

## Understanding the flux of nitrous oxide from the eucalypt soil in monoculture and Integrated Crop–Livestock–Forest systems

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### Abstract

The Brazilian agricultural sector is largely responsible for nitrous oxide (N<sub>2</sub>O) soil emissions, mainly due to beef cattle and the use of synthetic nitrogen fertilizers. Therefore, Brazil is looking for measures, such as integrated crop–livestock–forestry (ICLF), to increase productivity and reduce greenhouse gas emissions in this sector. The forest component within this system plays a positive role in the context of climate change, soil conservation, carbon dioxide (CO<sub>2</sub>) sequestration, and biodiversity protection. The aim of this study was to evaluate the effect of management and rainfall on N<sub>2</sub>O emissions in eucalypt monoculture soils and eucalypt soils in ICLF systems. Manual static chambers were used to collect gas samples, from November 2013 to October 2014, in four treatments, i.e., one eucalypt monoculture (F) and three modalities of ICLF (livestock–forest [LF], livestock–crop–forest [LCF], and integrated crop–livestock–forest [ICLF]). A gas chromatograph with an electron capture detector was used to measure the N<sub>2</sub>O concentrations. The results showed that rainfall considerably affected N<sub>2</sub>O fluxes across all the treatments, indicating that rainfall is the main factor in increasing emissions. During the wet season, the N<sub>2</sub>O levels ranged from 0.158 to 0.482 kg N-N<sub>2</sub>O ha<sup>-1</sup> across all treatments. During the dry season, all treatments behaved like sinks of N<sub>2</sub>O. Moreover, N<sub>2</sub>O flux did not differ between the soils in the eucalypt monoculture and ICLF systems. This indicates that the forestry component in the ICLF systems did not affect N<sub>2</sub>O soil fluxes.

**Keywords:** agroforestry system, greenhouse gases, integrated systems, mitigation, sustainability.

**Abbreviations:** GHG\_greenhouse gases; CO<sub>2</sub>\_carbon dioxide; N<sub>2</sub>O\_nitrous oxide; CH<sub>4</sub>\_methane; N\_nitrogen; NDC\_Nationally Determined Contribution; CO<sub>2</sub>eq\_Carbon dioxide equivalent; ICLF\_integrated crop-livestock-forest; NO<sup>3-</sup>\_Nitrate; LF\_livestock-forest; LCF\_livestock-crop-forest; F\_eucalypt monoculture; WFPS\_water filled pore spaces; N<sub>2</sub>\_elementar nitrogen.

### Introduction

Atmospheric concentrations of the three main greenhouse gases (GHG), carbon dioxide (CO<sub>2</sub>), nitrous oxide (N<sub>2</sub>O), and methane (CH<sub>4</sub>), increased by 146%, 257%, and 122%, respectively, compared to pre-industrial levels (WMO, 2018) due to land use change and agricultural practices, among other causes (Muller, 2005; Hartmann et al., 2013; Rodrigues et al., 2017; Tian et al., 2020).

In Brazil, the agricultural sector has the largest share (33.6%) of the net GHG emissions in 2016, reaching up to 439.213 Gg CO<sub>2</sub>eq, showing an increase of 2.3% compared to that in 2015 (Brasil, 2020). In terms of the regions associated with

high emissions, the Midwest and Mato Grosso State are leading, accounting for 34% and 12% of the total emissions, respectively, with the main sources of emissions being beef cattle and the use of synthetic nitrogen fertilizers (Silveira et al., 2018).

Although atmospheric concentrations of all three gases are relevant in agriculture, major emphasis should be placed on N<sub>2</sub>O, as its global warming potential is 265 times higher than that of CO<sub>2</sub> (Myhre et al., 2013). N<sub>2</sub>O emissions tend to increase with the expansion of agricultural sector emerging economies, such as Brazil, where the agriculture sector is the

largest contributor to GHG emissions (Brasil, 2020; Tian et al., 2020). This gas is the result of soil nitrification and denitrification processes, which vary with the dynamics of nitrogen (N) (Saggar, 2010; Ussiri and Lal, 2013) and is also emitted due to the decomposition of plant residues or application of nitrogen fertilizers (Sato et al., 2017; Scott et al., 2018). Other agronomic management practices (e.g., irrigation and the use of manure) (Reay et al., 2012; Bayer et al., 2015; Tian et al., 2015; Martins et al., 2015), as well as cultivated crops and local rainfall regimes may also add to the affect (Li et al., 2016).

