How Soil Organic Carbon Fractions Affect N_2O Emissions in a Long-Term Integrated Crop-Livestock System: A Case Study

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Contents

Abstract

The nitrous oxide (N_2O) emissions in agricultural systems are influenced by edaphoclimatic conditions, and the availability of soil organic matter (SOM) is a key factor in this process. Understand the relationship between SOM fractions

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and N_2O emissions in cultivated soils is fundamental to the sustainable management of tropical soils. However, this relationship remains unclear. The objective of this study was to evaluate the accumulation of labile and stable fractions of SOM and their relations with N_2O emissions in a 24-year field study that represents farm conditions in the Cerrado region. The following hypotheses were considered: (i) conservation systems protect SOM, avoiding its rapid decomposition and, consequently, reducing losses of N_2O to the atmosphere; (ii) conservation systems favor the increase of labile and stable fractions of SOM, which has the effect of reducing the N_2O emission in the soil. The following land-use systems were assessed: no-tillage with integrated crop-livestock system (NT1); no-tillage with continuous cropping (NT2); and conventional system (CT). An area of native vegetation of Cerrado was used as a reference. Nitrous oxide emissions were quantified over a period of 509 days, covering two agricultural years with soybean crop followed by sorghum and corn as a second crop in 2014/2015 and 2015/2016 agricultural years, respectively. Soil carbon fractions (labile and stable) and carbon in different classes of soil aggregates were also determined. The cumulative N_2O emissions were larger in CT, intermediate in NT systems, and smaller in the Cerrado area. Among the agricultural systems, lower cumulative N_2O emissions were observed in NT1, because of the greatest buildup of carbon in its most stable fractions and occluded in aggregates. From PCA results, it is possible to conclude that aggregation is a key factor that correlates with N_2O emissions from soil. Thus, NT1 showed the largest average diameter of aggregates and presented the lowest N_2O emissions among agroecosystems. Although the conservation systems show a greater microbial population, stable fractions of carbon are predominant, which decreases availability for the soil microbiota, which justifies lower rates of SOM mineralization and, consequently, the lowest N_2O emissions.

Keywords

Soil organic matter · Carbon stability · No tillage · Greenhouse gases · Brazilian Cerrado

Abbreviations

N_2O	Nitrous oxide
SOM	Soil organic matter
NT1	No-tillage with crop-livestock in the pasture phase
NT2	No-tillage with crop-livestock in the crop phase
CT	Conventional tillage
CER	Cerrado area
PCA	Principal component analysis
GHG	Greenhouse gases
CO_2	Carbon dioxide
CH_4	Methane

IPCC	International panel on climate change
COP	Conference of the parties
iNDC	Intended Nationally Determined Contributions
CLS	Crop-livestock integrated system
C	Carbon
N	Nitrogen
C:N	Carbon nitrogen ratio
TOC	Total carbon
TN	Total nitrogen
LC	Labile carbon
ADFS	Air-dried fine soil
POC	Particulate organic carbon
HA	Humic acid
FA	Fulvic acid
HI	Humification index
HUM	Humin
IC	Inert carbon
MWD	Mean weight diameter
MBC	Microbial biomass carbon
C-MACRO	Carbon in macroaggregates
C-MICRO	Carbon in microaggregates
N-MACRO	Nitrogen in macroaggregates
N-MICRO	Nitrogen in microaggregates

1 Introduction

The discussions on global climate change and greenhouse gas emissions (GHG) took on significant proportions during the 1990s and early 2000s due to increased anthropic concentrations of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) in the atmosphere. This discussion was highlighted after the publication of the IPCC report (2018) which pointed to the urgent reduction in GHG emissions to contain the average temperature increase on the planet below 2 °C. The trend of increasing GHG concentrations is expected to continue in the coming decades, and, if urgent measures are not adopted, it should be twice as high until 2050 when compared to the 1990s (Meinshausen et al. 2009). This increase is a result of both industrial development and food production with the expansion of the agricultural area in countries where the consumption of nitrogen fertilizers is growing and intensive (Smith et al. 2007). The need to increase food production has led to an increase in anthropogenic GHG emissions into the atmosphere because of the increased use of synthetic fertilizers in agriculture and livestock. In the case of N_2O, the agricultural sector is the main responsible for its emissions to the atmosphere, as a consequence of the oxidation of organic matter and complex microbial processes associated with the management practices of plant residues (Carvalho et al. 2016; Santos et al. 2016; Sato et al. 2017; Figueiredo et al. 2018).

During COP 21 in Paris, the Brazilian government, through its Intended Nationally Determined Contributions (INDC), pledged to reduce by 43% the emission greenhouse gases (GHG) in Brazil until 2030. In the agricultural and livestock sector, among other nations, the government established, as its goal, to recover 15 million hectares degraded pastures and to incorporate 5 million hectares of crop-livestock integrated systems (CLS) (Brazil 2015).

The integrated cropping systems are considered more efficient in recycling soil nutrients (Salton et al. 2014), as they improve soil quality (Salton et al. 2014), increase diversity of the fauna (Marchão et al. 2009a), and represent efficient carbon drainage, contributing to the mitigation of GHG emissions (Buller et al. 2015).

Among the GHGs, nitrous oxide (N_2O) gained notoriety for being potentially more harmful than CO_2, because of its greater warming capacity and longer time of permanence in the atmosphere, approximately 100 years. In Latin America, Brazil is the largest emitter of N_2O (Bustamante et al. 2014). The N_2O emission from soil in agricultural systems is affected by several factors such as content of water, which favors anaerobiosis processes (Butterbach-Ball et al. 2013); the soil acidity, which alters the nitrification and denitrification processes (Martins et al. 2015); N fertilizers, which affect the N availability (Metay et al. 2011; Martins et al. 2015); the tillage system, which changes the soil porosity and microbial communities (Bayer et al. 2015); the animal excrements (Buller et al. 2015); and the C:N ratio of SOM, which favor soil microorganisms (Bhattacharyya et al. 2013; Meena et al. 2020b).

Land-use systems may build up stable SOM from plant residues, which alter the dynamics of denitrification and, consequently, the N_2O production (Miller et al. 2008). However, the manner in which C accumulates in the soil in the different agricultural systems is variable, influencing its availability and the N dynamics, in function of the C:N ratio, and therefore in the N_2O released from the soil (Kong et al. 2009).

The greatest buildup of C in its recalcitrant fractions is normally associated to a greater degree of stability of SOM (Plaza-Bonilla et al. 2014). The chemical and physical fractionation techniques of SOM have been developed and supplied information on stability and location of SOM fractions in soil compartments. The C fractions indicate sensibility to alterations in soil management, whether they are short-term, as in labile carbon and microbial biomass C (Guimarães et al. 2013; Meena et al. 2018) or long-term, as in organic mineral-associated C (Trigalet et al. 2014).

The accumulation of different fractions of organic C is influenced by climatic conditions and soil management (Bayer et al. 2011). In contrast to conventional tillage with intensive plough, conservation practices as no-till associated with the integration of crops lead to maintain or increase SOM, reducing GHG emissions (Six et al. 2004; Buller et al. 2015).

