



Soil nitrous oxide emissions after the introduction of integrated cropping systems in subtropical condition

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ABSTRACT

The assessment of impacts of GHG to mitigate emissions in Brazil is a significant challenge for the expansion of integrated cropping systems. In Brazil, most studies on integrated cropping systems were conducted in tropical regions and evaluated N₂O fluxes, important GHG due to its global warming potential. However, the dynamics of N₂O fluxes of these systems in subtropical climate conditions in Brazil are still unclear. Thus, we investigated N₂O emissions under integrated cropping systems and monoculture systems and evaluated N₂O fluxes in five consolidated systems: cropland, integrated crop-forest (ICF), pasture, integrated livestock-forest (ILF), and eucalyptus. N₂O emissions were monitored weekly using six manual static chambers for each agricultural system. Soil-weather variables were observed consecutively during N₂O sampling. We assessed the relation between soil moisture, water-filled porous space (WFPS), rainfall, soil NO₃⁻, and soil NH₄⁺ with N₂O. Our results showed that seasonal water availability influenced N₂O fluxes in all five systems. Fertilization with N increased N₂O daily fluxes in cropland and ICF (N₂O maximum from 30 to 50 μg N m⁻² h⁻¹). However, cumulative N₂O in the second season was lower than the first season to all evaluated systems. Cropland, ICF, and eucalyptus showed an increase of more than 50% of cumulative N₂O emissions compared to the dry to the rainy season, while pasture and ILF presented an increase of more than 200% of cumulative N₂O from one season to another. However, the absolute cumulative value was higher for cropland, ICF, and eucalyptus than pasture systems. Thus, the use of annual crops or just monoculture could increase N₂O fluxes due to the influence of weather-soil variables. The results showed that N₂O emissions were similar between ICF and ILF systems and between cropland, pasture, and eucalyptus. Therefore, integrated cropping systems offer potential for reduced N₂O losses to the atmosphere and may support national and international climate change initiatives to reduce GHG emissions in agriculture.

1. Introduction

N₂O is a greenhouse gas (GHG) that depletes stratospheric ozone (Portmann et al., 2012; Ravishankara et al., 2009) with global warming potentials (GWP) 298 times higher than carbon dioxide (CO₂) (IPCC, 2013). In this context, the agricultural sector accounts for more than 50% of nitrous oxide (N₂O) emission, due to N fertilizer use and manure management (Syakila and Kroeze, 2011). To reduce GHG emissions, in

compliance with the National Politics on Climate Change (Law No. 12, 187 of December 29th, 2009), Brazil aims to expand 9.5 million ha through integrated cropping systems and N-fixing plants to reduce more than 28 million Mg CO₂eq until 2025 (BRASIL, 2017).

Agricultural intercropped systems could provide an economic return, increase productivity per unit area and reduce environmental impacts due to the integration of agriculture, livestock, and forest activities (Alvarenga et al., 2010; Alves et al., 2017; Damian et al., 2021).

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Agricultural diversification can favor nutrients cycling and Organic Matter (OM) supply (Cordeiro et al., 2012; Ciaís et al., 2013), while monoculture intensification can contribute to increasing N_2O emissions due to intense soil degradation and excessive use of N sources (Stehfest and Bouwman, 2006). Agriculture accounts for 21.1% of the Brazilian Gross Domestic Product (GDP, CEPEA, 2018), mainly with production and exports of commodities, such as soybean, maize, beef, and cellulose (Sato et al., 2017), underscoring the concern to reduce N_2O emissions and diversifying activities.

Emissions of N_2O depend on soil, climatic factors, and the type of management in agricultural areas (Casanave Ponti et al., 2020). Thus, edaphoclimatic factors, such as rainfall, temperature, soil moisture and N sources could considerably increase N_2O emissions (Fracetto et al., 2017). Temperature and water inputs (e.g., precipitation and irrigation) greatly influence biochemical processes that increase N_2O emissions under agriculture systems (Schindlbacher et al., 2004; Lu et al., 2006; Del Grosso and Parton, 2012).

However, one of the most significant concerns with the increase of N_2O production is using N sources in agriculture (Stehfest and Bouwman, 2006). Pareja-Sánchez et al. (2020) found a positive relationship between the increases in fertilizer rate and N_2O emissions in an area under maize cultivation in the Mediterranean region. Bureau et al. (2017) reported that N_2O fluxes were driven by soil moisture and nitrate content.

In tropical and subtropical regions, integrated cropping systems [e.g., Integrated Crop-Forest (ICF) and Integrated Livestock-Forest (ILF)] have great potential to reduce N_2O emissions (Dieckow et al., 2015; Nogueira et al., 2016). In a tropical climate condition, de Carvalho et al. (2017) evaluated N_2O emissions in integrated cropping systems and observed that N_2O losses to the atmosphere were below $50 \mu\text{g m}^{-2} \text{h}^{-1}$. Furthermore, Sato et al. (2017) found that integrated cropping systems emitted less N_2O ($\sim 27 \mu\text{g N m}^{-2} \text{h}^{-1}$) than monoculture systems ($\sim 36 \mu\text{g N m}^{-2} \text{h}^{-1}$), despite the use of N fertilizer. Nogueira et al. (2016) also reported that N_2O emissions were three-fold lower in integrated cropping systems ($7.7 \mu\text{g N m}^{-2} \text{h}^{-1}$) during the soybean cycle, compared to only annual production ($25.5 \mu\text{g N m}^{-2} \text{h}^{-1}$), despite the influence of water input in the soil.

For Sato et al. (2017) and Carvalho et al. (2021), the greater capacity

of integrated systems to mitigate N_2O emissions can be attributed to the greater diversity of plant species, optimizing the use and reducing the N losses due to the characteristics of each crop component. In subtropical climate conditions, Dieckow et al. (2015) observed lower N_2O emissions in integrated cropping systems ($21 \mu\text{g N m}^{-2} \text{h}^{-1}$) compared to soybean production ($70 \mu\text{g N m}^{-2} \text{h}^{-1}$) after N fertilizer application. However, these same authors advised that further studies are still needed to evaluate the dynamics and seasonality (e.g., rainy and dry seasons) of N_2O emissions on monoculture systems and integrated cropping systems and subtropical climate condition to support consistent conclusions. In this context, we hypothesize that integrated cropping systems (ICF and ILF) can efficiently mitigate the N_2O emissions in Brazil. Our objective was to quantify and examine the controlling factors (soil-weather variables) on the N_2O emissions after introduction of integrated cropping systems. For this purpose, we evaluated five consolidated agricultural systems [cropland, ICF, pasture and ILF, and eucalyptus] in a subtropical climate condition.

2. Material and methods

2.1. Experimental site

N_2O emissions were monitored from September 2017 to September 2018 in the experimental field of Embrapa (Brazilian Agricultural Research Company), located in Londrina ($23^\circ 28' 52'' \text{ S}$ and $50^\circ 59' 08'' \text{ W}$, 479 m.a.s.l.), Paraná State, Brazil (Fig. 1).