To increase efforts to mitigate climate change, Brazil, through its Nationally Determined Contributions (NDCs), which include mitigation, adaptation, and implementation components, has committed to reducing GHG emissions by 37% by 2025 and 43% by 2030, below 2005 levels (BRASIL, 2015). By 2030, actions under the NDCs are anticipated to expand the area under integrated crop–livestock–forest (ICLF) system by 5 million hectares (BRASIL, 2015). Partial results of these public policies are already obvious, as according to the ICLF Network, Brazil went from having an area of 1.87 million hectares under ICLF systems in 2005 to 11.5 million hectares by the 2015/2016 harvest, with the potential to stock 35.1 million tons of CO<sub>2</sub>eq (Rede ILPF, 2016).

The ICLF system has been identified as an efficient land management strategy that provides environmental, economic, and social benefits, such as soil and water conservation, wood production, and improved animal welfare, which can increase milk and beef production (Cordeiro et al., 2015; Alves et al., 2017). Additionally, it can improve the restoration of degraded pastures, mitigate GHG emissions, and increase soil carbon (C) sequestration by increasing biomass above and below the ground (Cerri et al., 2007; Euclides et al., 2010; Nair, 2012; Dube et al., 2012; Paula et al., 2013; Carvalho et al., 2014; Salton et al., 2014; Paustian et al., 2016; Stocker et al., 2013; Zomer et al., 2016). Furthermore, the use of crop rotations in ICLF with variable nitrogen availability is also a strategy for mitigating N<sub>2</sub>O emissions (Benoit et al., 2015; Jain et al., 2016).

According to Figueiredo et al. (2017), ICLF can reduce GHG emissions mainly due to pasture improvements, increase in the livestock yield, and the potential for carbon sinks in soil and biomass to offset emissions related to livestock management. In addition, these authors report that emissions can be reduced in terms of CO<sub>2</sub>eq emitted per kg of bovine weight produced, increasing meat, grain, and wood production. According to Nogueira et al. (2016), the cumulative N<sub>2</sub>O emissions in ICLF (0.4 kg N ha<sup>-1</sup>) may be lower than that in a monoculture crop (1.4 kg N ha<sup>-1</sup>), and similar to pasture monoculture, when there is no livestock (0.35 kg N ha<sup>-1</sup>), thereby presenting a potential to mitigate N<sub>2</sub>O emissions. Moreover, the adoption of ICLF can help achieve both environmental protection and the development of more efficient and sustainable agriculture in states with highly intensive livestock systems, low stocking rates, and where the agricultural sector is expanding rapidly, such as the Mato Grosso State (the country's leading cattle and soy producer) (Gil et al., 2015).

The lower N<sub>2</sub>O emission from forest areas compared to the crop areas is related to the greater ability of the tree roots to absorb water and NO<sup>3-</sup> in depth, reducing denitrification and leaching (Amadi et al., 2016). The nitrogen absorbed by the tree roots is returned to the soil through the leaf litter, resulting in more efficient N cycling and thus, decreasing the demand for N and emission of N<sub>2</sub>O (Thevathasan et al.,

2012). Given the advantages of the ICLF, the forest component deserves attention as it plays a positive role in control of climate change and deforestation through restoration of degraded areas, soil conservation, CO<sub>2</sub> sequestration, biodiversity protection, and reduction of pressure for the use of native forests in industrial processes (Cuer et al., 2018).

Of the total planted forest area in Brazil, 5.7 million hectares are eucalypt forests (Cuer et al., 2018), which indicates the importance of these species for the sector. These are fast-growing trees with high carbon sequestration potential during development (Burrows et al., 2002; Du et al., 2015; Bauters et al., 2019). Eucalypt plantations have been reported as a source of N<sub>2</sub>O and CO<sub>2</sub> and a sink for CH<sub>4</sub> in semi-arid and subtropical climates, as observed in most forest ecosystems (Zhang et al., 2017).

However, GHG fluxes in eucalypt plantations have not been well described in ICLF systems. Thus, in the present study, two questions were posed:

1. Do the fluxes of soil N<sub>2</sub>O differ between eucalypt monocultures and eucalypt in ICLF systems?
2. Does rainfall affect N<sub>2</sub>O emissions in a similar manner in both eucalypt monocultures and ICLF systems?

The implementation of an integrated management system is expected to reduce N<sub>2</sub>O emissions due to the increased inclusion of different crops, favoring increased productivity and efficiency in nutrient utilization. Rainfall is also expected to have a positive effect on N<sub>2</sub>O emissions owing to the filling of porous soil spaces and the stimulation of microbial action, thereby altering N<sub>2</sub>O production.