The ability to protect and stabilize soil C depends on the management practices adopted and on the soil's intrinsic characteristics (Bayer et al. 2011). In Oxisols, chemical stabilization is highlighted by the strong organomineral interaction (Six et al. 2004). Physical protection (formation of aggregates), on the other hand, is considered as a stabilization mechanism that predominates in conservation systems in temperate soils and in most tropical soils (Six et al. 2004; Conceição et al. 2008). The C accumulation in its most stable forms is associated with a higher degree of SOM stabilization (Six et al. 2002; Plaza et al. 2013; Plaza-Bonilla et al. 2014), resulting from the less exposure of SOM to the mineralization process, due to the more difficult access of decomposing microorganisms (Jahangir et al. 2014; Meena and Lal 2018).

The soil aggregation and its dynamics are fundamental for the SOM stabilization (Plaza et al. 2013). According to the aggregate formation process proposed by Golchin et al. (1994), fresh plant material incorporated into the soil is colonized by microorganisms and encrusted by primary particles through the binding action of microbial agents (e.g., mucilage and polysaccharides), thus forming macroaggregate. Over time, fresh plant material within macroaggregates is selectively decomposed leaving recalcitrant vegetable structural materials, which are coated with microbial metabolites and mineral particles to form stable microaggregates. The process of formation of macroaggregates is dependent on the continuous supply of C to the soil and, therefore, is regulated by the agricultural system (Bayer et al. 2011). Management systems that favor the intense supply of C, therefore, will favor the formation of aggregates and, consequently, higher soil C stocks with greater SOM stability. In no-tillage systems, the C input is more protected than those from conventional till because macroaggregates have a longer residence time. In addition, crop rotation favors a greater C supply in intra-aggregates (Zotarelli et al. 2007). Thus, the formation of macroaggregates is a key process for C sequestration and GHG mitigation (Chung et al. 2008; Meena et al. 2020a).

Considering that 98% of the total N of the soil is in organic forms (Stevenson 1994), and the availability and dynamics of N are influenced by the C:N ratio of the soil (Kong et al. 2009), the C fractions can affect the N_2O emissions. According to Miller et al. (2008), the availability of C in the soil, when coming from less complex sources such as glucose, will favor the production of N_2O. In addition, agricultural systems with more complex C sources, from plant residues with a higher C:N ratio, affect the rates of nitrification and denitrification, influencing the production of N_2O by the soil (Dendooven et al. 1996; Miller et al. 2008).

In a study in California, Kong et al. (2009) concluded that conventional till presents higher N conversion and incorporation in less stable silt and clay fractions, which provides greater N_2O flows. In Brazil, few studies correlate N_2O emissions with different fractions of SOM in agricultural systems. The greatest emphasis was given in soil C and N stocks (Coutinho et al. 2010; Bayer et al. 2015, 2016) and labile fractions such as microbial biomass C (Carvalho et al. 2017) and labile C (Carmo et al. 2005). Furthermore, in long-term experiments, SOM evaluation consider only the total C content which does not express the changes resulting from the management systems (Figueiredo et al. 2013). Additionally, it is relevant to explore the role of SOM fractions from different agroecosystems and their relationship with N_2O emissions.

In the Brazilian Cerrado, various studies demonstrated that N_2O emission is lower in native areas in comparison to agricultural systems, even though there is a greater content of organic carbon (Santos et al. 2016; Carvalho et al. 2017; Sato et al. 2017, 2019). However, land-use systems that are capable of balancing the increase of SOM content, with greater availability of N, without increasing the N_2O emissions in the atmosphere, are still scarce. Kong et al. (2009) noticed that the conventional system not only showed the fastest N turnover and more fertilizer-N incorporation into the less stable silt-and-clay fraction, but also the highest N_2O fluxes among the three assessed cropping systems. In Brazil, studies that correlate the N_2O flow to the different SOM fractions in land-use systems are rare.

From these results, we elaborated the hypothesis that the buildup of C in stable fractions of SOM, with greater degree of physical and chemical protection, would provoke smaller N_2O emissions. Additionally, the comprehension of the role of SOM fractions resulting from land-use practices on N_2O emissions is crucial for GHG mitigating. Therefore, the objective of this study was to evaluate the accumulation of SOM fractions and their relation to the N_2O emissions from the soil in a 24-year field trial in the Cerrado region.

2 Material and Methods

2.1 Study Site

The field trial was conducted at the experimental area located at latitude 15°39′ S, longitude 47°44′ W, and elevation of 1200 m, in Planaltina, DF, Brazil. The regional climate is classified as tropical savanna-Aw (Köppen classification), with a rainy season from October to March and a dry season from April to September. The soil was classified as typical Oxisol and had 610.5 g kg^{-1}, 79.5 g kg^{-1}, and 309 g kg^{-1} of clay, silt, and sand, respectively. Details on soil mineralogy are showed in Marchão et al. (2009b). Soil chemical attributes are presented in Table 1.

The field experiment was setup in 1991, with four replicates. Three land-use systems were assessed: (1) CT, continuous cropping with conventional tillage in the off-season (without grasses as cover crops); (2) NT1, no-tillage with crop-livestock system in the pasture phase with *Brachiaria brizantha* cv. Piatã; and (3) NT2, no-tillage in continuous cropping with *Brachiaria brizantha* cv. Piatã as cover crop and sorghum as a main crop (without off-season cattle). An adjacent native Cerrado vegetation characterized as typical savanna was studied as a reference of natural conditions. Figure 1 shows the sequence of operations and cropping performed in the experimental area over the 24 years. Details on history of land-use systems were comprehensively explained in Sato et al. (2019).

2.2 Soil Analysis and Gas Sampling

Soil samples (0–20 cm) were collected in October 2013, 20 days following soy planting, which preceded the sorghum (2013–2014 crop). In each area, four samples were collected. Each soil sample was composed of five sub-samples. After

