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Integrated production systems in a Plinthosol: greenhouse gas emissions and soil quality

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Abstract

Integrated systems (crops, livestock, and forest) are tools to avoid increases in greenhouse gas (GHG) emissions, such as CO_2, CH_4, and N_2O. The objective of this study was to evaluate the GHG emissions and soil biological and chemical characteristics in an integrated system. The experiment was carried out in an area with crop-livestock-forest integration systems (CLFI), in Pindaré-Mirim, state of Maranhão, Brazil. The treatments consisted of maize (*Zea mays*) intercropped with forage (*Urochloa brizantha* cv. Marandú) between eucalyptus trees (*Eucalyptus urophylla* × *Eucalyptus tereticornis*) (S1); maize intercropped with forage (*Megathyrsus maximus* cv. Massai) (S2); and degraded pasture areas with no soil or forage management for more than 14 years (S3), which was used as reference treatment. The experiment was conducted with four replications of four trenches for soil collection or four static chambers for gas flow assessments. The GHG emissions were collected by static chambers and analyzed by gas chromatography, and the soil quality was determined by chemical analysis. The interaction between GHG emissions and soil characteristics was assessed for each treatment, using multivariate analysis and PCA. The soil of the degraded pasture presented higher GHG emissions. The integrated systems presented negative methane fluxes, which denote their mitigating effect on GHG emissions. The CLFI system with eucalyptus and maize intercropped with *U. brizantha* cv. Marandú was the best option to improve the soil biological characteristics and mitigate GHG emissions. Crop-livestock-forest integration with Eucalyptus, maize, and *U. brizantha* cv. Marandú is indicated to improve soil biological characteristics and mitigate GHG emissions in the Amazonian region of the state of Maranhão, Brazil.

Keywords: carbon dioxide; methane; nitrous oxide; organic matter; forage; forest; crop
Abbreviations:' ABC Plan_low carbon agriculture plan; MBC_soil microbial biomass carbon; CFI_crop-forest integration; CH_4_ methane; CLFI_crop-livestock-forest integration; CLI_crop-livestock integration; CO_2_carbon dioxide; MOC_mineral-associated organic carbon; POC_particulate organic carbon; TOC_total organic carbon; DEG_degraded pasture; GHG_greenhouse gases; LFI_ livestock-forest integration; N_2O_nitrous oxide; MBN_soil microbial biomass nitrogen; OM_organic matter; PCA_principal component analysis; BRS_basal soil respiration; AT_air temperature; CIT_chamber internal temperature; TRU_technological reference unit; qCO_2 _metabolic quotient; pH_hydrogen potential; CEC_cation exchange capacity.

Introduction

Sustainable production of foods, such as meat, milk, and grains, has become a worldwide demand. Brazil is one of the largest beef cattle and grain producing countries.

The production systems used in Brazil have been constantly improved to intensify livestock and agriculture productions, reach higher efficiencies, protect surrounding environments, or promote ecological recovery (Salton et al., 2014). However, the exhaustive land use for crop and livestock productions has resulted in degradation of large areas of soil and pasture in the country. This is a widely discussed issue in Amazonian regions, since the extensive livestock and monoculture practices promote deforestation, contribute to soil degradation, decrease livestock productivity (Salton et al., 2014), and increase greenhouse gas (GHG) emissions (Li, 2007). It is estimated that approximately 70% of the total pasture area in Brazil are degraded or under degradation process (Galdino et al., 2015). Pasture degradation is the evolutionary process of loss of vigor and productivity and decrease of recovery capacity of natural pastures (Dias-Filho, 2011). Degraded pastures present low forage productivity, soil organic matter, and animal productivity. Maranhão is a state in the Northeast region of Brazil that has areas within the Amazon biome. This state has the second largest commercial cattle herd of the Northeast region, with 7,687,695 million animals (IBGE, 2017) and an increasing grain production, which reached 1.8 million kg of maize and 2.8 million kg of soybean in 2019 (IBGE, 2019). This expansion causes environmental conflicts and raises the