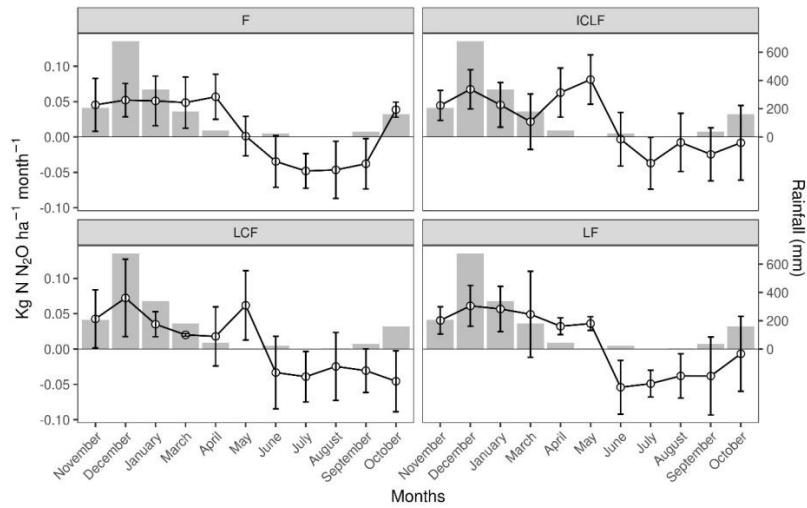
## Results

The N<sub>2</sub>O fluxes from eucalypt soil in the monoculture and in the integrated systems had the same variation throughout the year, as shown in Figure 1. The months from October to May showed positive N<sub>2</sub>O fluxes, while the months from June to September showed negative fluxes across all systems. In this study, the data show an evident seasonal effect of rainfall on N<sub>2</sub>O fluxes.

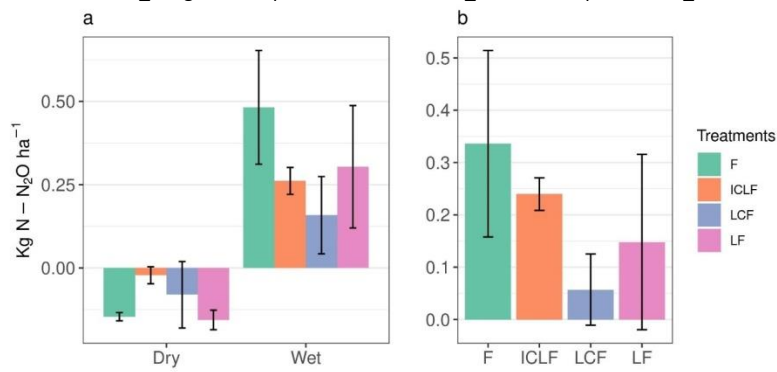
The monthly variation of N<sub>2</sub>O fluxes throughout the year in the study areas did not exceed 0.10 kg N-N<sub>2</sub>O month<sup>-1</sup>. In the dry season (from May to September), when soils have low humidity due to low rainfall, all systems acted as N<sub>2</sub>O sinks, except in May for ICLF, livestock–forest (LF), and livestock–crop–forest (LCF) (Figure 1). The cumulative highest average monthly N<sub>2</sub>O flux observed during the period was approximately 0.08 kg N-N<sub>2</sub>O month<sup>-1</sup> for the eucalypt soil in the ICLF system in May, while the highest consumption potential was -0.05 kg N-N<sub>2</sub>O month<sup>-1</sup> for the eucalypt soil in the LF system during June (Figure 1).

There was a significant difference between the cumulative emissions of the dry and wet seasons for all the treatments ( $p = 0.0000415$ ) (Figure 2a). Overall, the dry season shows negative emission values, indicating that the soil acts as a sink for N<sub>2</sub>O. Furthermore, there were no significant differences between the treatments in the dry season ( $p = 0.1129$ ), varying from -0.022 (ICLF) to -0.156 kg N-N<sub>2</sub>O ha<sup>-1</sup> (LF). Similarly, in the wet season, there was no significant difference between the treatments ( $p = 0.0987$ ), and the emission varied from 0.158 (LCF) to 0.482 kg N-N<sub>2</sub>O ha<sup>-1</sup> (F) (Figure 2a).