Table 1 Soil chemical properties of the experimental area

Land-use system	Layer (cm)	Al	Ca	H+Al	Mg	K	P	pH
		($cmol_c\ dm^{-3}$)				($mg\ dm^{-3}$)		
NT2	0–5	0.02 ± 0.0	3.97 ± 0.3	4.65 ± 0.7	1.06 ± 0.1	131.00 ± 44.1	14.41 ± 3.8	6.05 ± 0.2
	5–10	0.06 ± 0.0	2.04 ± 0.2	6.02 ± 0.9	0.50 ± 0.1	42.50 ± 25.0	9.00 ± 5.7	5.48 ± 0.2
	10–20	0.31 ± 0.1	0.69 ± 0.3	7.40 ± 0.6	0.18 ± 0.0	26.75 ± 5.6	3.06 ± 1.9	4.76 ± 0.1
	20–30	0.29 ± 0.1	0.58 ± 0.3	6.98 ± 0.6	0.15 ± 0.0	30.50 ± 3.3	2.63 ± 1.3	4.73 ± 0.0
CT	0–5	0.22 ± 0.1	1.31 ± 0.2	7.27 ± 0.8	0.20 ± 0.1	163.00 ± 57.7	8.59 ± 4.3	5.00 ± 0.2
	5–10	0.09 ± 0.1	2.18 ± 0.6	6.59 ± 0.1	0.36 ± 0.2	57.25 ± 26.4	7.92 ± 3.9	5.28 ± 0.2
	10–20	0.15 ± 0.0	1.27 ± 0.3	6.31 ± 0.5	0.26 ± 0.1	41.50 ± 10.6	2.35 ± 0.9	5.09 ± 0.1
	20–30	0.15 ± 0.1	1.08 ± 0.6	5.63 ± 0.2	0.23 ± 0.1	46.00 ± 9.4	1.58 ± 0.2	4.94 ± 0.1
NT1	0–5	0.05 ± 0.0	2.52 ± 0.2	5.40 ± 0.2	0.91 ± 0.2	123.00 ± 94.6	4.70 ± 2.4	5.60 ± 0.8
	5–10	0.14 ± 0.1	1.36 ± 0.3	5.82 ± 0.4	0.43 ± 0.1	48.75 ± 40.2	6.47 ± 5.6	5.22 ± 0.2
	10–20	0.19 ± 0.1	0.99 ± 0.3	5.87 ± 0.5	0.30 ± 0.1	21.50 ± 4.5	1.74 ± 0.3	4.98 ± 0.1
	20–30	0.19 ± 0.1	0.97 ± 0.1	5.73 ± 0.7	0.30 ± 0.1	20.00 ± 4.5	1.09 ± 0.2	5.03 ± 0.2
CER	0–5	0.65 ± 0.2	0.70 ± 0.3	10.22 ± 1.2	0.42 ± 0.2	74.00 ± 21.4	2.01 ± 0.2	4.88 ± 0.1
	5–10	0.71 ± 0.2	0.15 ± 0.2	8.54 ± 0.3	0.13 ± 0.2	44.00 ± 15.9	1.41 ± 0.2	4.74 ± 0.3
	10–20	0.46 ± 0.1	0.05 ± 0.1	7.16 ± 0.3	0.06 ± 0.1	31.00 ± 15.8	1.25 ± 0.1	4.91 ± 0.3
	20–30	0.34 ± 0.1	0.01 ± 0.0	6.24 ± 0.3	0.03 ± 0.0	22.50 ± 6.4	1.19 ± 0.1	4.98 ± 0.3

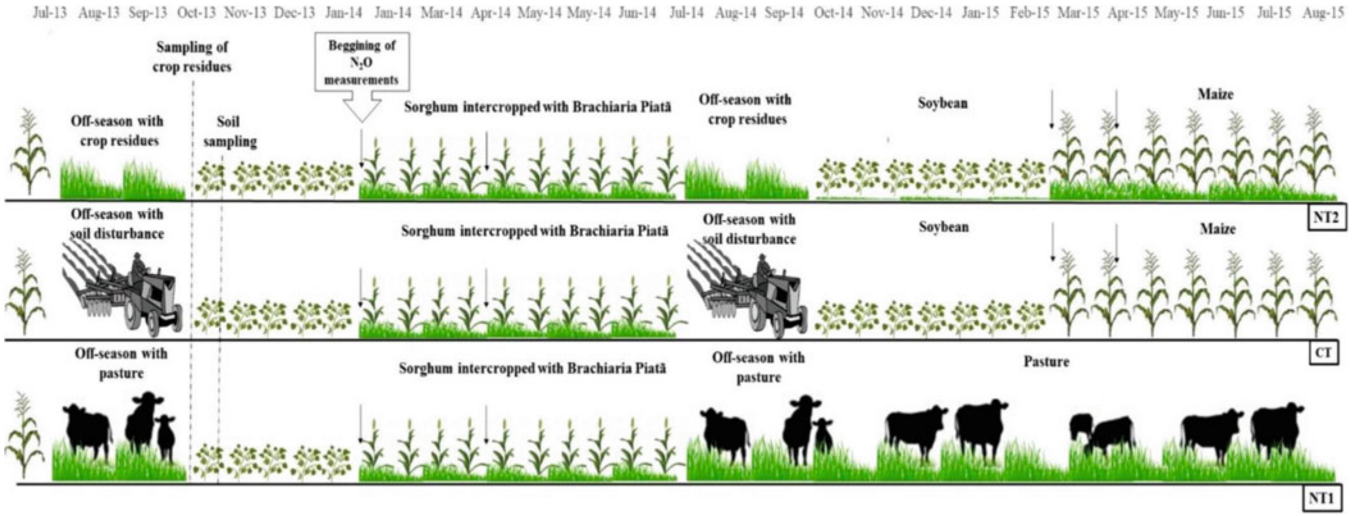

Fig. 1 Schematic representation of the experimental area and all operations performed along the cropping seasons

collection, the samples were air-dried and sieved (<2 mm). Background information on soil chemical analysis is available in our previous work (Sato et al. 2019).

2.2.1 Total Carbon and Total Nitrogen

The total N and C contents were determined using an elemental analyzer (Finnigan MAT, Bremen, Germany).

2.2.2 Labile Carbon

The labile C (LC) was considered as C susceptible to oxidation by a solution of $KMnO_4$ 0.033 mol L^{-1} (Blair et al. 1995). The samples were analyzed in spectrophotometer (565 nm).

2.2.3 Physical Granulometric Fractioning

Air-dried fine soil (ADFS) samples (20 g) were submitted to the physical granulometric fractioning (Cambardella and Elliot 1992). The C was determined through dry combustion in a Perkin Elmer Series II CHNS/O 2400 analyzer. The mineral-associated organic C (MOC) was obtained by the difference between TOC and particulate organic C (POC).

2.2.4 Microbial Biomass Carbon

The microbial biomass C (MBC) was determined through the irradiation-extraction method (Islam and Weil 1998), using 0.5 mol L^{-1} potassium sulfate as an extractor. The carbon quantification was determined by the method of oxi-reduction with 0.066 mol L^{-1} potassium dichromate and 0.033 mol L^{-1} ammonium iron(II) sulfate (Mohr's salt). The amount of MBC was estimated by the difference between C extracted from irradiated and non-irradiated soil samples (Mendonça and Matos 2005).

2.2.5 Chemical Fractioning of Soil Organic Matter

The differential solubility technique was used for the chemical fractioning of the SOM using 0.1 mol L^{-1} of NaOH (proportion of 1:20) as an extractor (Mendonça and Matos 2005). The following fractions were obtained-humic acid HA-C; fulvic acid FA-C; and humin HUM-C. The humification index (HI) was estimated as follows $\text{HI} = [(\text{HA-C} + \text{FA-C} + \text{HUM-C})/\text{TOC}] \times 100$.

2.2.6 Inert Carbon

Inert C was considered the fraction of SOM which remains after oxidation with hydrogen peroxide (H_2O_2) at 30% (v/v), according to Jackson (1958).