The study site has annual rainfall between 1300 and 1600 mm, with a rainy season from December to March. The climate in Paraná is classified as Cfa (Köppen), and our study site is characterized by hot summers (Alvares et al., 2013). The soil of the study site is classified as typical Hapludox (Soil Survey Staff, 2014) with 75% of clay, 20% of silt, and 5% of sand. The soil chemical attributes at the 0–20 cm layer are presented in Table 1.

The field trial was implemented in 2010, representing typical management systems for the subtropical climate conditions in Brazil, where soil correction was carried out with 1500 kg of limestone application per hectare and with the planting of *Eucalyptus grandis* GPC 23, *Brachiaria brizantha* cv. Piatã, and annual crops. In 2013, five agricultural systems

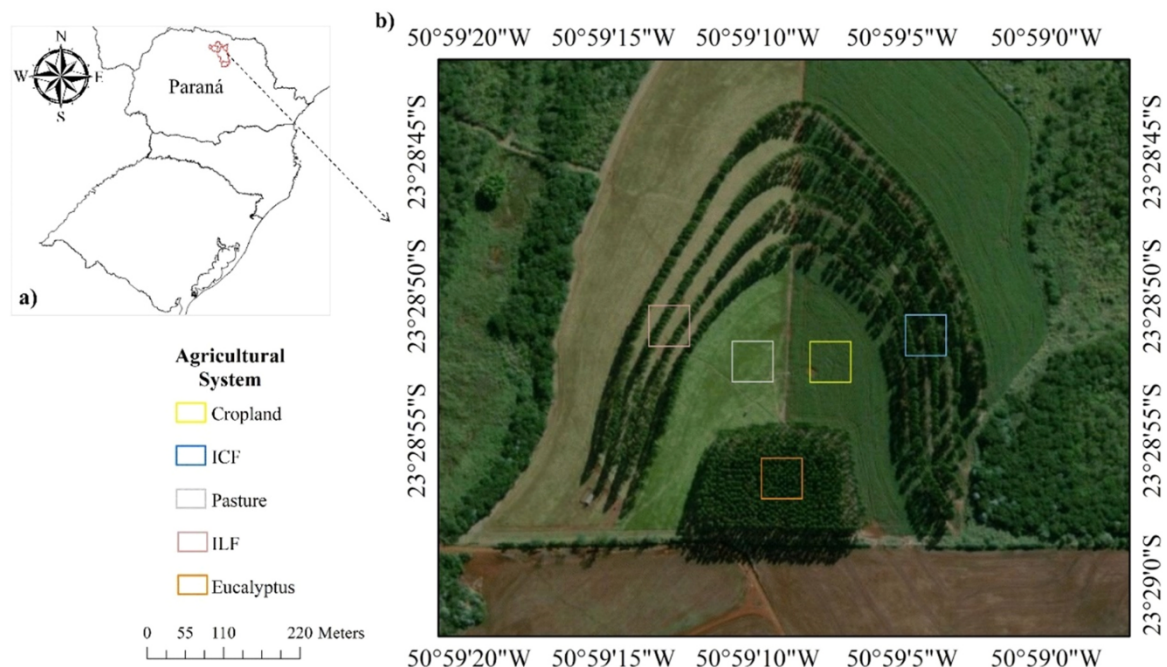


Fig. 1. Location of the study site in Paraná State (a) and in Londrina (b). The rectangles represent areas of measurements of N_2O fluxes from September 2017 to September 2018.

Table 1

Physicochemical characteristics of soil under the different agricultural systems in the experimental area for the 0–20 cm soil layer.

System	BD g cm ⁻³	C g kg ⁻¹	pH	avail-P mg dm ⁻³	Ca cmol dm ⁻³	Mg	K	H+Al	CEC	BS %
Cropland	1.33	16.6	5.5	21.5	6.7	2.0	0.7	4.5	13.9	67.8
Crop-Forest	1.32	15.8	5.4	22.4	5.9	2.0	0.7	4.8	13.4	64.1
Pasture	1.36	16.8	5.4	11.9	5.2	2.0	0.8	4.7	12.6	62.8
Livestock-Forest	1.36	16.1	5.2	9.2	4.7	1.9	0.6	5.1	12.4	58.5
Eucalyptus	1.25	15.4	5.3	8.4	5.5	2.3	0.5	4.8	13.1	63.1

BD = Bulk density, C = Soil Carbon, Avail-P = phosphorus available, CEC = cations exchange capacity, BS = Sum of Bases. Values represent average (n = 6).

were adopted, being three monoculture systems – cropland, pasture, and eucalyptus – and two integration systems called integrated livestock-forest (ILF) and integrated crop-forest (ICF) (Fig. 2 and Table S1). On September 29th, 2017, soil chiseling was carried out in the cropland and ICF systems before soybean sowing. Soybean was seeded on October 15th, 2017 and harvested on March 4th, 2018. Two applications of N fertilizer (urea) were carried out after the soybean harvest. First, on March 12th, 2018, before maize sowing (160 kg N ha⁻¹) and another on March 16th, 2018, during sowing (200 kg N ha⁻¹). Maize was harvested on September 4th, 2018. In the pasture and ILF systems, animals were submitted to the extensive stocking regime with one animal per hectare (1 AU ha⁻¹). The eucalyptus trees were thinned before September 2017 in the eucalyptus, ILF, and ICF systems. Nitrogen fertilizer was not applied to the pasture, eucalyptus, and ILF system.

2.2. Measurements of N₂O emissions

Ninety-two measurements were carried out to collect N₂O fluxes for one year, with a weekly frequency. In some weeks, more than one measurement was carried out (e.g., due to rain and fertilization events), which explains the total number of measurements proceed in one year (92 measurements). In each system, six manual chambers (replications) were randomly arranged, totaling 30 chambers in the experimental field. It is essential to highlight that we did not observe the lateral flow of soil water among the plots. Chambers for cropland and ICF were allocated to plants line of annual crops. As ICF and ILF were composed of four rows of eucalyptus, the arrangement of chambers occurred in the middle of the two central rows, and the eucalyptus system was placed in the central part of the plot. In pasture and ILF, the chambers were protected to prevent the influence of animals on monitoring.

Air samples were collected in static chambers (Mosier et al., 1998) of rectangular metallic measuring 40 × 60 × 10 cm (L × W × H) and mounted on a galvanized steel base 5 cm of height. The headspace volume of chambers was approximately 26.4 L. The metallic bases were

inserted in each system permanently at a soil depth of 5 cm and removed only at occurrence tillage and harvest operations for a short time. Chambers were closed and sealed with water to avoid air exchanges between atmospheres inside and outside. The chambers were equipped with a probe thermometer for monitoring air temperature inside and a rubber septum from where air samples were taken through a plastic tube closed by a three-way "Luer-lock" valve (Nicoloso et al., 2013). Sampling was performed at 0, 30, and 60 min after closing chambers and collected between 9 and 10 h, as indicated by Alves et al. (2012). Samples were collected with 60 mL polypropylene syringes, which were immediately stored in a cooler and conducted to the laboratory for the GC analysis. The samples were transferred to 20 mL glass pre-evacuated vials (-80 kPa) and closed with 2 mm⁻¹ butyl-rubber septa sealed with aluminum tops.