question of how to produce with less environmental impact. Brazil agreed to reduce GHG emissions until 2030 at the 24th United Nations Conference on Climate Change (COP 24) in 2018 (Brasil, 2018). Therefore, the Brazilian government has committed to strengthening the Low Carbon Agriculture Plan (ABC Plan). This plan includes the recovery of degraded pastures and the adoption of integrated production systems for the mitigation of GHG emissions. Crop-Livestock-Forest Integration (CLFI), or agrosilvopastoral systems and its variables: Crop-Livestock Integration (CLI) or agropastoral, Livestock-Forest Integration (LFI) or silvopastoral, and Crop-Forest Integration (CFI) have been used as strategies to achieve synergism and benefit from emergent properties resulting from the soil-plant-animal-atmosphere interactions (Moraes et al., 2013). The benefits of CLFI include greater soil carbon contents and stocks when compared to conventional and traditional systems (Silva et al., 2014), higher land and machinery use efficiency, and greater mitigation of GHG emissions (Bell and Moore, 2012; Ryschawy et al., 2012). It is estimated that 23% of the total anthropogenic GHG emissions (2007-2016) is derived from agriculture, forestry, and other land uses (IPCC 2019). Some land uses, such as livestock areas, and soil and nutrient managements can also contribute to anthropogenic GHG emissions (Sánchez et al., 2016). Thus, the quantification of impacts of mitigation strategies on soil GHG emission is essential to identify the best soil management practices for land uses such as agriculture or forestry (Li, 2007). In this context, the objective of this work was to evaluate the greenhouse gas flow in two integrated production systems, compared to a degraded pasture, and their ability to improve soil biological and chemical characteristics that mitigate GHG effects, in the Amazonian region of the state of Maranhão, Brazil.

Results and discussion

GHG emissions

N_2O flux in the degraded pastures was 1.6-fold higher than that in CLFI systems, and 20-fold higher than that in CLI systems (Fig. 1). These are similar results to those found by Piva et al. (2019) and Siqueira Neto et al. (2011) in areas under similar climate and crop conditions. CH_4 fluxes were 12.9, -5.9, and -8.2 µg C m^2 h^{-1} for the DEG, CLFI, and CLI systems, respectively (Fig. 2). CO_2 fluxes were higher in the degraded pasture.

The CLFI system presented higher values for basal respiration of the microbial biomass and higher OM and NO_3^- contents (Table 1), indicating that the CLFI system has a higher mineralization capacity.

N_2O formation in the soil occurs mainly during the incomplete N-NO_3^- denitrification process and simultaneously to the mineralization process of soil N organic forms (Bouwman, 2010). In the present study, a higher N mineralization of organic matter may had occurred because the soil of the pasture area (which was not prepared) had a larger number of anaerobic sites and a higher denitrification potential (Siqueira Neto et al., 2010), resulting in a high denitrification activity. Thus, the high concentration of mineral N available in the soil due to increases in soil N contents in the forms NO_3^- and NH_4^+ may have resulted in higher emissions of N_2O to the atmosphere, in the DEG system. The tillage in the CLFI and CLI areas increased the soil aeration, reducing anaerobic sites and the denitrification process. Although nitrogen fertilizer was used in these systems, it was split, which may have caused a synchronism in nutrient release and plant absorption, thus reducing N losses due to leaching or volatilization. This may have also reduced emissions of N_2O to the atmosphere (Azeem et al., 2014; Timilsena et al., 2015). The integrated production systems showed negative methane balance, which denotes methane consumption in the soil. Methane dynamics in agricultural soils are defined as a function of changes in the communities of methanogenic and methanotrophic bacteria, which act as a source or sink of atmospheric CH_4, respectively (Meyer et al., 2020). The higher CH_4 uptake in the CLI and CLFI systems may be connected to the better soil physical conditions, such as better structure and higher porosity due to recent soil tillage. Aerated soils act as CH_4 sinks through microbial oxidation (Dobbie and Smith, 2003). Many studies correlate higher CH_4 emission with higher NH_4^+ availability in the soil because ammonium ions would oxidize into CH_4 (Besen, 2015; Zanatta et al., 2010). In integrated production systems, plants with high nutrient demand, mainly nitrogen, make them unable to store NH_4^+, reducing their availability in the soil. In addition, crops with high water demand reduce the amount of water available in soil pores, improving drainage conditions and O_2 diffusion, thus increasing the soil ability to oxidize CH_4 (Saggar, 2003); this makes integrated production systems to drain atmospheric CH_4.

In degraded pastures, the soil surface cover is low, causing economic and environmental losses by the rapid degradation of OM and, consequently, releases of CO_2 caused by OM exposure (Costa et al., 2008), explaining the high CO_2 emission found in this environment. This is a parameter used to quantify the microbial activity; in the case of degraded pastures, the soil aerobic microorganisms oxidize the OM using O_2 as the final electron acceptor of CO_2 (Moreira and Siqueira, 2006).