2.2.7 Carbon and Nitrogen Contents in Macro- and Microaggregates

The soil samples were collected from mini-trenches, following the methodology proposed by Madari et al. (2005), for quantification of C in different classes of aggregates that are stable in water. In each installment, mini-trenches were dug in four random locations. These samples were sifted, still in the field, in a 19 mm mesh sieve with 210 mm in diameter, with the intention of preserving the natural characteristics of the soil. After sifting in the field, the material was stored in plastic recipients and sent to the laboratory to be air-dried in a shady location. Subsequently, the samples underwent water aggregate stability analysis in a vertical oscillation shaker (Yoder sieve shaker), according to the method proposed by Embrapa (1997).

After separation of aggregate classes, two classes were utilized for the quantification of total C in macroaggregates (>25 mm) and microaggregates (<25 smm). The mean weight diameter (MWD) of the aggregates was also calculated through the Kemper and Rosenau method (1986). To determine total C and N, the samples were grouped as macroaggregates and microaggregates. The quantification of C and N was carried out in an elemental analyzer in Soil Laboratory at Embrapa Cerrados.

2.3 Cumulative N_2O Emissions

N_2O fluxes measurements were performed 114 times from March 21, 2014, to August 12, 2015, over a period of 509 days (Fig. 1). The static chamber method was used (Alves et al. 2012).

The N_2O concentration was determined by gas chromatography (Trace GC Ultra, Thermo Scientific). Details on N_2O calculation are showed in Sato et al. (2019).

2.4 Statistical Analysis

The data were submitted to ANOVA, and the comparison of means was conducted with Tukey-Kramer test ($P < 0.05$) by using the GLIMMIX procedure of SAS. Descriptive statistical analyses were also performed for the attributes of SOM, and boxplots were used to the data display of each treatment.

A principal component analysis (PCA) was applied in a data matrix with 12 lines composed by 3 land-use systems and 4 repetitions per treatment and 16 columns comprising the organic matter attributes and cumulative N_2O emissions. The PCA was performed using XLSTAT software.

3 Results

3.1 Total Carbon and Nitrogen on Land-Use Systems

3.1.1 Total Carbon and Nitrogen

The TOC and TN contents are shown in Fig. 2. CER had the highest levels of TOC in soil (26.32 g kg^{-1}). The NT1 and NT2 systems presented an intermediary condition (25.31 and 24.53 g kg^{-1}, respectively). CT was the system that exhibited the lowest levels of TOC (19.81 g kg^{-1}), significantly different from the other land-use systems ($p < 0.05$).

The integrated system (NT1) had similar content of TN (1.58 g kg^{-1}) to the CER soil (1.50 g kg^{-1}) and larger than the CT and NT2 systems. The continuous crops

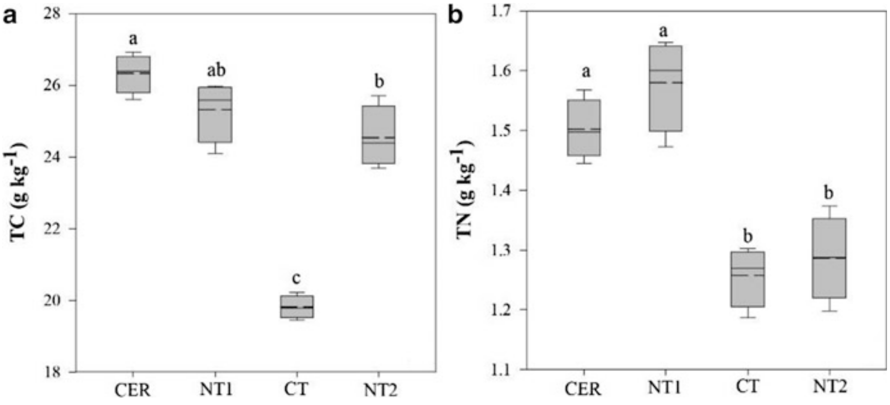

Fig. 2 (**a**) Total organic carbon (TOC) in soil; and (**b**), total nitrogen (TN) in soil submitted to different land-use system. Cerrado (CER); No-tillage with integrated crop-livestock (NT1); No-tillage with continuous cropping system (NT2); Continuous cropping system under annual heavy disc harrow (CT). Same letters within treatments indicate no difference (Tukey-Kramer; $p < 0.05$)

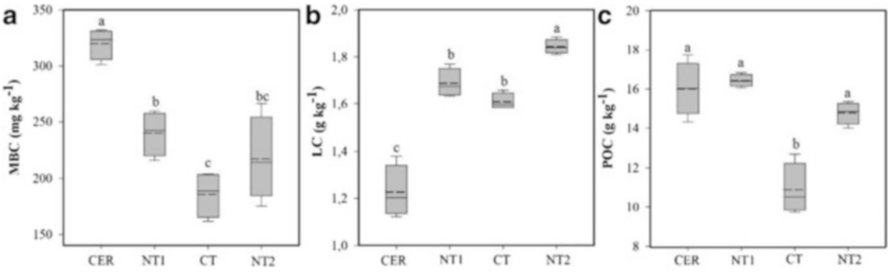

Fig. 3 (**a**) Microbial biomass carbon (MBC) in soil; (**b**). labile carbon (LC); and (**c**) particulate organic carbon (POC) in the different land-use systems. Descriptions of treatments are shown in the caption of Fig. 2. Same letters within treatments indicate no difference (Tukey-Kramer; $p < 0.05$)

were similar and presented the lowest levels of NT in the soil (1.26 g kg^{-1} in CT and 1.29 in NT2 g kg^{-1}).

3.1.2 Carbon Labile Fractions

The CER presented the highest levels of microbial biomass carbon (MBC) in the soil (320.13 mg kg^{-1}), the NT1 had intermediary levels (239.94 mg kg^{-1}), and the continuous cropping systems (NT2 and CT) had the lowest contents of MBC in the soil (Fig. 3a). Compared to the CER, the CT reduced the MBC in 42%, and NT1 reduced it in 25%.

Regarding the levels of LC (Fig. 3b), the CER soil presented the lowest levels (1.23 g kg^{-1}) compared to the cropping systems. There was no difference between the CT and NT1 ($p < 0.05$), with averages of 1.61 and 1.69 g kg^{-1}, respectively. The NT2 was the system with highest levels of POC in the soil (1.84 g kg^{-1}).

The highest levels of POC in the soil were observed in the NT1 (16.43 g kg^{-1}). In the CER soil, the POC showed a high coefficient of variation of 42%. Conventional system promoted the lowest value of POC (10.88 g kg^{-1}, on average) being different from the other agroecosystems and CER ($p < 0.05$).

3.1.3 Carbon Stable Fractions

The levels of carbon in humic fractions are shown in Fig. 4. The fulvic acid fraction (FA-C) in the soil did not present differences between the cropping systems and CER ($p < 0.05$). CER showed the lowest content of HA-C (3.03 g kg^{-1}).