N₂O concentration was determined by gas chromatography (Trace GC Ultra, Thermo Scientific) with columns filled with "Porapak Q", and an electron capture detector. The calculation is established on the linear relationship between N₂O incubation time and gas concentrations following the recommendations of Livingston and Hutchinson (1995) and Nicoloso et al. (2013). N₂O fluxes were calculated using Eq. (1),

$$N_2O = (\Delta Q \times P \times V) / (\Delta t \times R \times T \times A) \quad (1)$$

Where: N₂O is nitrous oxide fluxes (μg N m⁻² h⁻¹), ΔQ is the mass of the gas (μg N) inside the chamber at a given sampling time t, P is the atmospheric pressure (atm) assumed as 1 atm, V is the chamber volume (L), Δt is incubation time, R is the constant for ideal gases (0,08205 atm L mol⁻¹ K⁻¹), T is the temperature within the chamber at sampling time (K) and A is the basal area of the chamber (m²).

Cumulative N₂O emissions were estimated through linear interpolation between average N₂O daily fluxes by time scale, calculating the resulting area under the integration curve by trapezium rule (Whittaker and Robinson, 1967). The N₂O emission factor (EF) was determined to cropland and ICF with N fertilizer applications in the soil, according to the methodology proposed by IPCC (2013). The EF was calculated

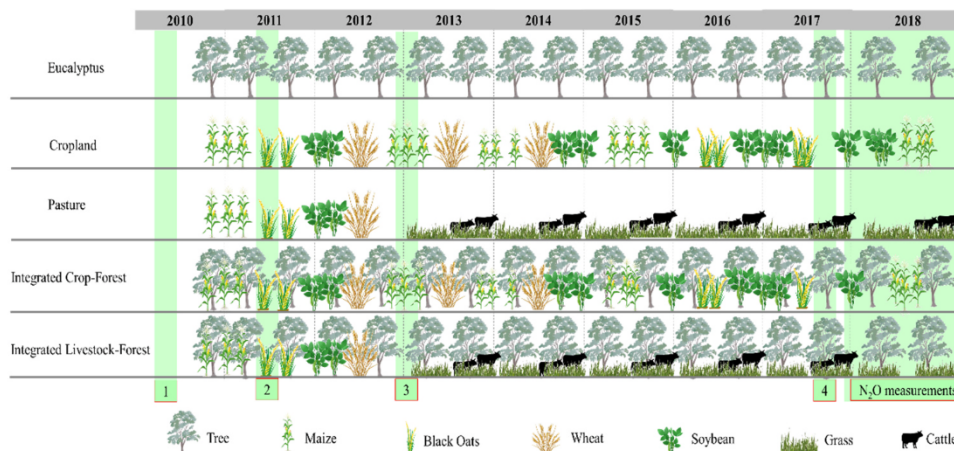


Fig. 2. History of implementation of the agricultural systems in the experimental area. 1: Preparation of the soil, 2: Thinning of Eucalyptus, 3: Implementation of *Brachiaria brizantha* cv *piatã*, 4: Thinning of eucalyptus and soil scarification.

according to Eq. (2),

$$EF = [(E_i - E_o)/N] \times 100 \quad (2)$$

Where: EF is the percentage of the total N fertilizer applied that was emitted as N₂O (%), E_i is the total N₂O emitted at the agricultural system (kg N) for an N fertilizer rate, E_o is the total N₂O emitted at the agricultural system before fertilization and N is the amount of N fertilizer applied (kg N).

2.3. Soil and weather variables

Soil samples were collected on days when N₂O emissions were monitored to determine rainfall (mm), soil moisture (%), and mineral N (NH₄⁺ N as soil NH₄⁺ and NO₃⁻ N as soil NO₃⁻) in 0–10 cm soil layer. The soil samples were collected in six replications, close to the area of static chambers. The rainfall was monitored by three replications using glass rain gauges.

Soil moisture was measured by the gravimetric method with soil drying at 105 °C for 24 h (Embrapa, 1997). The soil NO₃⁻ content was measured by the Delta-Absorbance method (4500- NO₃⁻- Nitrogen – Nitrate) (APHA, AWWA, WEF, 2006). The soil NH₄⁺ content was determined using the modified green salicylate method (Searle, 1984). Subsequently, the water-filled porous space (WFPS) was calculated by Eq. (3),

$$WFPS = U/(1 - \rho/ps) \times 100 \quad (3)$$

Where: WFPS is the water-filled porous space (%), U is soil moisture (%), ρ is soil bulk density (g cm⁻³), and ps is soil particle density (g cm⁻³). The soil bulk density (ρ) and soil particle density (ps) were measured according to Embrapa (1997), with samples collected in three campaigns throughout the experiment with six replications.

2.4. Statistical analyses

Statistical analyses were performed with software R (R Development Core Team, 2020). The data frequency histogram is presented in Fig. S1. The normal distribution (95%) to N₂O emissions and soil variables were checked using a quantile-quantile plot (Fig. S2, Supplementary material). No variables presented normality. Thus, we carried out descriptive statistics of data for each system in the rainy and dry seasons. The Kruskal-Wallis test ($p < 0.05$) was performed to analyze the variation of N₂O emissions, soil NO₃⁻ content, soil NH₄⁺ content, soil moisture and WFPS. If there was heterogeneity of variances, we performed the Man-Whitney U test ($p < 0.05$) to verify the mean differences between systems in each season. Finally, we investigated the effect of soil-weather variables under N₂O emissions for each system in the rainy and dry seasons using Spearman's correlation coefficient ($p < 0.05$) and linear regression.

3. Results

3.1. Weather variables

The total rainfall in the period studied reached 2057 mm, with 129 precipitation events, 75% of the precipitations concentrated between September 2017 and March 2018 (Fig. S3). The months with fewer rainfall events were April to September 2018 (with a cumulative value lower than 40 mm). Three months were drier, May to July, with only 10 mm of rainfall. In the sampling days, the mean air temperature was around 27.8 °C, with a maximum of 32.4 °C and a minimum of 17.5 °C. Months with the lowest mean temperatures were June and July.

3.2. Soil variables

The dynamics of WFPS followed the variations of rainfall events, mainly in the rainy season, with higher values to the systems (Fig. S3).

WFPS and soil moisture ranged between 25–75% and 20–40%, respectively (Fig. 3, and Table S2). However, the average WFPS values were very dynamic between systems, both in the rainy (September 06th, 2017 to March 08, 2018) and dry seasons (March 12th, 2018 to September 14th, 2018 – Fig. 2). Pasture presented the highest average WFPS in both seasons differing from the other systems (> 55%); however, in the dry season, pasture was similar to ILF (Fig. 4h). Cropland had the lowest average WFPS in both seasons (< 51%). Soil moisture showed no significant variation ($p < 0.05$) between systems (Fig. 4b and g).