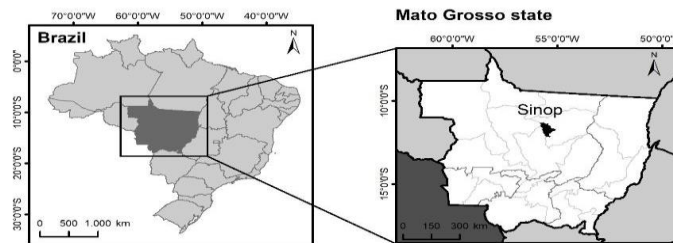
The results from analyses of the net cumulative emissions during the entire period shows that eucalypt soil from all the



**Figure 1.** Cumulative average monthly N-N<sub>2</sub>O fluxes (lines), and standard deviation (whiskers), and rainfall (bars) throughout the year for each treatment. F\_eucalypt monoculture; ICLF\_integrated crop-livestock-forest; LCF\_livestock-crop-forest; LF\_livestock-forest.



**Figure 2.** Cumulative N-N<sub>2</sub>O soil emissions between dry and wet seasons for each treatment (a) and net cumulative emission between the wet and dry period for each treatment (b). The vertical bars represent the standard deviation. F\_eucalypt monoculture; ICLF\_integrated crop-livestock-forest; LCF\_livestock-crop-forest; LF\_livestock-forest.



**Figure 3:** Location of the study site in Mato Grosso, Brazil.



**Figure 4.** Aerial image of the four treatments and three replications evaluated in Sinop, Mato Grosso, Brazil. Source: Google Earth. ICLF\_integrated crop-livestock-forest; LF\_livestock-forest; LCF\_livestock-crop-forest; F\_eucalypt monoculture.

		F	LF	LCF	ICLF	
Sowing	Oct - 2011				50.0 Kg ha <sup>-1</sup> Soybean ( <i>Glycine max</i> L.) BRSGO 8560 RR 400 kg ha <sup>-1</sup> NPK (00-20-20)	2011 – 2012 Harvest
	Nov - 2011				Hybrid <i>E. urophylla</i> x <i>E. grandis</i> 200 kg ha <sup>-1</sup> NPK (00-20-20) 350 kg ha <sup>-1</sup> (Phosphate fertilizer) 400 Kg ha <sup>-1</sup> NPK (04-30-16) <i>Brachiaria brizantha</i> cv. Marandú (6 kg ha <sup>-1</sup> )	
Fertilization after planting	Dec - 2011				96.40 Kg ha <sup>-1</sup> NPK (20-00-20)	
	Jan - 2012					
Harvest	Mar - 2012	20 Kg ha <sup>-1</sup> NPK (20-00-20)			20 Kg ha <sup>-1</sup> NPK (20-00-20)	
Sowing	Feb - 2012				Soybean (John Deere STS 9470) <i>Brachiaria brizantha</i> cv. Marandú (5.50 kg ha <sup>-1</sup> ) 10 Kg ha <sup>-1</sup> Com ( <i>Zea mays</i> L.) DKB 175 Pro 375 Kg ha <sup>-1</sup> NPK (04-30-16) 300 kg ha <sup>-1</sup> Urea	
					Com (John Deere STS 9470)	
Harvest	Jul - 2012				25 Kg ha <sup>-1</sup> Soybean ( <i>Glycine max</i> L.) BRSGO 8560 RR 350 kg ha <sup>-1</sup> NPK (00-20-20)	
Sowing	Oct - 2012					
Fertilizing	Jan - 2013				119.0 Kg ha <sup>-1</sup> NPK (20-00-20)	
Harvest	Feb - 2013				Soybean (John Deere STS 9470) 30 Kg ha <sup>-1</sup> Com ( <i>Zea mays</i> L.) 5.50 kg ha <sup>-1</sup> Semente <i>Brachiaria brizantha</i> cv. Marandú 300 Kg ha <sup>-1</sup> NPK (08-28-16)	
Sowing	Mar - 2013				96.0 Kg ha <sup>-1</sup> NPK (20-00-20) 210 Kg ha <sup>-1</sup> (Urea)	
Fertilizing	Jul - 2013				Com (John Deere STS 9470)	
Harvest	Oct - 2013				25 Kg ha <sup>-1</sup> Soybean ( <i>Glycine max</i> L.) BRSGO 8560RR Liming (1500 kg ha <sup>-1</sup> ) 400 kg ha <sup>-1</sup> NPK (00-20-20)	
Sowing	Feb - 2014				Soybean (John Deere STS 9470)	
Harvest					13.72 kg ha <sup>-1</sup> Com ( <i>Zea mays</i> L.) DKB 390 Pro 6 kg ha <sup>-1</sup> Semente <i>Brachiaria brizantha</i> cv. Marandú	
Sowing	Mar - 2014				450 Kg ha <sup>-1</sup> NPK (04-30-16) 300 Kg ha <sup>-1</sup> (Urea)	
Fertilizing	Jul - 2014				Com (John Deere STS 9470)	
Harvest	Oct - 2014				25 Kg ha <sup>-1</sup> Soja ( <i>Glycine max</i> L.) BRSGO 560RR 400 kg ha <sup>-1</sup> NPK (00-20-20)	
Sowing						