The CER showed highest level of C in the humic fraction (HUM-C) (7.10 g kg^{-1}), being higher than the other agroecosystems ($p < 0.05$). The three agroecosystems had similar content of HUM-C. The CER soil presented the highest levels of MOC (18.26 g kg^{-1}). Compared to CER, on average, all agroecosystems decreased 50% of the MOC content.

With regard to the levels of inert carbon (IC) in the soil, the NT1 system showed the highest content (8.20 g kg^{-1}) and the CT the smallest content (5.48 g kg^{-1}), reducing in 22% the IC content in comparison to the CER soil. The CER had similar content of IC to CT.

The HA-C/FA-C ratio was not affected by agroecosystems ($p < 0.05$). The CT system presented the highest humification index (HI) (74% on average). The CER exhibited the smallest HI (53%), and the NT1 and NT2 systems exhibited intermediary values of 60% and 58%, respectively.

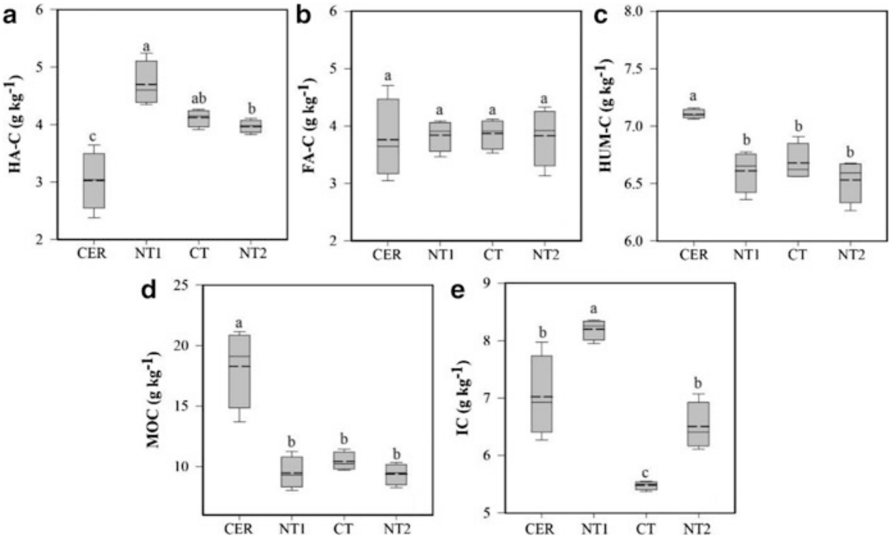

Fig. 4 (**a**) Fulvic acid (FA-C); (**b**) humic acid (HA-C); (**c**) humin (HUM-C) in soil; (**d**) mineral-associated organic carbon (MOC); (**e**) inert carbon (IC), in the different land-use systems. Descriptions of treatments are shown in the caption of Fig. 2. Same letters within treatments indicate no difference (Tukey-Kramer; $p < 0.05$)

3.1.4 Soil Aggregation, Carbon and Nitrogen Content in Macro and Microaggregates

NT2 presented in the Fig. 6 shows lowest levels of C in macroaggregates (C_{MACRO}) (20.71 g kg^{-1}) in the soil, 21% lower than the CER. Concerning the C in microaggregates (C_{MICRO}) in the soil, the NT1 showed the highest content (20.02 g kg^{-1}), with no differences between the other agroecosystems. The lowest content was found in the CER (17.34 g kg^{-1}).

The NT1 system exhibited the highest values of mean weight diameter (MWD), with average of 4.57 mm. The MWD of NT1 was 17% greater than the CER soil (3.90 mm). The continuous cropping showed a MWD value around 30–39% lower than the CER soil, with an average of 2.37 mm in CT and 2.75 mm in NT2.

With regard to the N content in soil aggregates, the highest content of N was found in the cropping systems soil, in both macroaggregates (N_{MACRO}) and microaggregates (N_{MICRO}). For the results, the CT exhibited a rate of N_{MACRO} (18.42 g kg^{-1}) 10% greater than the CER soil. NT2 had lowest N_{MACRO} content ($p < 0.05$). For the N_{MICRO} results, NT1 and NT2 showed higher contents than CER ($p < 0.05$).

3.2 Cumulative N_2O Emissions

Figure 7 shows the dynamics of the N_2O daily and cumulative fluxes for a period of a year and a half, which were correlated to the different C fractions in the soil (Fig. 8). The CT exhibited the highest fluxes of N_2O throughout a period of 509 days, with a cumulative emission of 4.56 kg ha^{-1}, while in the NT2 system the N_2O emission at the same period was 3.73 kg ha^{-1}. The NT1 had the lowest emission of N_2O from the soil (1.75 kg ha^{-1}). The CER had the lowest cumulative emission of N_2O (0.63 kg ha^{-1}), and the NT1 was similar to the reference area.

Two principal component analyses were performed. In the first, we considered all land-use systems including the Cerrado (Fig. 8a) and in the second only agricultural systems during the sorghum crop cycle were included (Fig. 8b).

The first PCA, performed with the data of SOM fractions and the N_2O accumulated in 509 days, revealed that the two first factors explained 69.71% of total data variability, of which 41.64% were explained by factor 1 and 28.07% by factor 2.

It is possible to observe that the factor 1 is associated with a SOM gradient in the areas, while factor 2 is related to soil aggregation and structure. Factor 1 is primarily correlated to the MBC, MOC, and HUM-C variables with positive eigenvectors and to N_2O and LC with negative eigenvectors. Factor 2 was correlated to the MWD, C_{MICRO}, N_{MICRO}, and POC variables, all of them with positive eigenvectors. A cluster of the areas may be observed in relation to the quadrants of the biplot. The cumulative N_2O emission was grouped in the quadrant of the CT, the opposite of the NT1 system, which correlated to the soil aggregation (MWD) and total C and N contents.

In the second PCA, with exception of the data from the Cerrado area and considering only N_2O cumulative emissions from agricultural systems during the sorghum cycle, the results revealed that the two first factors explained 66.09% of total data variability, of which 46.20% were explained by factor 1 and 19.89% by factor 2. The factor 1 clearly shows a positive relation between N_2O and carbon stable fractions, represented by the humification index (HI). In an opposite way, one can see a higher distance between N_2O and labile carbon fractions. Continuous crop under conventional tillage was the systems that better correlates with low contents of labile fractions and N_2O emissions.

4 Discussion

4.1 Overall Effects of Land-Use Systems on C and N Contents

The soil organic matter (SOM) dynamics is influenced by the management, soil preparation, fertilizers, cover crops, and the organic residues generated in the cultivation. In this study, the C fractions accumulation in the soil varied between cropping systems. The conventional tillage favored the greatest loss of total organic C, with 25% reductions in the CT in comparison to the Cerrado. The total organic C values obtained in the current study, which varied from 19 to 26 g kg^{-1}, are similar to other results collected from other long-term experiments carried out in the Cerrado (Figueiredo et al. 2013; Ferreira et al. 2016). The lowest levels of TOC in the system with soil till may be attributed to the increase in decomposition promoted by tillage of the soil and exposition of the SOM protected in the aggregates (Tivet et al. 2013). It is known that tillage induces the processes of decomposition by breakdown and soil exposure (Sheehy et al. 2015), promoting carbon loss (Sá et al. 2014) and increasing GHG emissions (Jantalia et al. 2008; Bayer et al. 2015; Martins et al. 2015) by reducing biological activity. Due to the harmful effects to the soil, there has been an increase in the use of conservation management of soil based on the absence of soil preparation (no-tillage) as it has been considered the best management practice. Corbeels et al. (2016) evaluated the C stocks in areas under no tillage after 20 years and observed that there is a tendency of C saturation in the superficial layer of the soil in this period. The authors also observed that after a period of 11–14 years, the stocks regained higher values than those found in the natural vegetation of the Cerrado.