Soil NO₃⁻ content showed a wide range of variation between 0 and 93 $\mu\text{g N g}^{-1}$ (Fig. 3, Table S2). Soil NH₄⁺ content ranged between 0 and 29 $\mu\text{g N g}^{-1}$, and values remained below 10 $\mu\text{g N g}^{-1}$ during most of the trial period (Fig. 3, Table S2). Soil NO₃⁻ and NH₄⁺ increased after the application of N sources in cropland and ICF during the dry season. From March to June 2018 (Fig. 3), the soil NO₃⁻ content remained high in cropland and ICF, and reduced near the maize harvest in July 2018.

The average of soil mineral N (NO₃⁻ and NH₄⁺) for cropland and ICF increased from the soybean to the maize harvest, from 15 $\mu\text{g N g}^{-1}$ to 40 $\mu\text{g N g}^{-1}$ in NO₃⁻ and less pronounced in NH₄⁺, from 2 $\mu\text{g N g}^{-1}$ to 5 $\mu\text{g N g}^{-1}$ (Table S2). Pasture, ILF, and eucalyptus showed a reduction from the rainy to the dry season. NH₄⁺ and NO₃⁻ contents were significantly different between pasture and ILF and the other systems in both seasons. During the rainy season (Fig. 4d), the average soil NO₃⁻ content was higher for cropland, ICF, and eucalyptus (> 15 $\mu\text{g N g}^{-1}$) compared to pasture and ILF (< 12 $\mu\text{g N g}^{-1}$). However, the behavior of soil NH₄⁺ (Fig. 4e) was inverse and statistically higher for pasture, and ILF (> 4 $\mu\text{g N g}^{-1}$) compared to the other systems (< 2.4 $\mu\text{g N g}^{-1}$). For the dry season, the soil NO₃⁻ content was statistically higher for ICF than for the other systems, followed by cropland and ILF, which differed between systems (Fig. 4i). Regardless of N fertilization, the soil NH₄⁺ content continued higher in pasture; however, similar to systems with annual crops (Fig. 4j) with the lowest values observed for ILF and eucalyptus, which differed from each other.

3.3. Effects of soil-weather variables in the N₂O emissions

Daily N₂O fluxes ranged from 0.8 to 50 $\mu\text{g N m}^{-2} \text{h}^{-1}$ (Fig. 5), with a variation between 0.94 and 36 $\mu\text{g N m}^{-2} \text{h}^{-1}$ during the rainy season and from 0.8 to 50 $\mu\text{g N m}^{-2} \text{h}^{-1}$ for the dry season (Table S2). The highest daily peak occurred in the dry season, after urea application (Fig. 1, event 4) in ICF and cropland (~ 50 $\mu\text{g N m}^{-2} \text{h}^{-1}$) (Fig. 5).

During the rainy and dry seasons, N₂O fluxes in the systems presented statistical differences (Fig. 4a and f). According to the boxplot, the average N₂O emissions were higher in the rainy than in the dry season. In both seasons, the annual crop systems presented higher N₂O emissions ($p < 0.05$). Nevertheless, emissions were similar to pasture (> 10 $\mu\text{g N m}^{-2} \text{h}^{-1}$) in the rainy season, while N₂O fluxes were equal for cropland, ICF, and eucalyptus (> 3.8 $\mu\text{g N m}^{-2} \text{h}^{-1}$) in the dry season. N₂O emissions of ILF were similar in both seasons (< 4.3 $\mu\text{g N m}^{-2} \text{h}^{-1}$), statistically equal to emissions in eucalyptus in the rainy season and in the pasture in the dry season ($p < 0.05$).

The Spearman's rank correlation showed which soil-weather variables related to the increase in N₂O emissions in both seasons (Table 2). In the rainy season, the increase in N₂O emissions was influenced by the water effect (WFPS, rainfall events, and soil moisture). The rainy season is characterized by high temperatures with frequent and higher rainfall events (Fig. 2), common in subtropical regions. Thus, in this period, N₂O fluxes had the most influence by soil water in cropland, showing a significant rise ($p < 0.05$) with increased rainfall (30%) and soil moisture (33%), more evident in WFPS (N₂O × WFPS 44%). Besides, in the rainy season, N₂O emissions in ICF had a significant and positive influence by increasing soil moisture and WFPS (31% and 40%, respectively) (Table 2). N₂O fluxes in eucalyptus increased (+ 28%) with rainfall events, with a relatively weak but significant correlation.

The dry season has few rainfall events and lower temperatures, but this season showed a strong influence of water on increase N₂O

