effects of sugarcane expansion on soil carbon dynamics and establish sustainable strategies in Brazil. Hence, we tested the hypothesis that: 1) the SOM changes in areas of sugarcane expansion depend on soil texture; 2) the conversion to sugarcane of pasture areas increases C inputs, causing a positive effect on C stocks over time, even if the physical protection of C conferred by soil aggregation is not fully reestablished; and 3) in the long-term, the increase in C stocks occurs mainly in the MAOM, given the effects of tillage in the less protected SOM fractions (i.e., POM). This study aimed to quantify the impact of LUC on soil organic matter fractions (POM and MAOM) in soils with contrasting texture and explore the correlations between possible alterations in soil aggregation and the effects on C stocks and SOM fractions.

MATERIALS AND METHODS

Description of study areas

The study was conducted in soils with contrasting texture from central-southern Brazil, which is the main sugarcane producing region in the country. The first area is located in Brotas, São Paulo State, (22° 17’ S and 48° 07’ W), and the second area is in Manduri, SP (23° 00’ S and 49° 19’ W), with an average annual rainfall of 1337 and 1249 mm, respectively. The climate in both areas is subtropical with hot summers – Cfa (Köppen and Geiger).

In Brotas, the soil is classified as *Neossolo Quartzarênico* órtico with a sandy texture, and in Manduri, the soil is classified as *Latossolo Vermelho* distrófico (SiBCS) with a clayey texture. To facilitate the presentation and understanding of the results, Brotas is called “Sandy site” and Manduri “Clayey site”, according to their granulometric characterization by densimeter method (Table 1). The predominant minerals in the clay fraction are 1:1 clay minerals and Fe and Al oxides.

To assess the effects of land-use change (LUC) on both soils, a chronosequence approach was established with the following uses: i) Native Vegetation (NV); ii) Pasture (PA) characterized by low productivity in clayey site and high productivity in sandy site (Strassburg et al., 2014); Short-term sugarcane (SC1), the area analyzed during the sugarcane plant cycle, with a short period since conversion; Long-term sugarcane (SC2), area analyzed during the ratoon sugarcane cycle, many years after conversion. This conversion order represents the main LUC scenario in central-southern Brazil (Cherubin et al., 2021), and more details related to the history of the areas and management practices can be found in table 2.

Table 1. Distribution of soil particle size at the layer of 0.00-0.10 m in the two soils that have contrasting texture with land-use change (native vegetation – NV, pasture – PA, short-term sugarcane – SC1 and long-term sugarcane – SC2)

Site	Land-use	Clay	Silt	Sand
		g kg^{-1}		
Sandy site	NV	50	11	939
	PA	50	20	930
	SC1	75	10	915
	SC2	75	01	924
Clayey site	NV	592	306	102
	PA	472	265	263
	SC1	620	286	94
	SC2	577	316	107

Table 2. Land-use change [native vegetation (NV), pasture (PA), short-term sugarcane (SC1) and long-term sugarcane – (SC2)] history and description of adopted management practices

Site	Land-use	Description
Sandy site	NV	Secondary vegetation and seasonal semideciduous forest composed mostly of *Trichillia clausenii, Euterpe edulis*, and *Aspidosperma polyneuron*.
	PA	The conversion of NV to PA occurred in 1975. The PA was cultivated with brachiaria (*Brachiaria decumbens*) cv. Basilik, without applying mineral fertilizer and with an animal stocking rate of ~7 animal units (AU) (7 AU ha^{-1}) until 2018. *B. decumbens* was replaced by *Brachiaria brizanta* cv. Marandu in 2018. During this conversion, 2 Mg ha^{-1} of lime, and 200 kg ha^{-1} of nitrogen, 135 kg ha^{-1} of phosphorus, and 115 kg ha^{-1} of potassium were applied to the soil surface. For weed control, 1.5 L ha^{-1} of 2.4-D (a.i.) was applied. The stocking rate remained the same as in the previous year. Duration of 45 years at sample collection.
	SC1	The conversion from PA to SC1 occurred in 2018, employing conventional tillage with plowing and harrowing. To the soil surface, 2 Mg ha^{-1} of lime was applied, and 60 kg ha^{-1} of nitrogen, 150 kg ha^{-1} of phosphorus, and 120 kg ha^{-1} of potassium were applied to the planting rows. The planted cultivar was IAC SP 97-4039. Duration of 2 years at sample collection.
	SC2	The conversion of PA to SC2 occurred in 2002. The harvest was done mechanically in the following years without burning or removing the straw. Sugarcane was replanted once every 5 years with tilling and harrowing. The last replanting was in 2017 with the cultivar IAC SP 97-4039. After the 2018 harvest, 155 kg ha^{-1} of nitrogen, 41 of kg ha^{-1} phosphorus, and 86 of kg ha^{-1} potassium were applied. Duration of 18 years at sample collection.

Continue

Continuation

Site	Land-use	Description
Clayey site	NV	Same description as in the sandy site.
	PA	The conversion from NV to PA occurred in 1970. The pasture contained *Brachiaria decumbens* without the addition of mineral fertilizer. Grazing was continuous with a stocking rate of 1.2 AU ha^{-1}. Duration of 50 years at sample collection.
	SC1	The conversion from PA to SC2 occurred in 2018. The conversion was done through conventional tillage with plowing and harrowing. At that time, 2 Mg ha^{-1} of lime were applied to the soil surface, and 50 kg ha^{-1} of nitrogen, 150 kg ha^{-1} of phosphorus, and 50 kg ha^{-1} potassium were applied to the planting rows. Duration of two years at sample collection.
	SC2	The conversion from PA to SC2 occurred in 2016. In 2017 and 2018, mechanical harvesting was done without burning or straw removal. After each harvest, 90 kg ha^{-1} of nitrogen and 80 kg ha^{-1} of potassium were applied. Duration of 4 years at sample collection.

Adapted from Luz et al. (2020).

Collection, sample preparation, and laboratory analysis

In each land-use, disturbed and undisturbed samples were collected from four points ($n = 4$) in July 2020, spaced approximately 50 m apart, totaling 32 samples (i.e., 2 soils × 4 uses × 4 points × 1 depth) at a layer of 0.00-0.10 m, where the highest levels of SOM are concentrated, which was sensitive to the LUC and the resulting changes in SOM fractions.