In the current study, the NT1 system also presented a tendency of C recuperation, with values close to the original levels of the reference area. These results are attributed to the combined effect of the plant residues of the agricultural cultures and the radicle root systems and residues of the forage plants which, when compared to the conventional continuous cropping, exhibits a more positive effect (Piva et al. 2014).

The NT1 system had the highest content of total N (TN). This result may be explained by the greater accumulation of organic residue in the soil of the NT1, promoted by the alternating crops and the use of tropical grass such as Brachiaria, which favors a greater buildup of carbon by the radicle root system. These results indicate the importance of the soil carbon fractionation, where management systems effects may be more easily detectable in tropical Oxisols.

4.2 Effects on Labile Fractions of SOM

The NT2 exhibited the highest contents of LC in the soil, which may be related to the N_2O emissions during the soy cycle, period in which the soil was collected. Sá et al. (2014), evaluating different land-use systems (conventional and no-tillage), also observed that the areas under no-tillage exhibited the highest contents of LC, which varied from 1.99 to 3.52 g kg^{-1} in the superficial layer of the soil (0–25 cm). Our studies have shown that the soil aggregation and the content of total organic C are related (Fig. 8). Therefore, the labile fractions, such as the LC, may increase the formation of aggregates and protect the organic C in the soil (Tivet et al. 2013), favoring the buildup of C in the different SOM pools.

The highest levels of MBC were found in the area under natural vegetation, with a decrease of up to 42% in the area under CT, while systems under no-tillage, compared to the CER, presented reductions which varied between 25 and 32% of MBC. Considering that no-tillage systems do not present soil disturbance, they increase the MBC in comparison to soils which are revolved (Stieven et al. 2014; Meena et al. 2020). Ferreira et al. (2016) observed decreases of up to 40% in MBC concentrations in the conversion of native areas into long-term conventional management systems. The authors attributed this loss of microbial biomass to the pulverization of macroaggregates caused by soil inversion. The assessment of the MBC is a parameter sensitive to changes caused by the land-use systems (Sousa et al. 2015), and it is utilized as a soil quality indicator (Mi et al. 2016). In the NT1, the MBC contents were higher than in the CT, which indicates that the preservation of macroaggregates positively affects the soil microbial population.

Another fraction of SOM which presents high sensibility to soil management is the particulate organic C (POC) (Plaza et al. 2013; Mi et al. 2016), since its buildup is associated with the recent input of plant material of rapid availability to be decomposed by the microorganisms (Duxbury et al. 1989). The current study observed that the levels of POC in the CER varied greatly due to the diversity of the plant material in the sample harvesting areas of the native Cerrado. With respect to the agricultural systems, the soil disturbance affects the TOC buildup and, consequently, the POC content in the soil. Kibet et al. (2016) evaluated the POC in different forms of soil preparation in a long-term experiment (33 years) and concluded that the soil under no-tillage presented the highest POC content, confirming the results of the study, where the highest values of POC were verified in the NT1 and NT2 systems. In these systems, the utilization of the Brachiaria as a cover crop in the interim harvest period propitiates a greater accumulation of POC in the soil, which was also observed in the previous study of Rossi et al. (2012).

In the NT1 and NT2 systems, it should be pointed out that the only difference between them during the crop phase is that in the NT1, there is the occurrence of grazing and excretion on soil by animals in the interim harvest period, all the other practices being very similar. Mi et al. (2016) studied different plant residues applied to the soil and observed that the residues from animal excreta influence the levels of POC in the soil due to its C/N ratio being lower than the residues of plant husks. The intensity of grazing is another factor that also affects the input of C to the soil for TOC and POC, since the greater sources of C are the plant forage residues. Assmann et al. (2014), in a long-term study of integrated crop-livestock system (15 years) in the south of Brazil, proved that intense grazing causes the decrease of 17–33% of the annual addition of C to the soil when compared to areas without grazing. Nicoloso et al. (2008) concluded that the areas managed with grazing in the interim harvest (CT) show a larger addition of C compared to the integrated crop-livestock areas, which have a high grazing frequency.

4.3 Effects on Stable Fractions of SOM

The MOC exhibited lower content in the cropping systems compared to the CER. These results show that the proportions of the stable forms of C are larger where there is no anthropic intervention, resulting in a greater stability of the mineral fraction (Rossi et al. 2012). As its cycling rate is lower, the MOC may be considered a “long-term C storage” (Pinheiro et al. 2015). The buildup of C in the humic fractions depends on high ratios of C/N and lignin/N of the plant residues, which have a slower decomposition speed, favoring the increase of recalcitrant fractions in the soil (Zhongkui et al. 2010).

The humin fraction (HUM-C) presented the highest content of TOC, varying from 26 to 33% among the systems. The results by Silva et al. (2011) report higher values of HUM-C, contributing with 45–75% of the TOC. The native area presented the largest HUM-C content, while the three cropping systems showed no significant differences. However, HUM-C content for the CT system represents around 33% of the TOC, compared to 26% of the other systems, indicating that the CT exhibits an elevated humification (Fig. 5b). These results demonstrate that, although there is less content of TOC in the CT system when compared to the other systems, the largest part of C in this system is humified in the form of HUM-C, seeing that the labile organic residues were rapidly mineralized, promoted by the breakdown of aggregates during soil preparation, forming a readily available deposit of C in the soil (Figueiredo et al. 2013).The smaller content of the humic acid (HA-C) in CER may be a consequence of its plant composition, which is rich in lignin compared to agricultural areas.