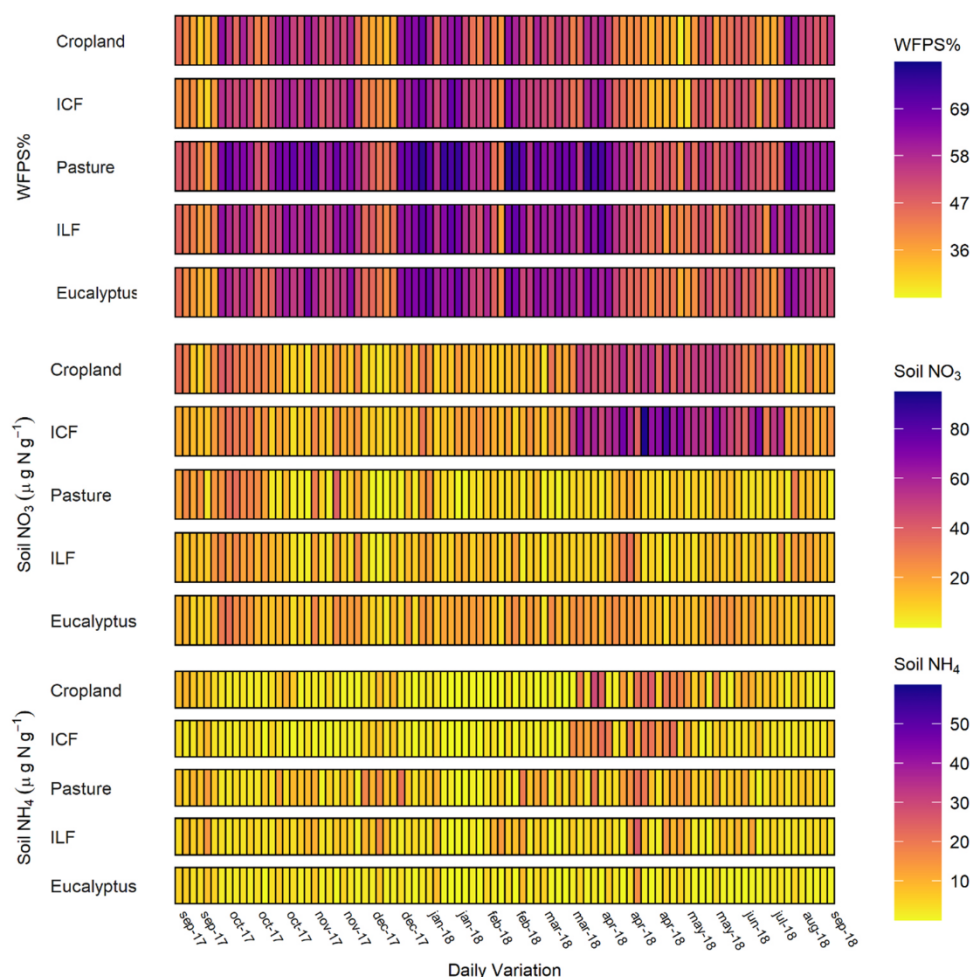


Fig. 3. WFPS, soil NO_3^- and soil NH_4^+ contents under cropland, crop-forest (ICF), pasture, livestock-forest (ILF) and eucalyptus systems during the rainy (September 06, 2017 – March 08, 2018, $n = 53$) and dry (March 12, 2018 – September 14, 2018, $n = 39$) seasons.

emissions in all systems (Fig. S3). Monoculture production systems (cropland, pasture, and eucalyptus) correlated with soil moisture and N_2O fluxes when the soil was wet, with more than 45% compared to integrated systems (ICF and ILF, correlation below 40%). Cropland and pasture had higher N_2O fluxes when pores were filled with water. This WFPS did not significantly influence any of the systems with eucalyptus (ICF, ILF, and eucalyptus) in the dry season. On the other hand, with the rainfall event for the dry season, there was a significant increase in N_2O emissions of 53%, 44%, and 34% in ILF, ICF, cropland and pasture, respectively. None of the systems showed a significant influence of N_2O emissions with mineral N, except for eucalyptus, where N_2O emissions increased in the dry season, probably due to an increase in soil moisture (+ 58%) and soil NO_3^- content (+36%) (Table 2).

The linear regression confirmed the influence of the soil moisture and WFPS on the N_2O fluxes of the different agricultural systems (Fig. 6). While the correlation of the equations ranged from 15% to 41% for the soil moisture (Fig. 6a) and WFPS (Fig. 6b), in general for the rainfall (Fig. 6c), NO_3^- (Fig. 6d) and NH_4^+ (Fig. 6e) the correlation ranged – 7% to 28%. The major part of the correlations were positive and negative correlations only verified between the N_2O fluxes with NO_3^- and NH_4^+ in the under cropland. However, the correlations were low what difficulting to generate robust conclusions about the effects of the interrelation of these soil variables with the N_2O fluxes.

3.4. Cumulative N_2O emissions

Cropland and ICF showed the highest cumulative N_2O emissions in

the rainy and dry seasons (Fig. 7). In the rainy season (Fig. 7a), annual crops had cumulative emissions of $0.50 \text{ kg N ha}^{-1}$, statistically higher than ILF and eucalyptus, but similar to pasture ($0.49 \text{ kg N ha}^{-1}$). In the dry season (Fig. 7b), cropland and ICF showed higher cumulative N_2O ($> 0.30 \text{ kg N ha}^{-1}$), while pasture and ILF presented values below $0.11 \text{ kg N ha}^{-1}$ (statistically less than annual crops). During the monitoring period, 82% and 78% of cumulative N_2O for pasture and ILF were concentrated in the rainy season. The same occurred for eucalyptus, which presented 61% of cumulative N_2O emissions in the rainy season. Although peaks in emissions were observed in cropland and ICF after N input, cumulative N_2O was 60% and 62% in the rainy season. Despite N fertilization, N_2O emissions were considerably higher in the rainy season.

During the rainy season, systems with annual crops (February 26th, 2018, cropland, and ICF) displayed an increase in cumulative N_2O emissions before soybean harvest (0.49 and $0.51 \text{ kg N}_2\text{O ha}^{-1}$, respectively) (Fig. S4). Pasture showed an increase throughout the rainy season, except when soybean senescence occurred in crop systems. However, after soybean harvest and maize sowing, daily cumulative N_2O emissions remained high only for cropland and ICF, decreasing for the other systems with an EF of 0.07%.