Nitrous oxide (N_2O) was positively correlated (0.99) with microbial biomass carbon (CBMS). According to Coutinho et al. (2010), increasing soil organic C content may increase N_2O production, and increasing CBMS may provide the organic carbon needed for soil denitrification (Cameron et al., 2013) (Table 2). The strong correlation (0.94) between N_2O and soil microbial biomass nitrogen (NBMS) occurred due to the increase of NBMS, which resulted in higher N_2O emissions through the NBMS recycling process combined with the higher NO_3^- availability in the soil (Magiero et al., 2011). In Amazonian soils, soil N_2O emission peaks when plant residues are left after grain harvest (Passianoto et al. 2003), which was the case of the present study, in which samples were collected after the grain harvest. A high correlation (0.99) was found between fluxes of methane (CH4) and carbon dioxide (CO_2). The availability of organic carbon in soils requires methanogenesis, and organic C content is a C availability indicator for methanogenesis (Silva et al., 2011). CO_2 and TOC, and CO_2 and MOC presented strong correlation because CO_2 emissions are related to soil organic C oxidation in forest areas converted into agriculture areas and when changing agricultural practices (Grutzmacher, 2016).

Table 1. Mean particulate organic carbon (POC), mineral-associated organic carbon (MOC), total organic carbon (TOC), nitrogen (MBN), carbon (MBC), and basal respiration (BRS) of microbial biomass, pH, ammonium (NH_4) and nitrate (NO_3^-) in the soil as a function of different land management systems, in the dry season. Pindaré-Mirim, Maranhão, Brazil.

System	POC	MOC	TOC	MBN	MBC	BRS	pH	NH_4	NO_3
	------ g kg $^{-1}$ ------			mg kg $^{-1}$ soil		mg C-CO_2 kg^{-1} day^{-1}	-	mg kg^{-1}	
CLFI	0.1	7.4	7.5	6.8	537.0	28.8	6.5	0.8	2,3
CLI	0.2	7.3	7.5	4.5	420.9	8.4	6.7	1.3	1.0
DEG	0.3	7.9	8.2	7.0	660.8	25.5	6.7	1.8	1.2
Mean	0.2	7.6	7.8	6.1	539.5	20.9	6.6	1.3	1.5
SEM	0.05	0.19	0.23	0.80	69.28	6.32	0.04	0.27	0.42

CLFI: eucalyptus, maize, and grass integration system; CLI: maize and grass integration system; DEG: degraded pasture - reference system. SEM: standard error of the mean.

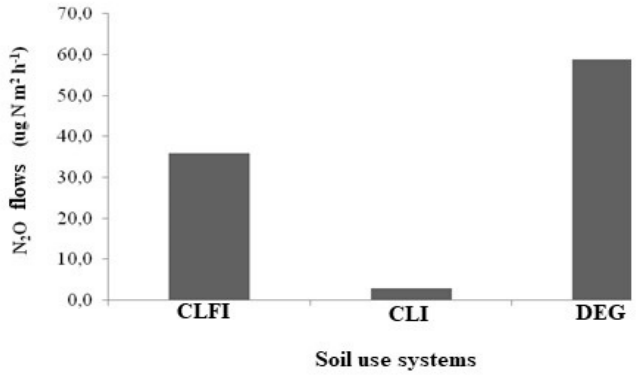

Fig 1. N_2O flows (µg N m^{-2} h^{-1}) in soils under integrated production and degraded pasture systems in Pindaré Mirim, Maranhão, Brazil. CLFI: eucalyptus, maize, and grass integration system; CLI: maize and grass integration system; DEG: degraded pasture.

Table 2. Correlation matrix for GHG, chemical and microbiological attributes, and carbon fractions in the 0-10 cm layer of soils under integrated production systems.