The samples were separated into two groups in the laboratory and broken up at the weak points. The first group was passed through 8000 µm sieve, while the second group of samples was passed through 2000 µm sieve, resulting in: (i) samples <2000 µm (bulk soil), which were subjected to the physical fractionation procedure of the SOM; and samples of (ii) aggregates <8000 µm, which were subjected to wet sieving. The samples <8000 µm (ii) were submitted to the wet sieving procedure to obtain the aggregate classes (macroaggregates and microaggregates), and sequentially these two aggregate classes were submitted to the physical fractionation of the SOM (Figure 1).

To separate the macroaggregate and microaggregate classes, the methodology proposed by Elliott (1986) was used, in which 50 g of aggregates (<8000 µm) were placed in contact with a slide of water to wet by capillarity for 16 h. The previously moistened samples were distributed in a set of mesh-opening sieves: 2000, 250, and 53 µm, which oscillated vertically at 30 cycles per minute for 15 min in a Yoder MA 148/3 shaker (Marconi, Piracicaba, SP, Brazil). This analysis separated the classes: large macroaggregates (2000–8000 µm), small macroaggregates (250–2000 µm), microaggregates (53–250 µm), and silt + clay particles (≤53 µm). The aggregate content retained in each sieve was transferred to a pot and dried in an oven with forced air circulation (40 °C) for five days and the mass was measured. Then, the distribution of aggregate classes was calculated.

Soil samples (<2000 µm), macroaggregates (small and large macroagreggates), and microaggregates were passed through a 2000 µm sieve and subjected to physical fractionation of SOM according to Cambardella and Elliott (1992). Then, 5 g of soil were added to 50 mL Falcon tubes with 30 mL of distilled water and 5 g L^{-1} of sodium hexametaphosphate. These samples were sent to horizontal mechanical agitation for 16 h and then passed through a 53 µm sieve. The material retained on the sieve (>53 µm) was considered POM and the material that passed through the sieve (<53 µm) was considered MAOM. And the sum of fractions (POM and MAOM) corresponds to more than 93 % of the mass of the initial sample recovered after fractionation.

The C and N contents of each fraction (POM and MAOM) were determined by dry combustion (Nelson and Sommers, 1982) in a CN-628 elemental analyzer (LECO, St. Joseph, MI, USA). Soil C and N stocks were calculated using equation 1:

$$\text{Soil carbon or nitrogen stock (Mg ha}^{-1}\text{)} = \text{C or N} \times \text{BD} \times \text{Prof} \qquad \text{Eq. 1}$$

in which: C is the carbon content (%); N is the nitrogen content (%); BD is the soil bulk density (Mg m^{-3}) (Table 3); and Prof is the thickness of the sampled layer (cm).

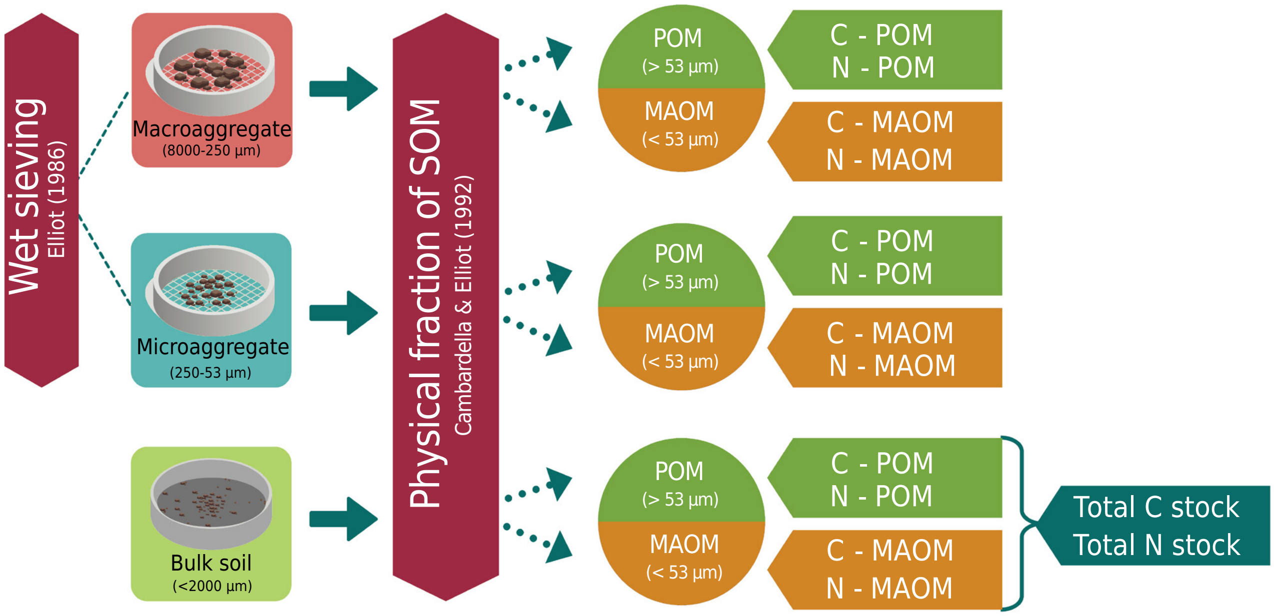

Figure 1. Workflow steps and analytical methods used in the wet sieving procedure and physical fractionation of soil organic matter.

Table 3. Soil bulk densities used to calculate C stock in a land-use change scenario [native vegetation (NV), pasture (PA), short-term sugarcane (SC1) and long-term sugarcane (SC2)]

Use	Site	
	Sandy	Clayey
	Mg m^{-3}	
NV	1.51 ± 0.04	0.90 ± 0.06
PA	1.82 ± 0.04	1.27 ± 0.17
SC1	1.64 ± 0.07	1.13 ± 0.07
SC2	1.72 ± 0.11	1.26 ± 0.05

More details can be seen in Luz et al. (2020).