4. Discussion

4.1. Linkages among soil-weather variables and N_2O emissions

Temporal N_2O emissions exhibited pronounced seasonal fluctuations

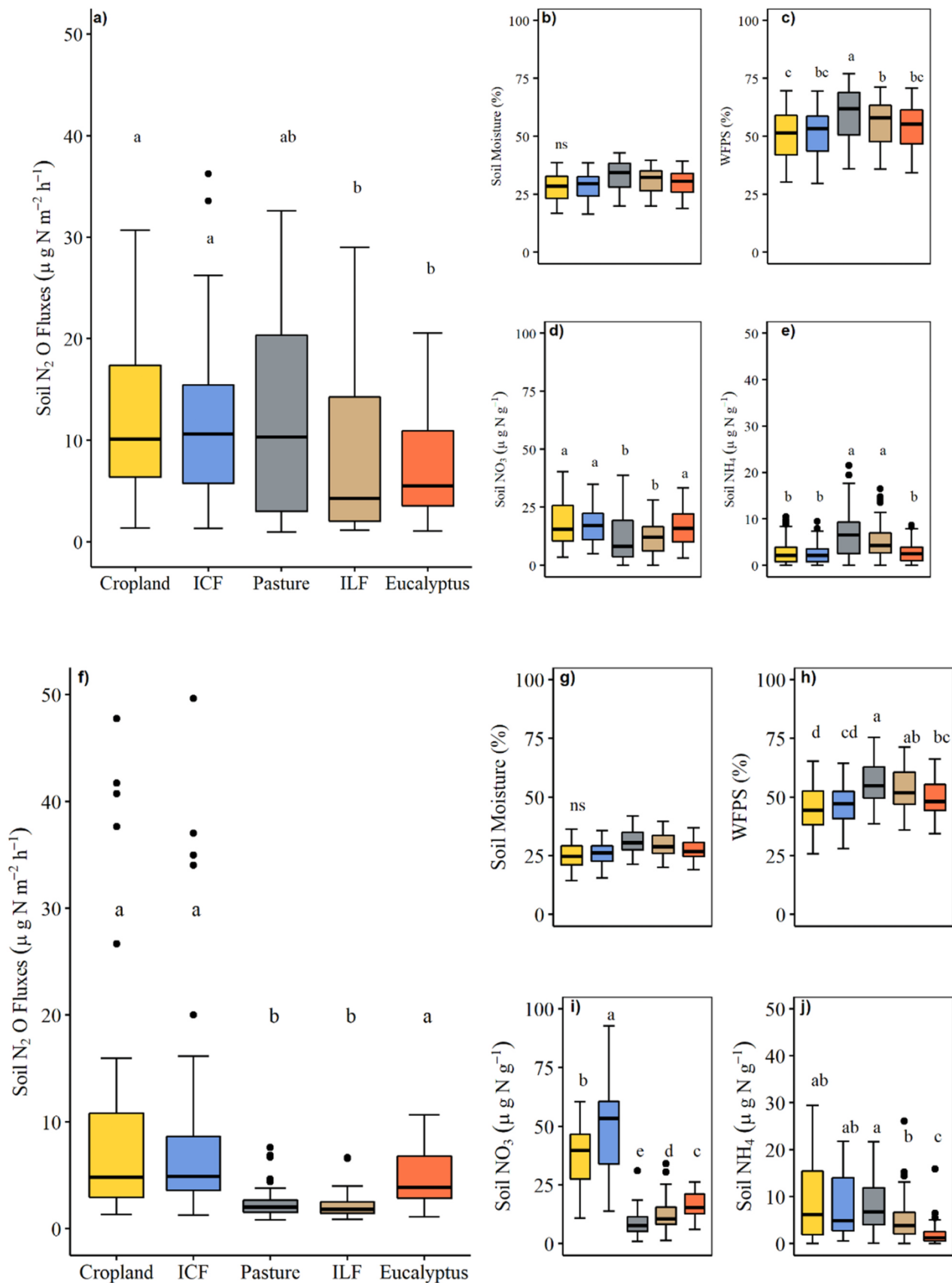


Fig. 4. Effects of cropland, crop-forest (ICF), pasture, livestock-forest (ILF) and eucalyptus systems under soil N₂O fluxes (a, f), soil moisture (b, g), WFPS (c, h), soil NO₃⁻ (d, i) and NH₄⁺ contents (e, j) grouped by the rainy (September 06, 2017 to March 08, 2018, n = 53) and dry (March 12, 2018 to September 14, 2018, n = 39) seasons. Central boxplot lines indicate the average values. The 1st and 3rd quartiles are represented by the limit of boxes, whiskers indicate maximum and minimum values. Different lower-case letters indicate significant differences between systems by the Mann-Whitney *U* test (*p* < 0.05). ns = not significant.

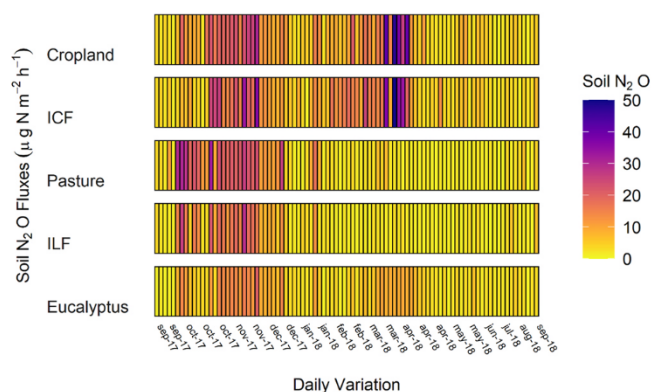


Fig. 5. Soil N_2O fluxes ($\mu\text{g N m}^{-2} \text{h}^{-1}$) from cropland, crop-forest (ICF), pasture, livestock-forest (ILF), and eucalyptus systems grouped by the rainy (September 06, 2017 – March 08, 2018, $n = 53$) and dry (March 12, 2018 – September 14, 2018, $n = 39$) seasons.

Table 2

The Spearman's rank correlation coefficient between N_2O fluxes and weather-soil parameters grouped by the rainy (September 06, 2017 – March 08, 2018, $n = 53$) and dry (March 12, 2018 – September 14, 2018, $n = 39$) seasons.

Soil-Weather Variables	Soil N_2O fluxes ($\mu\text{g N m}^{-2} \text{h}^{-1}$)				
	Rainy season				
	Cropland	ICF	Pasture	ILF	Eucalyptus
Soil Moisture (%)	0.33*	0.31*	-0.03	0.04	0.16
WFPS (%)	0.44*	0.40*	0.22	0.38	0.38
Rainfall (mm^{-1})	0.30*	0.16	0.17	0.21	0.28*
Soil NO_3^- ($\mu\text{g N g}^{-1}$)	-0.12	-0.10	0.16	0.07	0.14
Soil NH_4^+ ($\mu\text{g N g}^{-1}$)	-0.14	-0.26	-0.10	-0.11	-0.11
	Dry season				
Soil Moisture (%)	0.58*	0.39*	0.46*	0.35*	0.58*
WFPS (%)	0.42*	0.10	0.45*	-0.10	0.32
Rainfall (mm^{-1})	0.44*	0.44*	0.34*	0.53*	0.27
Soil NO_3^- ($\mu\text{g N g}^{-1}$)	0.30	0.43	-0.10	-0.13	0.36*
Soil NH_4^+ ($\mu\text{g N g}^{-1}$)	-0.16	0.13	-0.01	-0.26	-0.10

* If p -value < 0.05 is significative by t -test.

between agricultural production systems. The increase in N_2O emissions was related to weather and soil water (Table 2 and Fig. 6). Studies reported that when N substrates are abundant and soil water content is optimal for microbial processes, weather conditions may boost N_2O production (Skiba and Smith, 2000; Liu et al., 2011). This process may be related to the effect of seasonality in subtropical regions, also verified by Lessa et al. (2014).

Effects of soil moisture, WFPS, and rainfall events on N_2O fluxes were significant during the rainy and dry seasons for the cropland systems. Soil-weather variables influenced the pasture during the dry season. ILF and eucalyptus had significant interaction in at least one of the soil-weather variables in N_2O emissions for this season (Table 2 and Fig. 4). Therefore, soil moisture was an essential determinant of N_2O emissions from the soil (Table 2 and Fig. 6), also reported by other studies (Linn and Doran, 1984; Butterbach-Bahl et al., 2013). Soil moisture regulates oxygen availability for soil microorganisms, determines N_2O fluxes, and conditioning diffusivity and its subsequent loss to the atmosphere (Bollmann and Conrad, 1998). In our study, different from the rainy season, the dry season showed a significant effect of soil moisture and rainfall on pasture, ILF, and eucalyptus.

Some authors report WFPS as the primary regulator of N_2O fluxes in the soil (Davidson, 1991; Dobbie and Smith, 2003; and Huang et al., 2014). According to Bouwman (1998), WFPS between 50% and 80% helps increase the denitrification process, boosting soil N_2O production. However, Davidson et al. (2000) observed that depending of the soil type, the optimum level of WFPS for the denitrification process ranges

between 70% and 80%, contributing to N_2O emissions.

Availability of soil surface water in response to the reduction of soil porosity accelerates the denitrification process that rapidly produces N_2O (Sato et al., 2017). Thus, WFPS reduction in cropland could be attributed to soil tillage performed in the sites with annual crops (Fig. 1, event 1). The soil management with soil tillage can influence N_2O dynamics (Signor and Cerri, 2013). Soil tillage could reduce soil micro-porosity and decrease porous water retention (Teixeira et al., 2016), reducing WFPS for the cropland and ILF systems in the rainy season. Despite reduced WFPS, cropland presented WFPS > 50% in 56% of the days, influenced by many rainfall events that resulted in the highest N_2O fluxes in the rainy season (Table 2, Fig. 3), similar to studies of Shelton et al. (2018) conducted in a temperate climate.