	N_2O	CH_4	CO_2	CIT	AT	POC	MOC	TOC	MBN	MBC	BRS	pH	NH_4
CH_4	0.86												
CO_2	0.79	£ 0.99											
CIT	-0.74	-0.30	-0.18										
AT	-0.37	0.15	0.27	0.90									
COP	0.44	0.83	0.89	0.28	0.67								
WITH	0.86	1.00 *	£ 0.99	-0.31	0.15	0.83							
TOC	0.80	£ 0.99	1.00 *	-0.19	0.27	0.89	£ 0.99						
NBMS	0.94	0.64	0.54	-0.93	-0.67	0.11	0.64	0.54					
CBMS	£ 0.99	0.92	0.86	-0.65	-0.25	0.55	0.92	0.87	0.89				
RBS	0.84	0.45	0.34	-0.99	-0.81	-0.11	0.46	0.35	0.98	0.77			
pH	-0.04	0.47	0.57	0.70	0.94	0.88	0.47	0.57	-0.38	0.09	-0.57		
NH_4	0.37	0.79	0.86	0.35	0.73	1.00 *	0.79	0.86	0.03	0.48	-0.19	0.91	
NO_3	0.30	-0.23	-0.34	-0.86	-1.00 *	-0.73	-0.22	-0.34	0.61	0.18	0.76	-0.97	-0.78

Note: Nitrous oxide (N_2O), methane (CH_4), carbon dioxide (CO_2), internal chamber temperature (CIT), air temperature (AT), particulate organic carbon (COP), mineral associated organic carbon (COM), total organic carbon (TOC), microbial biomass nitrogen (NBMS), microbial biomass carbon (CBMS), basal respiration (RBS), hydrogen potential (pH), ammonium (NH_4) and nitrate (NO_3^-).
£ and * - significant at 10% and 5% probability, respectively.

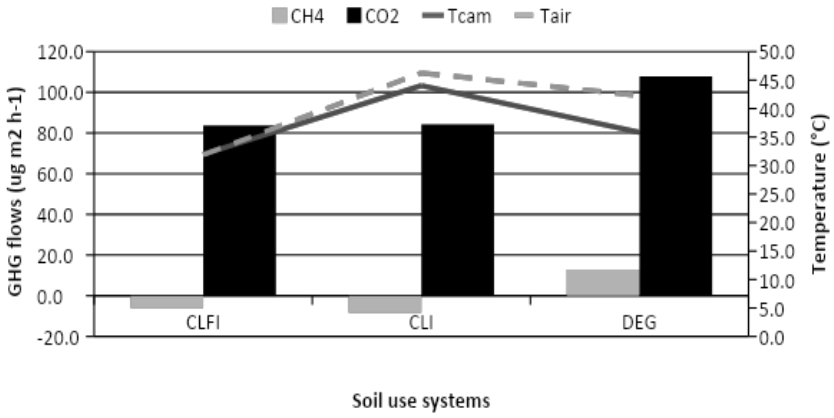

Fig 2. CH_4 (µg C m^{-2} h^{-1}) and CO_2 (µg C m^{-2} h^{-1}) flow, air temperatures, and temperatures inside static chambers during gas collection in soils under integrated production systems and degraded pasture in Pindaré Mirim, Maranhão, Brazil. CLFI: eucalyptus, maize, and grass integration system; CLI: maize and grass integration system; DEG: degraded pasture; CIT: internal temperature of the chamber; AT: air temperature.

Table 3. Principal component analysis (PCA), eigenvalues (λi), and percentage of variance explained by principal components (VPC%) for GHG characteristics and chemical, microbiological attributes, and C fractions of soils under integrated production systems.

Attributes	PC1	PC2
N_2O	0.30	-0.10
CH_4	0.21	-0.26
CO_2	0.18	-0.29
CIT	-0.28	-0.15
Tar	-0.20	-0.28
ST	-0.12	-0.33
POC	0.03	-0.36
MOC	0.13	-0.33
TOC	0.11	-0.33
MBN	0.31	-0.01
MBC	0.29	-0.13
BRS	0.28	0.16
qCO_2	0.20	0.28
pH	-0.31	-0.03
CEC	0.30	0.11
NH_4	0.20	-0.28
NO_3	0.21	0.26
Ninorg	0.31	-0.02
λi	8.06	6.94
PCV (%)	53.7	46.3
PCV accumulated (%)	53.7	100.0

Note: Nitrous oxide (N_2O), methane (CH_4), carbon dioxide (CO_2), internal chamber temperature (CIT), air temperature (AT), soil temperature (ST), particulate organic carbon (POC), mineral associated organic carbon (MOC), total organic carbon (TOC), microbial biomass nitrogen (MBN), microbial biomass carbon (MBC), basal respiration (BRS), metabolic quotient (qCO_2), hydrogen potential (pH), cation exchange capacity (CEC), ammonium (NH_4), nitrate (NO_3^-), inorganic nitrogen (Ninorg) eigenvalue (λi), and principal component value (PCV).