**Figure 5.** Timeline and description of all management (i.e., Fertilizing, Sowing, Harvest) applied in the treatments, including all species planted and rotation used. The crop timeline for this study starts in the year 2011 (light gray) and finishes in 2015 (dark gray). F\_eucalypt monoculture; LF\_livestock-forest; LCF\_livestock-crop-forest; ICLF\_integrated crop-livestock-forest.

treatments had positive emission values, with no significant differences in the treatments ( $p = 0.1473$ ) (Figure 2b). The emissions varied from 0.057 (LCF) to 0.336 kg N-N<sub>2</sub>O ha<sup>-1</sup> (F).

## Discussion

The predominantly positive N<sub>2</sub>O fluxes observed during rainy season across all treatments may be a reflection of the availability of inorganic N due to fertilizer application and mineralization of plant residues (Bayer et al., 2015; Piva et al., 2019) from the other components of the integrated systems. In addition, the anaerobic conditions of the highly humid soil can directly influence the soil's water-filled pore spaces (WFPS), which are important in the denitrification processes and are the main factor in the formation of N<sub>2</sub>O (Rosenkranz et al., 2006; Konda et al., 2008; Fang et al.,

2012; Butterbach-Bahl et al., 2013). These processes may be related to the higher activity of microorganisms in soaked soils, potentiated when saturation level of WFPS is more than 70% (Weerden et al., 2012; Butterbach-Bahl et al., 2013; Corrêa et al., 2016). However, when the WFPS saturation is equal to or greater than 80%, the denitrification process is intensified, and the end product under these conditions will be mainly N<sub>2</sub> (and not N<sub>2</sub>O) (Linn and Doran, 1984; Davidson et al., 2000; Bateman and Baggs, 2005; Butterbach-Bahl et al., 2013). Thus, the reduction in emissions after December (Figure 1) in ICLF, LCF, and LF may be related to high WFPS saturation due to high rainfall. Changes in soil moisture and temperature maybe responsible for approximately 95% of the temporal variations in field N<sub>2</sub>O emissions (Bouwman, 1998). Nevertheless, higher N<sub>2</sub>O consumption potential during periods of low rainfall may be associated with the low N

content available in the soil, reducing the nitrification and denitrification activities, which are the main processes for N<sub>2</sub>O emission (Chapuis-Lardy et al., 2007). Furthermore, low mineral nitrogen and rapid soil water drainage may not offer favorable conditions for high N<sub>2</sub>O fluxes (Neves, 2016; Carvalho et al., 2017). Under these circumstances, air can diffuse into the macro- and micro-pores of the soil and allow microorganisms to use N<sub>2</sub>O as a source of N (Rosenkranz et al., 2006). In their study evaluating GHG emissions with different N rates in eucalypt areas in China, Zhang et al. (2017) found higher emissions of nitrous oxide in the wet season and lower emissions in the dry season, with the highest emissions always observed for the treatments with higher N rates, even in the dry season. Thus, the low N<sub>2</sub>O fluxes found in this study during the dry season may be related to the high soil porosity, drought conditions, and low soil N content. Our results showed a high standard deviation (Figure 2), as soil N<sub>2</sub>O fluxes normally have high spatial and temporal variability due to the heterogeneity of environmental parameters and soil characteristics (McDaniel et al., 2017; Rivera et al., 2019; Charteris et al., 2020). The climate seasons also impact the N<sub>2</sub>O emission, as found by Nascimento and Rodrigues (2019) who found positive cumulative fluxes in the dry period with approximately 0.25 kg N-N<sub>2</sub>O ha<sup>-1</sup> and 0.50 kg N-N<sub>2</sub>O ha<sup>-1</sup> in soils with eucalypt monoculture and in native forests, respectively. Their results differ from the results of this study, in which we showed that the eucalypt soil from all the treatments acted as a sink, with negative values (N<sub>2</sub>O consumption) in the dry period (Figure 2a). In the study by Nascimento and Rodrigues (2019), the maximum cumulative value during the rainy season was 0.45 kg N-N<sub>2</sub>O ha<sup>-1</sup> for the eucalypt monoculture and 0.50 kg N-N<sub>2</sub>O ha<sup>-1</sup> for the native forest. A comparison between the above results and the results of this study showed that the soil of a eucalypt forest planted in an integrated system has a lower potential N<sub>2</sub>O emission than that of a eucalypt monoculture (F).