Carbon and N stocks from each land use (PA, SC1 and SC2) were corrected by equivalent mass, considering native vegetation as a reference (Lee et al., 2009). Additionally, the contribution of each SOM fraction (POM and MAOM) to the total C stock (soil, macroaggregates, and microaggregates) was calculated. Soil bulk density were sampled using cylindrical rings of ~100 cm^{-3} (5 cm in diameter by 5 cm in height).

The annual rate of C accumulation or loss associated with the processes of converting native vegetation into pasture and pasture into sugarcane in the short (SC1) and long-term (SC2) was calculated according to equation 2. Positive values refer to C accumulation and negative values refer to C losses due to LUC.

$$C_{accum/loss} = \frac{\left(C_{final} - C_{initial}\right)}{T_{LUC}} \qquad \text{Eq. 2}$$

in which: $C_{accum/loss}$ is the annual rate of accumulation or loss of C due to LUC (Mg C ha^{-1} yr^{-1}); C_{final} is the total C stock after conversion (PA, SC1, and SC2) (Mg C ha^{-1}); $C_{initial}$ is the C stock before conversion (NV and PA) (Mg C ha^{-1}); T_{LUC} is the conversion time (years).

To understand the magnitude of C loss or gain from the fractions (POM and MAOM) in soil samples, macroaggregates, and microaggregates after LUC processes, the rate of change in soil C stocks was calculated, based on conversion sequences: native vegetation converted to pasture (NV-PA) and pasture converted to sugarcane in the short (PA-SC1) and long (PA-SC2) term (Equation 3).

$$\Delta C\% = \left[\left(\frac{E_2}{E_1}\right) - 1\right] \times 100 \qquad \text{Eq. 3}$$

in which: ΔC% is the Rate of change in soil C stocks; E_1 is the C stock of the previous land-use (Mg C ha^{-1}); E_2 is the C stock of the current land use (Mg C ha^{-1}).

Data analysis

Total carbon and nitrogen stocks; organic matter fractions (POM and MAOM) and SOM fractions within aggregate classes (macroaggregates and microaggregates); C:N ratios; and aggregate classes were submitted to analysis of variance (ANOVA) in completely randomized design, given the assumption of normality (Shapiro-Wilk, $p>0.05$) and when significant (F test <0.05), the means were compared using Tukey's test ($p<0.05$).

Principal component analysis (PCA) was performed to determine the relationship between SOM fractions and aggregate classes with land uses. All analyzes were conducted using RStudio software, version 4.0.4 (R Development Core Team, 2021), and packages "ExpDes" (Ferreira et al., 2021), "Hmisc" (Harrell Jr, 2021) and "Factoextra" (Kassambara and Mundt, 2020).

RESULTS

Total C and N stocks

The dynamics of LUC altered C and N stocks in both soils (Figures 2a and 2b). In sandy site, the conversion of native vegetation (NV) to pasture (PA) increased C and N stocks more than 50 % and 60 %, respectively ($p<0.05$), with a mean rate of 0.10 Mg of C ha^{-1} yr^{-1}, for this study site. Likewise, the LUC NV-PA in the clayey soil significantly reduced about 30 % of C and N stocks associated with C losses at a rate of 0.25 Mg of C ha^{-1} yr^{-1} (Figure 2c).