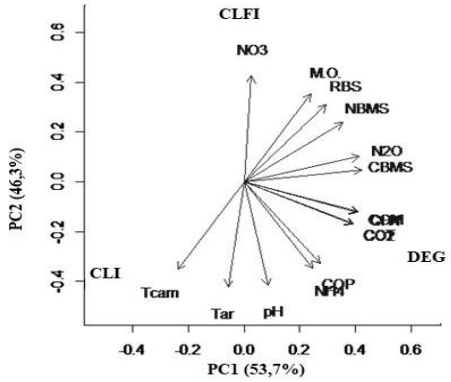

Fig 3. Biplot graph of principal components (PC1 and PC2) for GHG characteristics, microbiological attributes, and carbon stock in the 0-10 cm layer of soils under integrated production systems (CLFI: eucalyptus, maize, and grass; CLI: maize and grass; DEG: degraded pasture).

Air temperature (AT) was strongly and negatively correlated with nitrate (NO_3^-). Temperature directly affects the mineralization process that generates NO_3^-. According to Siqueira (1997), nitrification (part of the mineralization process) is favored by good soil aeration, air temperatures within 26 and 32 °C, soil moisture close to the field capacity, pH between 6.0 and 6.5, and C to N ratio in the narrow range (lower than 30). The strong positive correlation between ammonium (NH_4^+) and particulate organic carbon (POC) is explained by the mineralization process of organic C, since POC is more available than other C forms because it is in the particulate fraction of the organic matter (Nunes et al., 2011). The positive values of PC1 found for CLFI (eucalyptus, maize, and grass) (Fig. 3) is related to high nitrate and organic matter contents, basal respiration, and nitrogen contents from microbial biomass. These results show the efficiency of this system for soil nitrogen fixation and organic matter increases, which favor the mineralization process.

Correlations and principal components for soil quality

Linear correlations were found between N_2O and CBMS, CH_4 and CO_2, COM and COT, CO_2 and COM, CO_{2+} and COT, AT and NO_3, and COP and NH_4 (Table 2); 18.6% of the 91 correlations were high (Table 2) according to the classification described by Asuero et al. (2006) and Mukaka (2012).

Considering the eigenvalues and the percentage of variance explained by the principal components (PC), PC1 and PC2 accounted for 53.7% and 46.3% of the variance, respectively (Table 3). PC1 showed high contribution to N_2O, CH_4, CO_2, COP, COM, TOC, NBMS, and CBMS. PC2 showed a negative contribution to pH, CH_4, CIT, AT, and COP, and a positive contribution to RBS, OM, and NO_3^-. The CLI system was negatively related to PC2, mainly due to the air temperature, chamber internal temperature, and soil pH; and its was negatively related to soil nitrate availability. The absence of trees in the CLI resulted in increases of 2 to 5 ºC in the average environment temperature. Regarding the soil microorganisms, air temperatures above 30 ºC increase bacterial respiration and activity (Pietikäinen et al., 2005) and accelerate organic matter degradation. Thus, the presence of postharvest maize straw on the soil increases the nutrient input to the soil microbiota, increasing its activity and releasing heat to the environment (Siqueira Neto et al., 2011).

The results found for the degraded pasture were related to pH, particulate organic carbon, mineral-associated organic carbon, total organic carbon, ammonium, methane, and carbon dioxide. The pasture areas presented higher organic carbon contents than the other treatments because the implementation of the systems with soil preparation for planting may have favored the loss of carbon and microbial nitrogen, and because they were new implemented systems (CLFI and CLI) that probably have not had time to show significant benefits (Souza, 2019). Soil turning is one of the practices that most affect soil organisms and their interaction by affecting their population balance (Balota, 2017). The soil of the pasture area was prepared with no soil turning; thus, the soil probably conserved the microbial carbon contents due to the large number of grass roots.

Methane and carbon dioxide emissions due to decomposition of plants on the degraded pasture decreased the soil C to N ratio, hindering the metabolism of microorganisms, which cannot complete mineralization processes, producing only ammonium (Nunes et al., 2011).