The total N<sub>2</sub>O emission for the entire period for the eucalypt soil is still low (Figure 2b) when compared to the total values of the native Amazon Forest soil (0.6 kg N-N<sub>2</sub>O ha<sup>-1</sup>) (Nascimento et al., 2020), native Atlantic Forest soil (0.82 kg N-N<sub>2</sub>O ha<sup>-1</sup>), and soil of eucalypt monoculture (0.55 kg N-N<sub>2</sub>O ha<sup>-1</sup>) (Silva, 2019). The results in this study are also moderate as compared to a study carried out in the transition biome Cerrado and Amazon Forest, which found cumulative values of approximately 1.0 kg N-N<sub>2</sub>O ha<sup>-1</sup> for native forest and 0.70 kg N-N<sub>2</sub>O ha<sup>-1</sup> for monoculture of eucalypt (Nascimento and Rodrigues, 2019).

Although we did not evaluate all the components of the integrated systems (crops, livestock, and forest), Nogueira et al. (2016) evaluated it in the same study area and found cumulative values of 0.4 kg N-N<sub>2</sub>O ha<sup>-1</sup> for ICLF and 1.4 kg N-N<sub>2</sub>O ha<sup>-1</sup> for monoculture crops, demonstrating the trend of low emissions in the eucalypt soil of integrated systems. In this study, animal non-grazing in the areas near eucalypt may also have been reflected in the low emissions, as there was no deposition of urine residues and cattle feces, which can influence emissions (Piva et al., 2014).

Overall, low N<sub>2</sub>O fluxes in the eucalypt component could be a consequence of the high demand for nitrogen by the plants, reducing the loss of mineral nitrogen as N<sub>2</sub>O, helping to mitigate the emissions (Figueiredo et al., 2018). Moreover, the low N<sub>2</sub>O fluxes in these forest component systems may be related to changes in the phenolic content of eucalypt litter (Soumare et al., 2015), which contributes to the decreasing microbial community size and enzymatic

activities and the increasing physiological microbial stress (Chen et al., 2013). Carvalho et al. (2017) evaluated ICLF and integrated crop–livestock (ICL) system over a 2-year period and found that soil N<sub>2</sub>O fluxes were lower in the ICLF system than in the ICL system associated with eucalypt litter, which is rich in phenolic compounds that leads to a low carbon microbial biomass.

## Materials and Methods

### Study area characterization

The experimental field was located in the municipality of Sinop, Mato Grosso, Brazil (11°51 'S, 55°35 'W, 384 m altitude) (Figure 3) in the experimental field of Embrapa Agrossilvipastoril. The climate is classified as Am (Köppen monsoon climate) (Alvares et al., 2013). The annual average air temperature is 25.8 °C with an accumulated rainfall of 2,250 mm (Embrapa, 2017), with a dry season that extends from May to September (Souza et al., 2013). The average annual relative humidity is 71% (Embrapa, 2017).

The soil of the experimental area was classified as a typical dystrophic red-yellow latosol according to the Brazilian Soil Taxonomy (Santos et al., 2018), which is equivalent to a Hapludox under the US Soil Taxonomy (Soil Survey Staff, 2014). It has a clayey texture, moderately flat relief, sub-perennial vegetation, and kaolinitic-gibbsite composition (Viana et al., 2015). The soil, in the 0–20 cm layer, presented the following features at the start of the experiment: 5.7 pH in H<sub>2</sub>O, 13.7 mg dm<sup>-3</sup> of P (Mehlich 1), 79 mg dm<sup>-3</sup> of K (Mehlich 1); 2.3 cmolc dm<sup>-3</sup> of Ca, 0.66 cmolc dm<sup>-3</sup> of Mg, 0.01 cmolc dm<sup>-3</sup> of Al, and 29.6 g kg<sup>-1</sup> of organic matter (Farias Neto et al., 2019). The soil texture was clayey with 28% sand, 16% silt, and 56% clay.