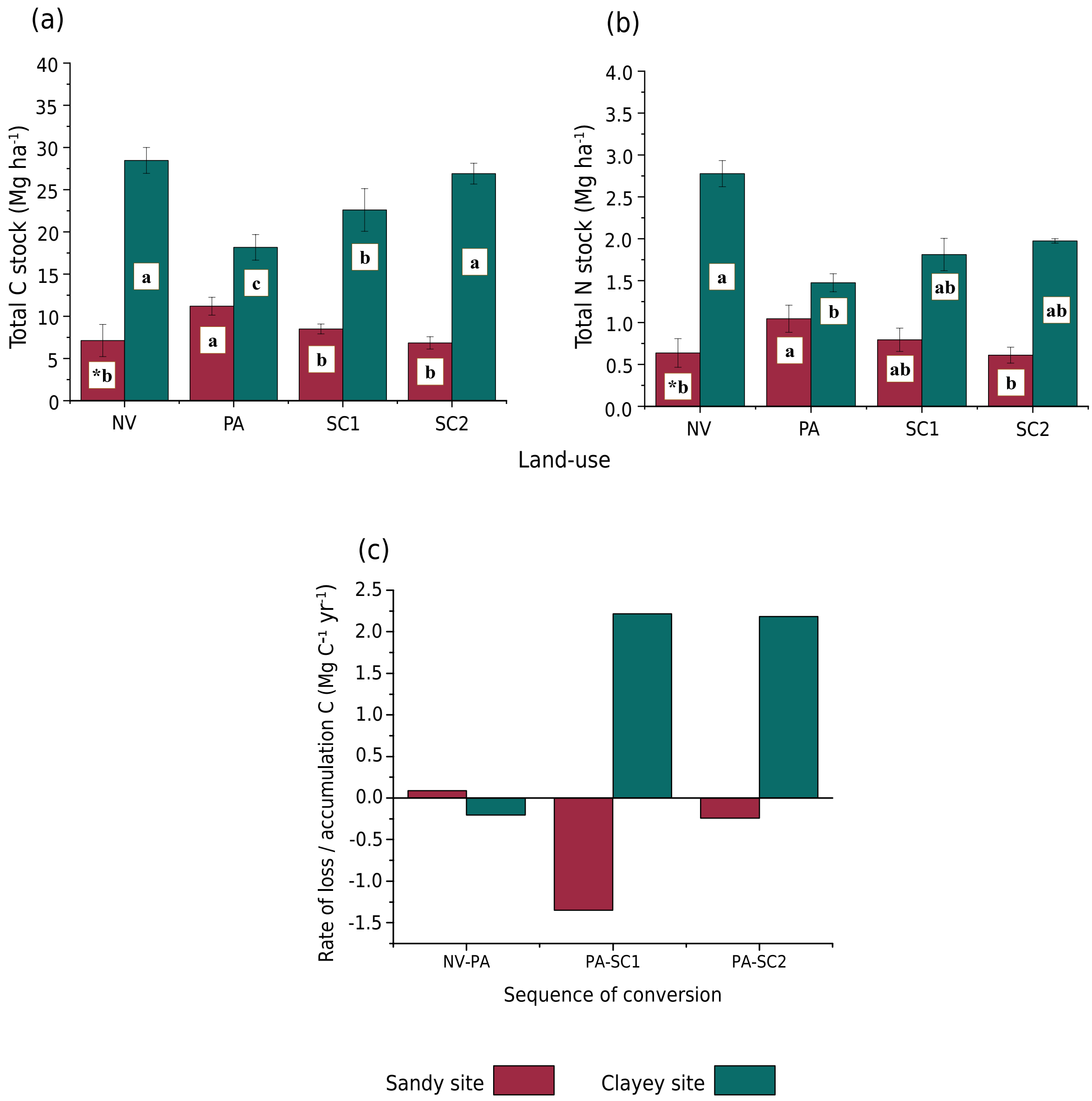

Figure 2. Carbon (a) and nitrogen (b) stocks and the rate of C accumulation or loss (C) in sandy and clayey site, in the 0.00–0.10 m soil layer in a land-use change scenario [native vegetation (NV), pasture (PA), short-term sugarcane (SC1) and long-term sugarcane (SC2)]. * Means followed by the same letter in the same soil do not differ ($p<0.05$) by Tukey's test.

The expansion of sugarcane into pasture areas in the short (SC1) and long (SC2) term showed different outcomes regarding soil C stocks behavior (Figure 2a). For sandy site, this conversion process reduced soil C and N stocks over time. In SC1 and SC2 sugarcane reduced stocks, associated with a loss rate of 1.30 Mg of C ha^{-1} yr^{-1} and 0.25 Mg ha^{-1} yr^{-1}, respectively. In clayey site, C and N stocks increased both in SC1(~ 20 %) and SC2 (~ 40 %) ($p < 0.05$) with soil C change rates of 2.16 and 2.18 Mg ha^{-1} yr^{-1}, respectively (Figure 2c).

Carbon and N stock and C:N ratio of SOM fractions

In areas of NV, MAOM (<53 µm) accounted for 76 and 93 % of the C stored in the sandy and in the clayey site, respectively (Table 4). However, LUC was associated with alterations in C partitioning between mineral-associated and particulate organic matter. In the sandy area, the conversion of NV into PA increased C and N stocks in MAOM by more than 1.7 times ($p<0.05$). However, this increase was lost with the long-term conversion of PA to SC2. No changes were observed in the C:N ratio due to the LUC process (Table 4).

In the clayey soil, the C and N stocks in the MAOM fraction were reduced ($p<0.05$) more than 1.6 times in the conversion of NV to PA. However, the SC1 and SC2 conversion recovered the C stocks in MAOM, and besides increasing the N stocks, the C:N ratio in both physical fractions was evaluated (Table 4).

Carbon and N stock and C:N ratio of SOM fractions associated with aggregate classes

The LUC altered the distribution of aggregates in the sandy site (Table 5). Such changes were detected in SC2, where soil had a lower proportion of macroaggregates and a higher proportion of microaggregates along with silt and clay particles than the NV and PA areas. In clayey soil, both LUC did not affect the distribution of aggregates ($p>0.05$) (Table 5).

In both sites and for all land-uses evaluated, more than 80 % of C and N stocks were stored in the MAOM fraction (<53 µm), irrespective of the aggregate size (Table 6). The LUC altered the C and N stocks and the C:N ratio of aggregate classes in both soils in the sandy site (Table 6). In the macroaggregate class, C and N stocks as well as the C:N ratio of MAOM reduced with the conversion of native vegetation to pasture. In microaggregates, the C and N stocks in the MAOM fraction increased by 60 % ($p<0.05$) in this conversion process. The expansion of sugarcane over pasture resulted in a reduction of C stocks in

Table 4. Carbon and N stocks, % of contribution (between parenthesis) and C:N ratio of soil organic matter fractions (POM - particulate organic matter, MAOM - mineral-associated organic matter) in the 0.00–0.10 m layer of bulk soil in a land-use change scenario [native vegetation (NV), pasture (PA), short-term sugarcane (SC1) and long-term sugarcane (SC2)]

Site	Use	POM			MAOM		
		C	N	C:N	C	N	C:N
		Mg ha^{-1}			Mg ha^{-1}		
Sandy site	NV	(24) 1.74^{a}	(22) 0.14^{ns}	16^{ns}	(76) 5.39^{b}	(78) 0.50^{b}	11^{ns}
	PA	(14) 1.60^{a}	(14) 0.15^{ns}	13^{ns}	(86) 9.59^{a}	(86) 0.89^{a}	11^{ns}
	SC1	(13) 1.13^{ab}	(9) 0.07^{ns}	19^{ns}	(87) 7.37^{b}	(91) 0.73^{ab}	10^{ns}
	SC2	(10) 0.71^{b}	(15) 0.09^{ns}	13^{ns}	(90) 6.13^{b}	(85) 0.52^{b}	12^{ns}
Clayey site	NV	(8) 2.34^{ns}	(6) 0.16^{a}	15^{b}	(92) 26.13^{a}	(94) 2.62^{a}	10^{c}
	PA	(12) 2.18^{ns}	(7) 0.11^{ab}	23^{a}	(88) 15.99^{c}	(93) 1.36^{c}	12^{b}
	SC1	(7) 1.67^{ns}	(4) 0.08^{b}	19^{ab}	(93) 20.93^{b}	(96) 1.73^{b}	12^{b}
	SC2	(9) 2.54^{ns}	(5) 0.10^{ab}	25^{a}	(91) 23.92^{ab}	(95) 1.86^{b}	13^{a}

* Means followed by the same letter in the columns do not differ by Tukey's test ($p<0.05$).