In our study, rainfall favored changes in soil moisture and WFPS (Figs. 2 and 3). A significant effect of rainfall events on N_2O emissions was observed in cropland and eucalyptus in the rainy season and during the dry season, which was observed in all systems, except for eucalyptus. According to Cosentino et al. (2013), topsoil temperature is a primary factor, while WFPS is secondary for N_2O emissions in non-tilled soils under different crops. This could be related to lower N_2O emissions observed in the dry season, including autumn and winter (Fig. S3). Bosco et al. (2019) report that temperature influences N_2O emissions in agricultural systems, such as crop rotation in integrated and organic management. Therefore, temperature and WFPS explain more than 95% of the temporal variability of N_2O emissions rates (Cosentino et al., 2013; Butterbach-Bahl et al., 2013).

The daily soil NH_4^+ and NO_3^- contents in the rainy season followed the seasonality of N_2O emissions. However, the NH_4^+ and NO_3^- contents were not related to emissions, except for the eucalyptus system in the dry season. Some authors found no relationship between N pools and N_2O fluxes (Rochette et al., 2004; Gelfand et al., 2016; Casanave Ponti et al., 2020). Eucalyptus, cropland, and ICF displayed higher soil NO_3^- contents; however, eucalyptus presented the lowest N_2O fluxes than annual crops in the rainy season. This occurs because soybean residues decompose rapidly, compared to eucalyptus litter, which has a slower decomposition (Nogueira et al., 2016). According to Rochette et al. (2018), inorganic N cycling in tree systems results from the efficient use of available N, because of their more extended growing season. The pasture systems presented a lower soil NO_3^- content for the rainy season. The reduction of soil NO_3^- content in the pasture systems refers to pasture growth that absorbs N sources and reduces the denitrification process (Piva et al., 2019), favoring the availability of soil NH_4^+ . Thus, grass roots exudate substances that inhibit nitrification bacteria, increasing NO_3^- absorption and reducing transformation of NH_4^+ due to the nitrification inhibiting process (Subbarao et al., 2009).

No significant correlation ($p < 0.05$) was observed between the availability of mineral N (NO_3^- and NH_4^+) and N_2O emissions; however, high emission peaks were observed in cropland and ICF with high mineral N soil content along with adequate WFPS in the dry season after fertilization (360 kg ha^{-1}). Combining these two factors and adequate temperature can trigger N_2O emissions, as proposed in the "hot moments" approach (Baggs et al., 2003; Snyder et al., 2014; Casanave Ponti et al., 2020).

According to Hickman et al. (2014), 60% of total N_2O fluxes occur up three weeks after N fertilization and may last up to two months. In our study, the availability of soil NO_3^- after fertilization with urea remained for four months, disappearing near the maize harvest. The availability of NO_3^- during the dry season resulted from N fertilization. Piva et al. (2019) found an increase in soil NO_3^- during 28 days after fertilization in a pasture area. The eucalyptus system did not receive N fertilization; nevertheless, it presented a significant correlation for NO_3^- and N_2O fluxes. The soil NO_3^- content was higher, while the soil NH_4^+ content presented an increase and remained for a few days in the annual crop systems in the dry season. Millar et al. (2018) showed that the formulation and application methods of fertilizers could influence the immediate production and availability of the soil NH_4^+ content. Casanave

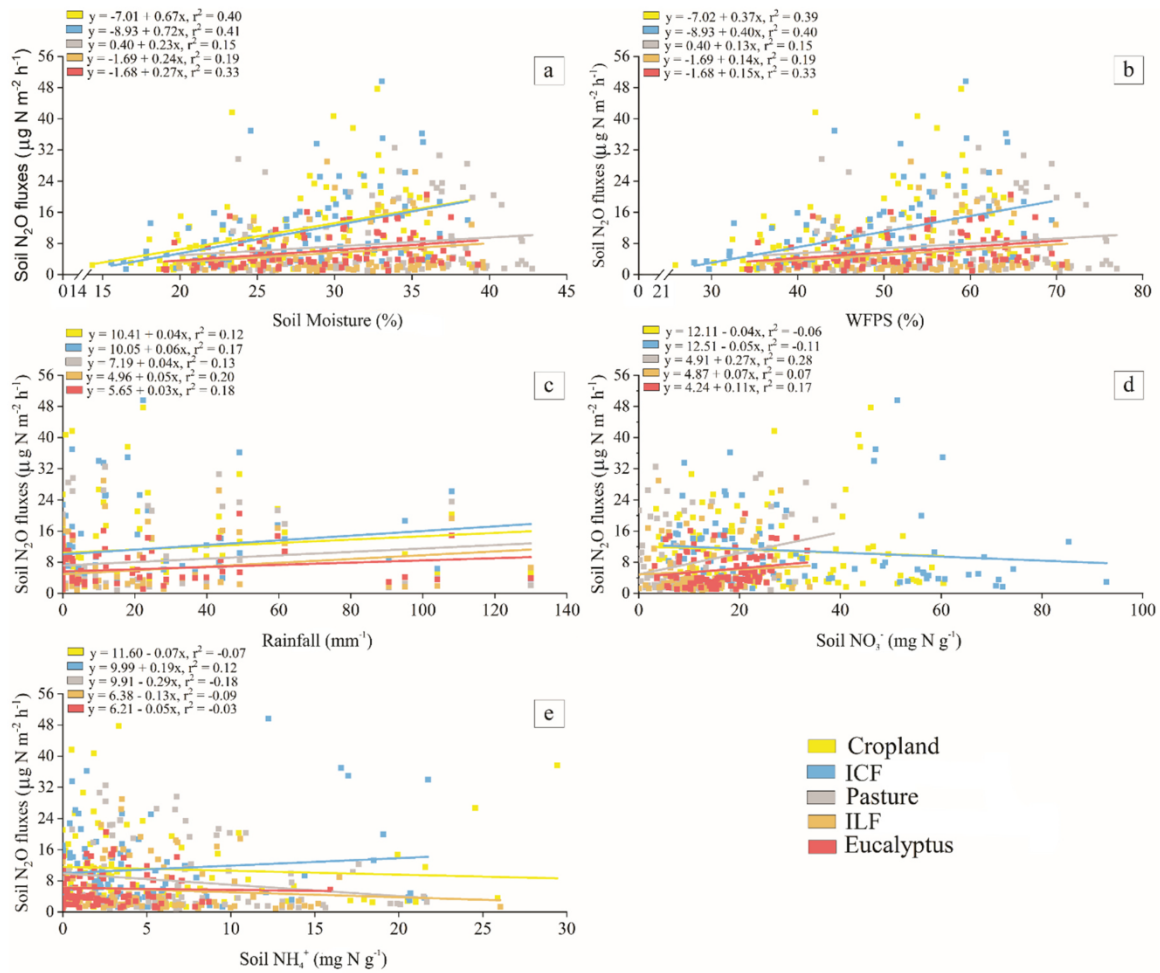


Fig. 6. Linear regression between soil N₂O fluxes (μg N m⁻² h⁻¹) and soil moisture (a), WFPS (b), soil NO₃⁻, soil NH₄⁺ (d), and rainfall (e) for the cropland, crop-forest (ICF), pasture, livestock-forest (ILF), and eucalyptus systems.