The experimental field originally showed a transition between the Cerrado and Amazon vegetation. Deforestation occurred in 1984 for the production of cassava (*Manihot esculenta* Crantz) (Araujo et al., 2009). In the 1990s, it was cultivated with rice (*Oryza sativa*) and later with soybean (*Glycine max* L.). Between 2002 and 2007, the area was cultivated with soybean and corn (*Zea mays* L.), and during the 2007–2009 harvests, soybean and cotton (*Gossypium hirsutum* L.) successions were conducted. During the 2010–2011 harvest, the area remained fallow (Diel et al., 2014).

In October 2011 (2011–2012 season), four treatments were implemented using a randomized complete block design with three replications. There were three types of integrated crop–livestock–forest (ICLF) systems: livestock–forest (LF), livestock–crop–forest (LCF), and ICLF, named in the order of entry of the agricultural component and livestock in each treatment, and a eucalypt monoculture (F). The F was evaluated in experimental plots of 1 ha and the other treatments (LF, LCF, and ICLF) were in plots of 2 ha, amounting to a total area of 21 ha with 12 experimental units (Figure 4).

The hybrid *Eucalyptus urophylla* × *E. grandis* clone H13 was used in the F treatment, and the clones were implanted with a spacing of 3.5 × 3.0 m and a density of 952 plants ha<sup>-1</sup>. In the other treatments (LF, LCF, and ICLF), the clones were planted with a spacing of 3.5 × 3.0 m, in the east–west direction, and with a distance of 30 m between each grove. Furthermore, each grove consisted of three rows of eucalypt trees, totaling 270 plants ha<sup>-1</sup>. LF treatment always consisted of *Brachiaria brizantha* (Hochst. ex A. Rich.) R. D. Webster grass between eucalypt groves. In the LCF treatment, crop and livestock components were alternated every 2 years. In the first two harvests (2011–2012 and 2012–2013), marandu

palisade grass (*Brachiaria brizantha* 'Marandu') was cultivated between eucalypt groves. In the third and fourth harvest years (2013–2014 and 2014–2015), the area between groves was planted with soybean [*Glycine max* L. (Merr.)] from October to February, followed by corn (*Zea mays* L.) in combination with marandu palisade grass (cover crop) from February to July. In the ICLF treatment, the crop and livestock components were alternated annually. Soybean was cultivated every year between October and February (2011–2012, 2013–2014), followed by corn in combination with marandu palisade grass (cover crop) from February to July (Figure 5).

The planting of eucalypt in all treatments was carried out in November 2011. Plowing was performed at a depth of 50 cm, and 350 kg ha<sup>-1</sup> of simple superphosphate was applied to the planting furrow.

The activities of each treatment varied over the first three years of the system in relation to culture and fertilization, as shown in Figure 5.

The treatments were established in 2011, and until 2015, there were no livestock insertions. That is, during the period of evaluation of this research, no animal grazing occurred in any of the treatments, as the intention was to use the pasture for cutting in the use of silage and as a cover crop during the first year. As the experimental plot is located in an Amazon region with high rainfall, soil cover and enhancement of organic matter in the soil for other crops is extremely essential. Therefore, in the beginning of the experiment the animals were not inserted into the area, and the marandu palisade grass was used for silage and hay, being harvested with mechanical machines.

N<sub>2</sub>O sampling of soil

Gas samples were collected every seven days during the 2013–2014 harvest (November 2013 to October 2014). Owing to logistics issues related to the circulation of agricultural machinery, it was not possible to perform sampling in February 2014. Thus, the fluxes from this month could not be measured.

N<sub>2</sub>O flux was collected through a manual static chamber in a base-top rectangular model, similar to that described by Nogueira et al. (2015). Metal bases (length × width × height) of 60 × 40 × 11 cm were installed in the field one week before the first collection in order to avoid disturbances in the soil. The bases were fixed at a depth of 5 cm in the soil and were maintained throughout the experiment. The top of the chamber consisted of a 60 × 40 × 9.2 cm (length × width × height) polyvinyl chloride (PVC) tray covered with a double-sided thermal blanket to decrease solar absorption and maintain internal temperature (Parkin and Venterea, 2010). Approximately 50 cm<sup>3</sup> of air that had accumulated in the chamber was removed through a three-way valve connected to a 60 cm<sup>3</sup> polypropylene syringe.

After chamber coupling (i.e., bottom-top), an air sample was immediately taken from the interior of the chamber, considered as time zero, and subsequent samples were taken at 20, 40, and 60 min (Parkin and Venterea, 2010). Consequently, the tops were removed from the base and the soil area was again exposed to the environmental conditions. N<sub>2</sub>O sampling was always performed between 8 and 11 h (Zuchello, 2010). At the time of gas collection, the internal chamber temperature used for the N<sub>2</sub>O flux calculation was monitored using thermohygrometers.