Table 5. Distribution of aggregate classes in the 0.00–0.10 m layer in land-use change scenario [native vegetation (NV), pasture (PA), short-term sugarcane (SC1) and long-term sugarcane (SC2)]

Site	Land-use	Distribution of aggregate classes		
		Macroaggregates (>250 µm)	Microaggregates (53-250 µm)	Silt + Clay (<0.53 µm)
		%		
Sandy site	NV	89^{a}	9^{b}	2^{ab}
	PA	91^{a}	8^{b}	1^{b}
	SC1	82^{ab}	16^{ab}	2^{ab}
	SC2	71^{b}	24^{a}	4^{a}
Clayey site	NV	97^{ns}	1^{ns}	2^{ns}
	PA	95^{ns}	3^{ns}	2^{ns}
	SC1	97^{ns}	2^{ns}	1^{ns}
	SC2	91^{ns}	6^{ns}	3^{ns}

* Means followed by the same letter in the columns did not differ by Tukey's test ($p<0.05$).

the POM fraction and a reduction of C and N stocks of the MAOM in the macroaggregate class, both in SC1 and SC2 (Table 6). The microaggregates showed an increment in the N stock from POM and losses of C and N (~30 %) in the MAOM due to sugarcane's long-term expansion (SC2).

In macroaggregates from the clayey site, the conversion of native vegetation to pasture increased C stocks and reduced N stocks in the POM by more than 20 % ($p<0.05$), while the C and N stocks in MAOM reduced by more than 30 % ($p<0.05$; Table 6). In this same LUC, the C:N ratio also increased in the POM (from 15 to 23) and MAOM (from 9 to 11) ($p<0.05$). In the microaggregates, the changes in C and N stocks in the MAOM and N in the POM were similar to the macroaggregates ($p<0.05$), whereas the C stock in the POM showed no changes with the LUC. Finale, there was an increase in the C:N ratio of POM (from 14 to 22) and MAOM (from 9 to 11) in the NV-PA transitions (Table 6).

The expansion of sugarcane on pasture reduced C and N stocks and the C:N ratio in the POM fraction from macroaggregates ($p<0.05$) in short and long-term (Table 6). However, C stocks in the MAOM fraction were partially restored after conversion ($p<0.05$). In the microaggregates, the conversion of pasture into sugarcane increased in C and N stocks by more than 50 % and increased the C:N ratio in SC1 and SC2 in both physical fractions ($p<0.05$), exceeding in some cases the values of C stocks of NV (Table 6).

Rate of changes in C stocks from POM and MAOM within aggregates

Conversion of NV to PA increased C stocks in the POM fraction (macro- and microaggregates) by up to 20 % and in the MAOM fraction (microaggregates) by more than 50 % in sandy site (Figure 3a). The C stocks in the MAOM (macroaggregates and microaggregates) reduced by up to 40 % in the clayey site (Figure 3b).

Expansion of sugarcane into pasture in SC1 resulted in the reduction of C stocks in the POM (macroaggregates) and MAOM (macroaggregates and microaggregates) fractions by up to 20 % in the sandy site (Figure 3a). In the long-term, this proved to be more

Table 6. C and N stocks, % of contribution (between parenthesis) and C:N ratio of SOM fractions within the aggregate classes, in the 0–10 cm layer in a land-use change scenario [native vegetation (NV), pasture (PA), short-term sugarcane (SC1) and long-term sugarcane (SC2)]

Site	Use	Macroaggregates						Microaggregates					
		POM			MAOM			POM			MAOM		
		C	N	C:N	C	N	C:N	C	N	C:N	C	N	C:N
		Mg ha^{-1}											
Sandy site	NV	(13) 1.27^{ab}	(16) 0.15^{a}	9^{b}	(87) 8.87^{a}	(84) 0.76^{a}	12^{a}	(15) 0.80^{b}	(19) 0.10^{b}	8^{bc}	(85) 4.62^{b}	(81) 0.42^{c}	11^{b}
	PA	(15) 1.40^{a}	(13) 0.11^{a}	15^{b}	(85) 7.87^{b}	(87) 0.71^{b}	11^{c}	(11) 0.94^{ab}	(7) 0.05^{c}	18^{a}	(89) 7.30^{a}	(93) 0.68^{a}	11^{b}
	SC1	(14) 1.12^{b}	(5) 0.03^{b}	41^{a}	(86) 6.79^{c}	(95) 0.59^{c}	11^{b}	(14) 1.14^{a}	(16) 0.12^{a}	10^{b}	(86) 7.07^{a}	(84) 0.64^{b}	11^{b}
	SC2	(12) 0.65^{c}	(20) 0.10^{a}	8^{b}	(88) 4.62^{d}	(80) 0.41^{d}	11^{b}	(15) 0.85^{b}	(21) 0.11^{a}	7^{c}	(85) 4.78^{b}	(79) 0.41^{c}	11^{a}
Clayey site	NV	(8) 2.05^{b}	(5) 0.14^{a}	15^{c}	(92) 23.46^{a}	(95) 2.55^{a}	9^{c}	(6) 0.94^{b}	(4) 0.06^{c}	14^{c}	(94) 13.94^{b}	(96) 1.59^{a}	9^{b}
	PA	(13) 2.40^{a}	(6) 0.10^{b}	23^{a}	(87) 16.15^{c}	(93) 1.46^{b}	11^{b}	(8) 0.93^{b}	(4) 0.04^{d}	22^{a}	(92) 10.10^{c}	(96) 0.88^{c}	11^{a}
	SC1	(7) 1.19^{c}	(4) 0.06^{c}	21^{b}	(93) 16.69^{c}	(96) 1.47^{b}	11^{b}	(10) 1.66^{a}	(7) 0.09^{a}	18^{b}	(90) 15.69^{a}	(93) 1.29^{b}	12^{a}
	SC2	(6) 1.11^{c}	(4) 0.06^{c}	20^{b}	(94) 17.51^{b}	(96) 1.48^{b}	12^{a}	(10) 1.69^{a}	(6) 0.08^{b}	21^{a}	(90) 15.05^{ab}	(94) 1.29^{b}	12^{a}

* Means followed by the same letter in the columns did not differ by Tukey's test ($p<0.05$).