Materials and Methods

Study site description

The study was conducted in a Crop-Livestock-Forest Integration (CLFI) Technological Reference Unit (TRU) of the Embrapa Cocais, in a Plinthosol (Plintossolo Argiluvico Distrofico tipico; Embrapa, 2006) of medium texture. The experimental areas were in the Muniz Farm, in Pindaré-Mirim, Maranhão, Brazil (3°46'13.3" S, 45°29'46.6" W, and average altitude of 38 m), within the limits of the Brazilian Legal Amazon, according to the Law No. 5,173 of October 27, 1966 (Brasil, 1966).

The region presents an Aw, hot and humid climate, according to classification of Köppen (1948), with an annual average temperature of 26 ºC (GEPLAN, 2002) and two well-defined seasons: a rainy season from October to July and a dry season from August to September. There was no precipitation, and the average air temperature during the study was 28 ºC (Supplementary Fig. 1).

Experimental design

Three land management systems were evaluated. The descriptions of the systems are available at Supplementary Table 1.

Information of areas and conduction of the study

The soils of the S1 and S2 areas were prepared using application of dolomitic limestone (1.8 Mg ha^{-1}), one plowing (disc plow), and one harrowing (disc harrow to incorporate the limestone) because a no-till seed drill was subsequently used. Maize seeds were sown on soil covered with Marandú grass straw (*Urochloa brizantha*) from plants that had been desiccated using glyphosate herbicide (4.5 L ha^{-1}) with a drying adhesive (0.9 L ha^{-1}). Soil fertilizer applications for the maize crop in these areas were carried out using 400 kg ha^{-1} of the NPK formulation 05-30-15 at sowing; and 200 kg ha^{-1} of the NPK formulation 36-00-30 as topdressing, at 10 and 20 days after the maize emergence.

Marandu grass (*Urochloa brizantha* cv. Marandú) was seeded as suggested by Dias-Filho (2012), using 10 kg ha^{-1} in the seed drill reservoir, simultaneously with the maize sowing, with no need for mixing with fertilizers. Seeds of Massai grass (*Megathirsus maximus* cv. Massai) were previously mixed with a NPK formulation 05-30-15 (400 kg ha^{-1}) and sowed simultaneously with maize. The fertilizers used for the eucalyptus were phosphate (0.075 kg) and a NPK formulation 36-00-30 (0.15 kg), which were applied to a depth of 0.30 m.

Fifteen days after maize emergence, post-emergent herbicides were applied, using atrazine (3.0 L ha^{-1}) and nicosulfuron (0.5 L ha^{-1}) for the initial control of the pasture and development of broad-leaf weeds. In addition, 3.5 L ha^{-1} of leaf fertilizer (Grap Nitro), 0.35 L ha^{-1} of fungicide (Abacus), and 1.5 L ha^{-1} of insecticide (Bazuka) were applied at 25 days after emergence.

Evaluated characteristics

The collection of soil samples for chemical and biological analyses and collection of gases emitted by the soil were carried out during the transition from the rainy to the dry season (August 2017), and the samples were evaluated at the Laboratory of Soil and Plants of the Embrapa Meio-Norte. The results of the initial soil chemical and particle-size analysis, before the implementation of the CLFI (Muniz Farm TRU) integration system, are shown in Supplementary Tables 2 and 3.

Physical and chemical fractionation of the soil organic matter and determination of biological attributes were done for all areas. Four trenches (0.5×0.5×0.5 m) were opened in randomly chosen locations. Twelve equidistant points were marked around each trench to collect deformed samples (0-10 cm layer), which were combined into one composite sample, sieved in a 2 mm mesh sieve, and stored under refrigeration (5 to 10 °C) until the microbiological analysis. In the CLFI system, single additional samples were collected in points at approximately 1.5 m from the eucalyptus canopy projection.

The physical particle-size fractionation of the organic matter was carried out using 20 g of air-dried fine soil, which passed through a 2 mm sieve; 100 mL of the 0.1 mol L^{-1} NaOH solution was added to the soil as a dispersing agent and the material was stirred on a shaker table for 3 hours, passed through a 0.053 mm mesh sieve using several washes under running water to remove the organic matter (OM), and then transferred to identified aluminum caps and dried in a forced air circulation oven at 60 °C. The material was then ground and sieved in a 60-mesh sieve, and 2.0 g of this soil was placed in digestion tubes and subjected to the same procedure used for extraction and titration of total organic carbon (TOC), using four whites, two heated and two unheated, as described in Embrapa (1997).