4.4 Carbon and Nitrogen in Soil Aggregates

The HA-C/FA-C ratio (Fig. 5a) allows to predict the degree of evolution of humification, as well as to evaluate the capacity of mobility of C in the soil (Kononova 1982). In the current study, the HA-C/FA-C ratio in the cropping areas varied

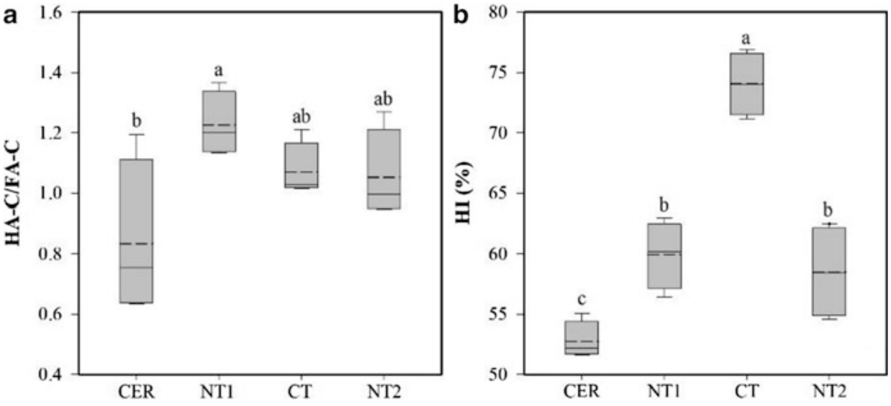

Fig. 5 (**a**) HA-C/FA-C ratio; and (**b**) humification index (HI) in the different land-use systems. Descriptions of treatments are shown in the caption of Fig. 2. Same letters within treatments indicate no difference (Tukey-Kramer; $p < 0.05$)

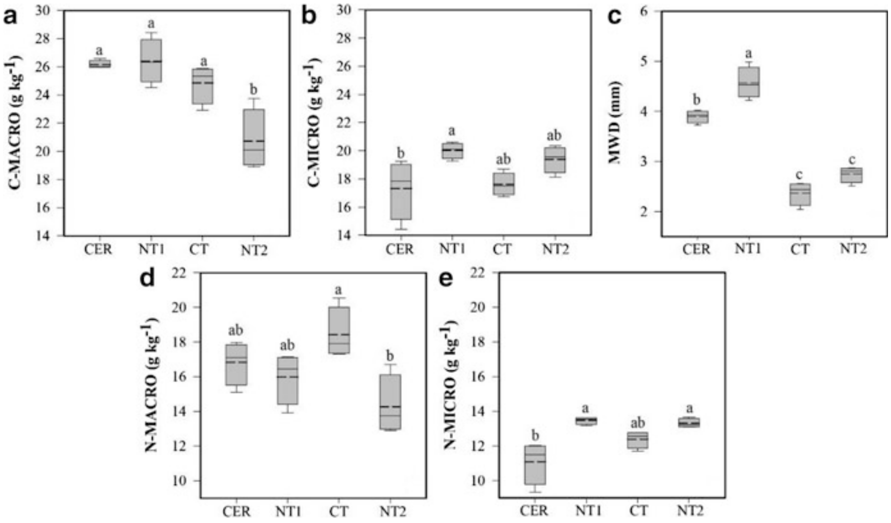

Fig. 6 (**a**) Carbon in macroaggregate soil (C_{MACRO}); (**b**) carbon in microaggregate soil (C_{MICRO}); (**c**) mean weight diameter (MWD) in soil; (**d**) nitrogen in macroaggregate soil (N_{MACRO}); and (**e**) nitrogen in microaggregates (N_{MICRO}) in the different land-use systems. Same letters within treatments indicate no difference (Tukey-Kramer; $p < 0.05$)

between 0.9 and 1.1. This indicates that the soil is in an intermediary process of humification for exhibiting intense mineralization of the plant residues. In the CER, this proportion was low (0.56). These results allow the inference that, over 24 years of experiment, the intense plant residue deposit favors the increase of HA-C

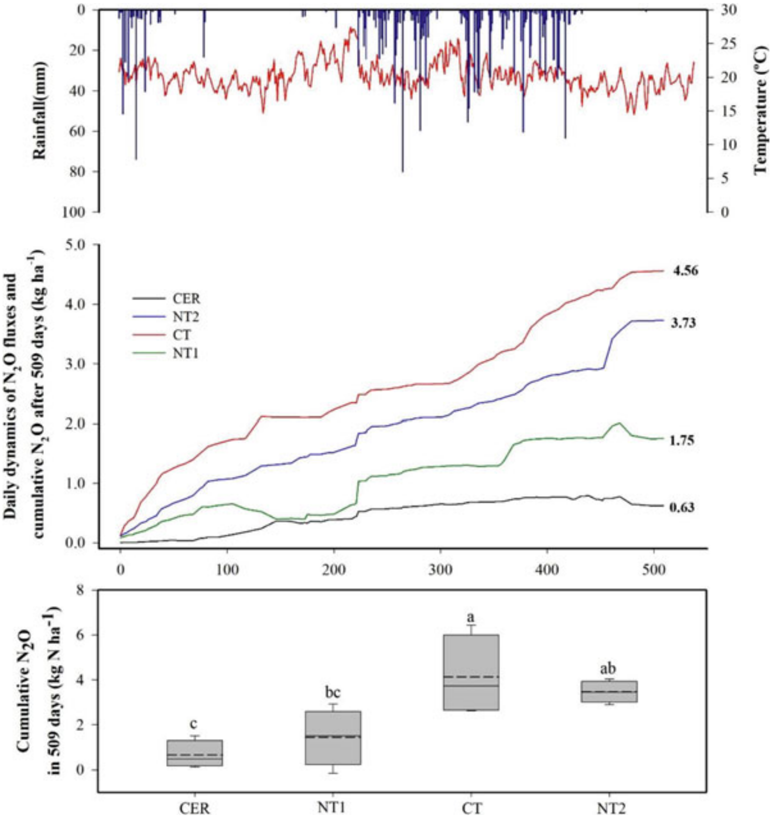

Fig. 7 Rainfall (mm) and average air temperature (°C); daily dynamics of N_2O fluxes and cumulative N_2O after 509 days and boxplot of cumulative N_2O. Same letters within treatments indicate no difference (Tukey-Kramer; $p < 0.05$)

fractions in the soil, due to the low content of lignin in the vegetation cover in the soil compared to the Cerrado area.

In the Cerrado region, there is a great quantity of inert carbon (IC), in charcoal form, derived from wildfires, typical of this region. However, the change of native areas for agricultural production reduced in up to 22% the IC content in continuous crops. In the NT1 system, there was an increase of 15% of this fraction.

The labile fractions of SOM are fundamental for C cycling between compartments and for short-term nutrient cycling, but also contribute to the transitory formation and stability of soil aggregates (Santos et al. 2013). The use of Brachiaria in the agricultural systems is being considered an important factor which favors soil aggregation (Loss et al. 2011; Salton et al. 2014). The results of these study show that the NT1 exhibited larger proportions of aggregates, with a mean weight diameter (MWD) of 4.57 mm. Nowadays, there is a consensus that soils with greater aggregation present a better soil quality than those with similar