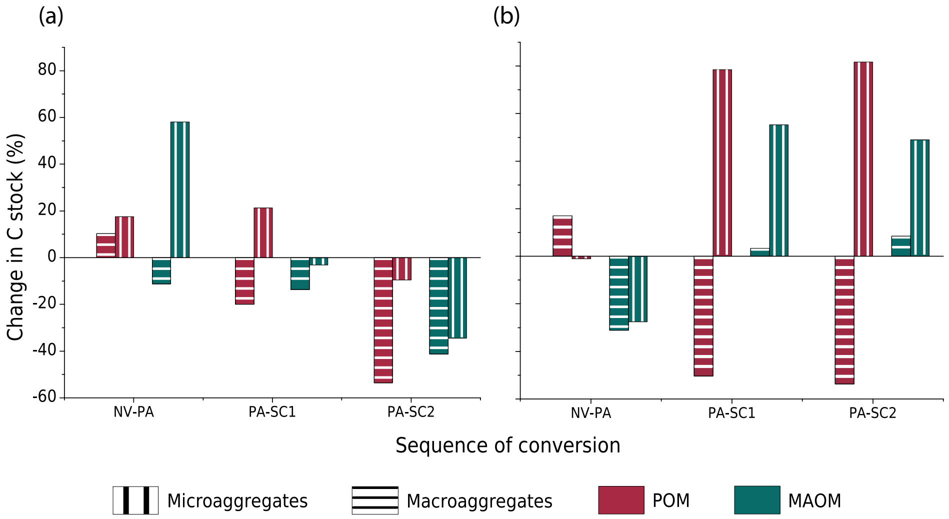

Figure 3. Relative rate of change in C stocks in the 0.00–0.10 m soil layer in conversions associated with land-use change scenario [native vegetation (NV), pasture (PA), short-term sugarcane (SC1) and long-term sugarcane (SC2)] in sandy (a) and clayey (b) site.

harmful, decreasing C stocks by up to 40 % in the POM and MAOM (macroaggregates and microaggregates). However, in the clayey site (Figure 3b), the expansion of sugarcane into the pasture area (SC1 and SC2) increased the C stocks in the POM (microaggregates) and MAOM (macroaggregates and microaggregates) by up to 60 %.

Relationship of the LUC with aggregate classes and SOM fraction

The relationships between land-uses with SOM fractions and aggregate classes were demonstrated by principal component analysis for sandy and clayey site. For sandy site (Figure 4a), the first two components (axes) correspond to 85 % of the data variance. Macroaggregates, POM, MAOM, N and C stocks positively correlated with each other, negatively with microaggregates and the increase in C and N stocks is related to the increase in POM fraction. The conversion of NV into PA is related to the increase in total C and N stocks as well as the POM and MAOM fractions. On the other hand, the sugarcane expansion is associated with a higher proportion of microaggregates in SC2 (Figure 4a).

For clayey site (Figure 4b), two components (axes) explain about 92 % of the data variance. The POM, MAOM, and N, C stocks are positively correlated and the increase in C and N stocks is related to the increase in both POM and MAOM fractions. The conversion of NV into PA reduced the total C and N stocks along with the POM and MAOM fractions. The sugarcane expansion, especially SC2, tends to re-establish the total C and N stocks and POM and MAOM fractions, despite increasing the proportion of microaggregates (Figure 4b).

DISCUSSION

LUC-induced variations of soil C stocks

The LUC affects soil C stocks depending on intrinsic soil characteristics such as soil mineralogy and texture, climatic conditions (Blanco-Canqui and Lal, 2004; Jiménez and Lal, 2006; Franco et al., 2015), and management practices adopted (Bordonal et al., 2017; Oliveira et al., 2019). Here, the clay content in both sites clearly modulated the effects of PA-SC land-use transitions on soil C stocks (Figure 2). Depending on soil texture, the expansion over pasturelands would increase the C stocks or even heighten the soil C debt in the sugarcane areas.

In the sandy site, the positive rates of change in soil C stocks (Figure 2) that occurred in converting NV to PA can be associated with high inputs of above-ground and root