In each treatment, a chamber was allocated to the soil under the eucalypt tree canopy. In the F treatment, the chamber was installed at the center of it. The chambers were installed in the central row of the central eucalypt grove in the

integrated systems (e.g., LF, LCF, and ICLF). The static chambers evaluated soils from within the eucalypt component of the respective systems, and not in areas with crop or livestock components.

The meteorological station of Embrapa (Embrapa, 2017) was used to obtain the daily accumulated rainfall (mm), and then the accumulated monthly rainfall was calculated.

#### Laboratory analysis

Gases were transferred from syringes to butyl rubber sealed vials (e.g., glass vial) with 20 cm<sup>3</sup> (Parkin and Venterea, 2010), previously vacuumed (approximately -100 kPa) using an electric vacuum pumping system for concentration analysis. After the transfer of the gases, the vials were left in an air-conditioned room at a temperature of 21 °C for approximately 24 h before analysis. Subsequently, the samples were analyzed using a gas chromatography (Shimadzu GC-2014) equipped with an automatic injector and electron capture detector (ECD) to measure N<sub>2</sub>O concentrations with 95% precision. The ECD detector temperature was maintained at 325 °C, and the column was maintained at 75 °C in the isothermal system. The columns were in the Hayesep series (1.0, 4.0 M; 1.5 M; 1.5 M; 0.7 M). Ultrapure nitrogen was used as the carrier gas at a flux rate of 25 mL min<sup>-1</sup>, and the injector pressure was maintained at 300 kPa. To determine the standard curve, three gaseous solutions containing the N<sub>2</sub>O standard (white Martins) were used. The default values were 382.8, 808, and 2080 mol mol<sup>-1</sup>, respectively. The sample run time was eight minutes.

From the sample concentrations, the gas concentration change rate was calculated by considering the linear fit model. After obtaining the best fit in the gas increment inside the chamber, the flux was determined according to Equation 1, proposed by Hutchinson and Livingston (Hutchinson and Livingston, 1993). From equation 1, the fluxes of N<sub>2</sub>O in µg N m<sup>-2</sup> h<sup>-1</sup> were obtained, and the cumulative emission (kg N-N<sub>2</sub>O ha<sup>-1</sup>) was obtained.

$$N_2O \text{ Fluxes } (\mu\text{g N m}^{-2} \text{ h}^{-1}) = \frac{\left(\frac{\Delta C}{\Delta t}\right) * V}{A} * \left(\frac{m}{V_m}\right)$$

where ΔC/Δt is the slope of a linear function adjusted to the gas concentration of the samples taken at 0, 20, 40, and 60 min after chamber closure (ppm hour<sup>-1</sup>); V is the chamber volume (L), A is the chamber area (m<sup>2</sup>), m is the gas molecular weight (g mol<sup>-1</sup>), and V<sub>m</sub> is the molar volume of the gas (m<sup>3</sup> mol<sup>-1</sup>) corrected for air temperature (K) from inside the chamber.

#### Data analysis

The cumulative N<sub>2</sub>O emissions (kg N-N<sub>2</sub>O ha<sup>-1</sup>) of dry (May to September) and wet (October to April) seasons were estimated using the trapezoidal integration method (Hergoualc'h et al., 2019). The net cumulative emissions from the entire period were estimated by subtracting the wet and dry period emissions. The data were not normally distributed, and nonparametric statistics were used. We used the Kruskal–Wallis (Kruskal and Wallis, 1952) test to perform comparisons among treatments by season, and Mann–Whitney U-test (Mann and Whitney, 1947) to perform comparisons between dry and wet seasons. All data analyses were performed using R Core Software (R Core Team, 2016).

#### Conclusions

Soil use and management intensification in other components from the ICLF system did not increase N<sub>2</sub>O

emission from the eucalypt soil, which was equal to that of eucalypt in monoculture (in which the total area is less intensive). It was observed that across all the treatments, eucalypt soil had positive emission values in the wet season, whereas in the dry season, the soil acted as a sink with negative emission values, thereby proving the influence of rainfall on the N<sub>2</sub>O emissions.

ICLF is considered a technological option that contributes to the objectives of the ABC Plan to Brazil to achieve its NDCs commitment under the Paris Agreement. However, to confirm the mitigation potential of ICLF systems, it is necessary to quantify N<sub>2</sub>O emissions from all components of the system (crop, livestock, and forests).

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