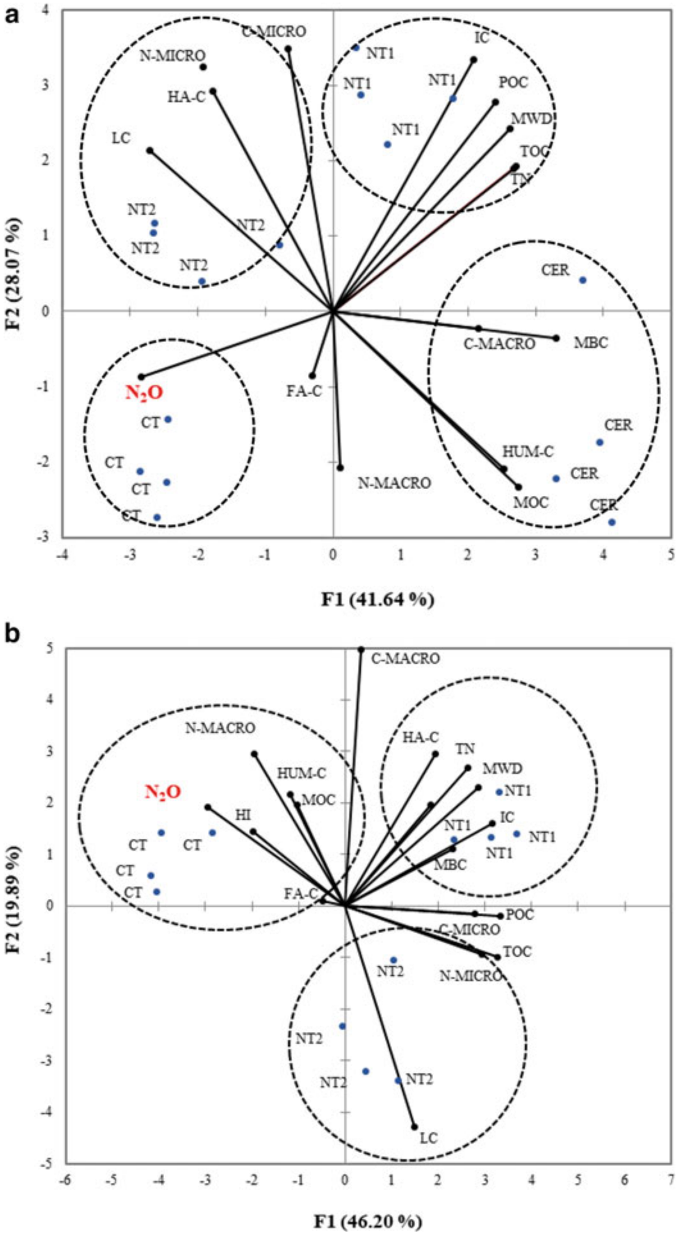

Fig. 8 Principal component analysis (PCA) of the C fractions in the soil and cumulative N$_2$O emissions after 509 days in the different evaluated systems (**a**) and PCA of C fractions in the soil and cumulative N$_2$O emissions in the sorghum crop in the agriculture systems (**b**)

characteristics and lesser aggregation, due to the physical protection of carbon provided by greater aggregation (Salton et al. 2014).

Finally, the results of microaggregate C, along with the results of MWD, indicate that the NT1 exhibits greater soil aggregation than the other evaluated systems and a larger C pool in microaggregates than that of the native area. According to Tivet et al. (2013), the greater concentration of organic labile fractions may increase the formation of aggregates, thus protecting the C from the soil physically and chemically impeding its loss to the atmosphere. This demonstrates the potential of the crop-livestock system for the mitigation of GHG, such as N_2O.

4.5 Relationship Between Soil Organic Matter Fractions and N_2O Emissions

The first two principal components of PCA (Fig. 8) explained around 66% of the data variability. PC1 distinguished mainly agricultural systems with a gradient of C and N contents with positive eigenvalues and accumulated N_2O, with negative eigenvalues. PC2 is mainly related to a gradient SOM fractions and aggregation with positive eigenvalues and LC, with negative eigenvalue. The coordinates of the agricultural systems (plots) plotted in the factorial plan shows a grouping (Fig. 8b). Axis 1 clearly separated the NT1 and NT2 from the CT system, which is related mainly to higher N_2O emissions. It is possible to observe that axis 2 distinguished mainly NT2 system from the others with a tendency to high levels of LC. From this result, it is possible to conclude that in the CT system C losses of the most labile fractions occur due to soil plough, which causes higher N_2O emissions. On the other hand, in the conservation systems (NT1 and NT2), these losses are smaller favored by the soil aggregation.

Furthermore, various studies indicate that high quantities of SOM are potentially related to higher N_2O emissions (Kong et al. 2009; Morley and Baggs 2010; Bhattacharyya et al. 2013). In the present study, the NT1 was the agricultural system that emitted the lowest amount of N_2O to the atmosphere and also presented the highest C content in the following fractions: C_{MACRO}, C_{MICRO}, POC, IC, and HA-C. These results show that the lower accumulated emissions of N_2O into the atmosphere may result from a balance between labile and stable fractions of SOM and better protection in aggregates (Sato et al. 2019).

The results obtained from the PCA (Fig. 8b) demonstrate that the N_2O emissions were associated with the CT system, with vector positioning opposite the LC and POC properties. It is possible to conclude that in the CT system, C losses of labile fractions of SOM occur due to soil rotation, which may have resulted in higher N_2O flows. In the conservation systems, these losses are smaller by favoring the aggregation of the soil. The most recalcitrant fractions, such as HUM-C, HA-C, IC, and MOC, in NT1 are possibly related to the low fluxes of N_2O.

The residues of crops with low C/N ratios, such as legumes, also trigger high rates of N_2O emissions (Huang et al. 2004; Millar et al. 2004), since they decompose easily and supply N composts readily available for the soil microorganisms (Miller et al. 2008). Morley and Baggs (2010) showed that the composition of plant residues interferes in N_2O emissions and demonstrate that N_2O emission is favored when the plant residues are more easily converted into simple carbohydrates.

Qiu et al. (2015), in studies with dissolved SOM and GHG emissions in soils in China, verified that the dissolved organic C and the temperature of the soil present a positive relation in N_2O emission from the soil. Therefore, in tropical soil, with a higher clay content, a higher N_2O emission was expected. In view of these results, the present study observed that the NT1, which exhibited the greater content of organic carbon associated with microaggregates (C_{MICRO}), resulted in lower cumulative N_2O emissions. Thus, it was observed that the NT1 is the cropping system with lower emission, proving to be more sustainable and efficient in this aspect, exhibiting a balance that favors mitigation of GHG emissions.

5 Conclusion and Future Prospects

Among the soil attributes that influence the emission of gases, those that are related to the structure and stability of the SOM are determinants of N_2O fluxes. In conservation systems such as NT1, the presence of Brachiaria as a cover crop, in addition to promoting greater total carbon accumulation, also contributes to the formation of soil aggregates, which promote greater protection of SOM and lower N_2O emissions. The results demonstrate that the conventional system reduced all fraction of SOM and decreased the physical protection of SOM and increased the humification index of SOM and, consequently, increased the emission of N_2O to the atmosphere. The NT1 system had the lowest cumulative N_2O emissions. This may be due to the greatest buildup of C in its most stable fractions and occluded in aggregates, confirming the hypothesis that the accumulation of C in the most stable fractions of the soil, unavailable to the microbiota, causes lower emissions of N_2O to the atmosphere. Despite the high SOM content in the crop-livestock system, the predominance of C and N in stable forms and physically protected in aggregates may reduce the emission of N_2O from soils.

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