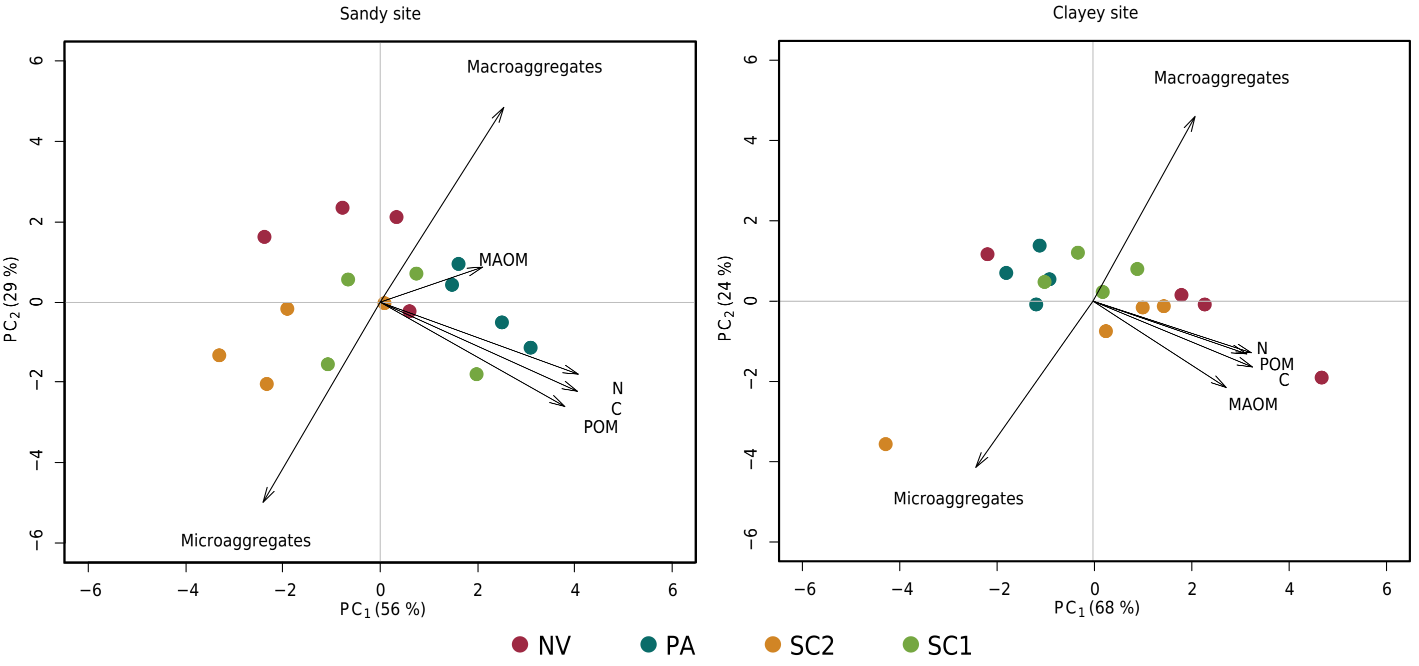

Figure 4. Principal component analysis in land-use change scenario [native vegetation (NV), pasture (PA), short-term sugarcane (SC1) and long-term sugarcane (SC2)] in sandy (a) and clayey (b) site. POM: particulate organic matter; MAOM: mineral-associated organic matter; C: total carbon stock; N: total nitrogen stock.

biomass from grasses in well-managed pastures (Gmach et al., 2018). Thus, the C accumulation in this land-use may have occurred due the deposition of grass residues and renewal of the root system, in addition to the absence of soil disturbances (i.e., tillage) (Table 2), preserving soil aggregates that protects SOM from decomposition, one of the main mechanisms for C persistence on soil (Sarkar et al., 2018). In addition, the baseline must be taken into account: soil C stocks in the NV was low (~7 Mg ha^{-1}) (Figure 2a), as observed in other studies with semideciduous forest in sandy soils (Gmach et al., 2018).

In the clayey site, the conversion to pasture was associated to C losses of 0.25 Mg ha^{-1} yr^{-1} (Figure 2c). Loss of C is likely attributed to the processes of turning over the soil during the conversion, which impacts the mechanisms of C sequestration, as the soil exposure accelerates C decomposition by microbial activity, reducing its accumulation (Blanco-Canqui and Lal, 2004). Oliveira et al. (2017a) estimated losses of 0.34 mg of C ha^{-1} yr^{-1} in this transition from NV to PA, which were attributed to deforestation and biomass burning, as well as the subsequent processes of soil degradation in pasture areas, mainly associated with the absence of fertilization practices (Segnini et al., 2019), as observed in the pasture area from the clayey site (Table 2).

The conversion of pasture to sugarcane was associated with C and N losses in the sandy site (Figure 2), which can be related to management practices during the LUC (plowing and harrowing) and the intrinsic characteristics of the soil (texture, mineralogy). C losses of ~ 1 Mg ha^{-1} yr^{-1} in SC1 (Figure 2c) may be associated with the increment of decomposition rate caused by interaction with oxygen and microbial attack through the breakdown of aggregates (Bordonal et al., 2017). In SC2, C losses are still observed, related to the renewal of the sugarcane field (once five years), where the operations of tilling and harrowing the soil are also carried out (Silva-Olaya et al., 2013; Bordonal et al., 2017; Tenelli et al., 2019).

On the other hand, the conversion of pasture into sugarcane in clayey site promoted C and N accumulation in both time span (Figure 2c). Such an outcome is a combined effect of the high C inputs in sugarcane areas without burning or straw removal (Morais et al., 2020) and the role of clay in the formation and persistence of SOM (Brandani et al., 2017). Moreover, areas previously occupied by low-productivity pastures usually have a notable C debt (Alkimim and Clarke, 2018) and are prone to storage soil C at high rates when converted to more sustainable land uses (Alkimim and Clarke, 2018) (Figure 2c). Furthermore, studies show that 3 to 4 Mg C ha^{-1} can be accumulated during the 5-year cycle of sugarcane, which corresponds to 0.74-0.80 Mg C ha^{-1} yr^{-1} (Silva-Olaya et al., 2017), leading to a positive balance of C in the soil when pastures are converted to sugarcane (Franco et al., 2015; Oliveira et al., 2016a, 2017b).

Physical fractions of SOM altered by LUC

To understand the sensitivity of the SOM fractions due to the LUC processes, the MAOM contributed about 80 % of the total C stocks in both soils (Table 4). These results are in line with other studies conducted in soils in tropical regions where POM, although important, contributes only a small part of C stocks (Brandani et al., 2017; Gmach et al., 2018; Morais et al., 2020). In sandy site, the reduction in POM after LUC (Table 4) is associated with the decomposition of less protected compounds after the soil disturbance (i.e., tillage) and disruption of poorly cemented aggregates, resulting in a SOM with a predominance of more persistent C fractions in sugarcane areas (Oliveira et al., 2016b, 2017a). One of the main inputs of C into POM is through above-ground (straw) biomass from plants (Mitchell et al., 2021); thus, the increase in SC2 C:N ratio in the clayey site is influenced by its high C:N ratio (close to 100) and biochemical composition, in which complex structures such as tannins and lignin predominate (Pimentel et al., 2019).

Although MAOM is less responsive to LUC (Rocci et al., 2021), the results showed that both the transition from NV to PA and pasture to sugarcane caused changes in this C fraction in both soils (Table 4). In the pasture from the sandy site, the root system of grasses, despite representing a small fraction of the total SOM, is associated with the formation of compounds in several stages of decomposition, and such C turnover is crucial to MAOM formation (Kögel-Knabner, 2017; Rocci et al., 2021). In the clayey site, the decrease in MAOM may be related to the degradation of the soil structure in the pasture (Luz et al., 2020) and increases accessibility for soil microorganisms to decompose this fraction.

Sugarcane expansion into pasture areas had two distinct consequences in the studied sites (Table 4 and Figures 4a and 4b). The reduction in SC2 of C and N stocks by more than 30 % in sandy site was caused by sugarcane cultivated in a conventional system that was highly susceptible to C loss over time (Tenelli et al., 2019, 2021). On the other hand, the increase in C and N stocks by more than 30 % in SC1 and SC2 in MAOM fraction in clayey site was probably due to the entry of low molecular weight compounds from the root biomass (when dead) and rhizodeposition, which also serve as food for soil microorganisms and supply MAOM (Kögel-Knabner, 2017; Rocci et al., 2021). Therefore, it is possible to promote short-term C sequestration in areas of sugarcane expansion since soils have a high silt and clay content as well as a high deficit of C saturation (Blanco-Canqui and Lal, 2004; Mitchell et al., 2021; Rodrigues et al., 2022).