The microbiological attributes evaluated in these samples were: carbon and nitrogen contents in the microbial biomass and basal respiration. Microbial biomass was determined by irradiation-extraction (Ferreira et al., 1999). Soil basal respiration was determined by quantifying the CO_2 released after seven days of incubation under aerobic conditions. The first evaluation was carried out after three, the second after six, and the third after seven days, as described by Alef (1995) and adapted by Embrapa (1997).

The GHG were collected immediately after the grain harvest in the integrated system areas and, at the same time, in the degraded pasture, on August 17 and 18, 2017. Gas flows were assessed using static chambers, as recommended by the protocol for measuring soil GHG flows, and development of static measurement chambers, as described by Steudler et al. (1991).

Four sample units (chambers) were used for the degraded pasture, four for the CLI system, and eight for the CLFI

system (four for the maize and grass areas and four for the eucalyptus), totaling 16 sample units.

The chambers consisted of two parts: a galvanized steel base of rectangular shape buried in the ground at approximately 5 cm depth, and a rectangular plastic lid (25×39×59 cm) with an aluminized thermal blanket. The bases were placed on the ground 24 hours before beginning the gas collection and remained in the field throughout the evaluation period (Steudler et al., 1991). At the time of collection, the lid was attached to the base, supported by a channel at the outer edge of the base. The lid was carefully sealed to the base by adding a small amount of water inside the channel.

Each chamber had a hole for the collection of gas samples and a digital thermometer to record the internal temperature during the collection at its upper end. After the chamber were closed, samples of the gases emitted by the soil were collected using four-time intervals: the time the lid was attached to the base (time zero), and 10, 30, and 45 minutes after the closure of the chamber. Samples were collected in 50 mL polypropylene syringes and immediately transferred to pre-evacuated vials (80 Kpa) and sealed with a rubber septum.

CO_2, CH_4, and N_2O concentrations in the samples were determined by gas chromatography (Agilent 7890A chromatograph, Wilmington, DE, USA) at the Chromatography Laboratory of the Embrapa Semi-Arid. The chromatographer was equipped with a Flame Ionization Detector (FID) to determine CO_2 and CH_4 concentrations in the samples, and with an Electron Capture Detector (μECD) to determine N_2O concentrations. The rate of changes in gas concentration inside the chamber was used to calculate the GHG emission flow, using the formula:

$$F\left(\mu g\ C - \frac{CO_2}{N} - \frac{H_2O}{N} - CH_4\ m^{-2}h^{-1}\right) - \left(\frac{\Delta C}{\Delta t}\right) * \left(\frac{m}{Vm}\right) * \frac{V}{A}$$

where $\Delta C/\Delta t$ is the rate of change of gas concentration inside the chamber at a given time (ppm hour^{-1}); m is the molecular mass of each gas (g); Vm is the molecular volume of the gas (1 mol occupies 22.4 L under normal temperature and pressure conditions); V is the chamber volume (76.81 L); A is the chamber area (0.22 m^2). The molecular volume of the gases was corrected for the temperature inside the chamber during the sampling by multiplying 22.4 by (273 + T / 273), where T is the average temperature inside the chamber (°C).

Concomitantly with the gas collections, soil samples (0-10 cm deep) were collected near the chambers. Nitrate and ammonium contents were determined in these samples, according to Cantarella and Trivelin (2001), using extraction with KCl 1 mol L^{-1}, followed by distillation in a Kjeldahl Distillation Apparatus and subsequent titration of the distillate. In these same samples, the soil pH in water was also determined, according to Embrapa (1997).

Statistical analysis

The results were subjected to multivariate analysis using principal component analysis (PCA) and presented as biplot graphs (Kroonenberg, 1997), which were developed considering the first two main components with the largest variances and eigenvalues greater than 1.0 (Moraaguilheira et al., 1993). The results were also subjected to Pearson correlation analysis, using the interpretation classification according to Pearson's correlation coefficients (r): insignificant (0.0-0.3); low (0.31-0.50), moderate (0.51-0.70), high (0.71-0.90), and very high (0.91-1.0) (Mukaka, 2012), considering significance α_0.05 and 0.10. The analyses were performed using the R program (R Core Team, 2018).

Conclusions

The use of crop-livestock-forest integration system with eucalyptus, maize, and grass (*Urochloa brizantha* cv. Marandú) is an alternative for the improvement of soil biological characteristics and mitigation of greenhouse gas emissions in Plinthosols of the Amazonian region of the state of Maranhão, Brazil.

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