Physical protection of SOM within aggregates

Soil organic matter is directly related to the formation and stabilization of aggregates, which are considered diagnostic indicators of the C responses due to LUC (Lavallee et al., 2020). In all land uses evaluated in both soils, macroaggregation predominated above 70 % (Table 5). In the sandy site, the NV-PA-SC2 transition reduced the proportion of macroaggregates and significantly increased the proportion of microaggregates (Table 5). In both the aggregates classes, MAOM is the dominant fraction, corresponding to about 80 % of the total C and N stock in both soils (Table 6).

The formation of macroaggregates is promoted mainly by plant debris from the soil surface and live roots, as well as fungal hyphae, which form a tangle that brings together and binds soil particles (Tisdall and Oades, 1982; Bronick and Lal, 2005). These C inputs are the fresh fraction of the POM (Lal, 2018; Lavallee et al., 2020), crucial to promoting the stabilization of aggregates, which in turn provide physical protection against the action of decomposing agents (Blanco-Canqui and Lal, 2004) as observed in the transition from native vegetation to pasture for both sites. However, this protection can be affected by the LUC due to disturbances caused by soil tillage and exposure of SOM to its decomposers, increasing its mineralization, and consequently, decreasing the binders related to macroaggregate formation (Six et al., 2000; Tivet et al., 2013; Franco et al., 2015, 2020; Bordonal et al., 2017). Those process may be related to the results obtained in the conversion of pasture to sugarcane, where decreasing POM are followed by a lower proportion of macroaggregates, mainly in the sandy site (Tables 5 and 6).

Soil aggregation process can change the fractions of SOM (POM transforms into MAOM), and C stabilization is closely associated with the presence of microaggregates formed within the macroaggregates (Table 6), thereby indicating that microaggregates significantly act in C sequestration by stabilizing SOM through organo-mineral complexes. In turn, macroaggregates physically protect POM against microbial decomposition (Six et al., 2002a; Six and Paustian, 2014; Hoffland et al., 2020; Cotrufo and Lavallee, 2022). Therefore, the disruption of macroaggregates caused by the LUC process alters the MAOM stocks within the macroaggregates, significantly reducing them in the long-term after successive conversions in the sandy site (Tables 5 and 6).

For the clayey site, the partially recovery of MAOM after pasture-sugarcane LUC may be associated with the combined effect of the high C inputs in sugarcane areas without burning or straw removal (Morais et al., 2020) and the role of clay in the formation of MAOM (Brandani et al., 2017).

SOM stabilization ability via physical protection and organo-mineral complex

Based in our data, we suggest two main mechanisms of SOM persistence related to the soil texture of each area. In the sandy site (Figure 4a), SOM persistence is associated with the physical protection provided by aggregates (macroaggregates and microaggregates) (Six et al., 2002a; Sarkar et al., 2018). Such mechanism promotes maintenance of aggregate stability and is associated to the SOM accretion in the pasture of the sandy area because of the presence of binders agents (i.e., plant roots, fungal hyphae, and partially decomposed plant residues), in addition to the absence of tillage. Accordingly, adopting management practices such as reduced tillage, in which only the planting furrow is disturbed, are strongly recommended to avoid SOM losses undergoing sugarcane expansion over sandy soils (Tenelli et al., 2019).

Another SOM stabilization mechanism that is extremely important in soils from tropical regions is the surface sorption capacity of minerals (i.e., Fe and Al sesquioxides and 1:1 clay minerals) in clayey soils (Six et al., 2002a; Blanco-Canqui and Lal, 2004; Mitchell et al., 2021), such as clayey study area (Table 1 and Figure 4b). The high affinity of the clay fraction with low molecular weight organic compounds makes them physically inaccessible to degradation by soil microorganisms (Bordonal et al., 2017; Tenelli et al., 2021) in addition to having an important role in the aggregation process (Barthès et al., 2008), contributing to the formation of stable aggregates (Six et al., 2002b). Despite the C provided by the straw, the large proportion of MAOM in sugarcane areas (Table 4) is also related tohe C inputs via roots and exudation and its favorable chemical composition for MAOM formation (Mitchell et al., 2021).

Finally, despite being less vulnerable than POM to decomposition, tillage operation may also accelerate MAOM losses, mainly in agricultural areas where POM is scarce and MAOM represents the main source of energy to microbes (Lugato et al., 2021). In this sense, adopting reduced tillage is a feasible and necessary management option to avoid future C losses in sugarcane areas cultivated in clayey soils (Figure 4b).

CONCLUSION

Sugarcane expansion over pasture areas leads to different behaviors related to soil texture. In sandy area, this conversion decreases the physical protection the soil aggregates provides, through their rupture and successive losses in both fractions of the SOM (POM and MAOM), causing losses in the total C and N stocks. On the other hand, clayey soils are less prone to C losses, and the conversion of low-productivity pasture areas into sugarcane, can be considered a promising strategy for C and N accumulation (MAOM fraction). Therefore, relating the intrinsic properties of the soil (i.e., texture) with the sequestration of C is crucial to guide management strategies and public policies that are premised on the sustainability of the production system of bioenergetic crops in Brazil.

Finally, expanding sugarcane areas, especially in areas of extensive pastures with low productivity located on clayey soils, can be considered a sustainable strategy in a scenario where multiple gains from C sequestration and improved soil quality occur. The main limitation of this study is related to the analyses occurring only in the 0.00-0.10 m soil layer, since the uses present different residue input and root system, generating opportunities for studies to explore the dynamics of the C fractions in the other layers. The main findings here are relevant for policymakers to improve initiatives such as the Renovabio and ABC+ programs by reducing CO_2 emissions through diversifying the energy matrix with ethanol production.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://www.rbcsjournal.org/wp-content/uploads/articles_xml/1806-9657-rbcs-47-e0220103/1806-9657-rbcs-47-e0220103-suppl01.pdf.

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