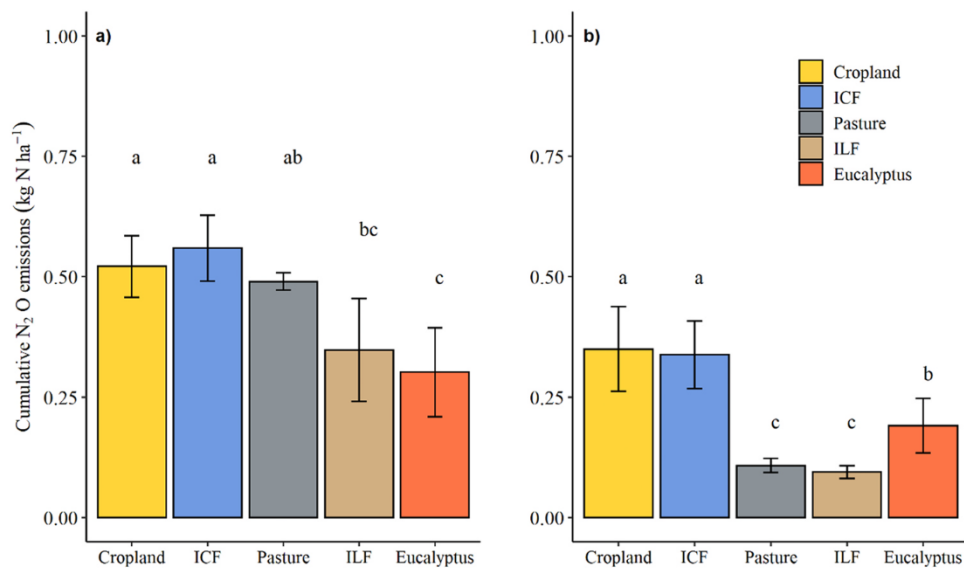


Fig. 7. Cumulative Soil N₂O emissions (kg N ha⁻¹) from cropland, crop-forest (ICF), pasture, livestock-forest (ILF) and eucalyptus systems grouped by a) rainy (September 06, 2017 – March 08, 2018, n = 53) and b) dry (March 12, 2018 – September 14, 2018, n = 39) seasons. Vertical bars represent standard errors (n = 6). Different lower-case letters indicate significant differences between systems by the Mann-Whitney U test (p < 0.05).

Ponti et al. (2020) also observed a slight increase followed by reducing the soil NH_4^+ content, while the soil NO_3^- remained the same due to the nitrification process. According to Casanave Ponti et al. (2020), this phenomenon refers to the input of soybean residues that may increase the mineral N contents. However, in our findings, the effect of fertilization may have masked the increase in soil NO_3^- and NH_4^+ contents due to soybean crop residues.

4.2. Cumulative N_2O emissions for different agricultural systems

Cumulative N_2O emissions were higher in the rainy than in the dry season, despite N application in the soil. During the rainy season, cumulative N_2O emissions displayed significant difference (Fig. 7a). In the dry season, cumulative N_2O emissions also differed statistically between the systems studied. Nogueira et al. (2016) evaluated agricultural integrated and monoculture systems in a tropical region and reduced cumulative N_2O emissions from monoculture to integration areas. Our results in a subtropical region showed that ILF had lower cumulative N_2O in the period with greater water availability; however, the behavior was similar to pasture in the dry period.

In subtropical climate conditions, in the rainy season on similar systems, the average N_2O fluxes were approximately $12 \mu\text{g N m}^{-2} \text{h}^{-1}$ for cropland, ICF, and pasture; $8.23 \mu\text{g N m}^{-2} \text{h}^{-1}$ for ILF, and $7.02 \mu\text{g N m}^{-2} \text{h}^{-1}$ for eucalyptus (Table S2). However, agricultural systems showed similarities in the average N_2O fluxes for the rainy season. In our findings, cropland presented lower emissions than systems under tropical climate conditions in Brazil. For the dry season, Nogueira et al. (2016) found average N_2O emissions of $\sim 1.5 \mu\text{g N m}^{-2} \text{h}^{-1}$ for cropland and ICF, and 4.1 and, $1.1 \mu\text{g N m}^{-2} \text{h}^{-1}$ for eucalyptus and pasture. In our study, cropland and ICF presented average N_2O fluxes around $9 \mu\text{g N m}^{-2} \text{h}^{-1}$, $\sim 2.2 \mu\text{g N m}^{-2} \text{h}^{-1}$ for pasture and ILF, and $4.8 \mu\text{g N m}^{-2} \text{h}^{-1}$ for eucalyptus.

In general, it is important to emphasize that despite our study being related to a one year of evaluation, the present results are important as a first step regarding the inventories of the N_2O emissions in different agricultural systems, mainly due to the introduction of integrated integration agricultural systems. Kim et al. (2016) stated that N_2O emissions for conventional agriculture and agroforestry were not different in tropical regions. In our study under subtropical climate condition, the integrated agricultural systems demonstrated to have potential to reduce the N_2O emissions when compared with the monoculture systems, mainly the ILF system. On the other hand, ICF was influenced by N fertilization to the soil, which ended up limiting its potential to reduce N_2O emissions. In this sense, we highlight that when the scope was to attend to national (e.g., “Low-carbon agriculture”) and international (“4 per 1000”) initiatives for reducing GHG emissions in agriculture, some management techniques as is the case of the use of urea as N source need to be adapted for integrated agricultural systems.

As the N_2O balance in agriculture systems is not completely clarified, the potential of the integrated agricultural systems for mitigating N_2O emissions needs to be carefully analyzed, mainly due to the strong interrelation of the soil C and N cycles. According to Guenet et al. (2021), changes in soil organic carbon (SOC) stocks may override the effects of N_2O reduction, but the GHG mitigation induced by increased SOC storage is generally overestimated if associated N_2O emissions are not considered. More studies regarding the trade-offs of SOC storage and N_2O emissions are still missing for these authors, especially in integrated agricultural systems.

5. Conclusions

This study demonstrated that N_2O emissions in integrated cropland, pasture, and eucalyptus production are similar to or lower than in non-integrated systems but never higher. The increase of daily N_2O fluxes was related to soil water. N_2O emissions were linked to higher water availability for the rainy season due to precipitation, soil moisture, and

WFPS. However, in the dry season, N_2O emissions were considerably reduced, showing that the use of N sources and soil water occurred in the systems. Even with urea application in annual crops, N_2O emissions in the dry season remained low, demonstrating that soil-weather variables greatly influence daily N_2O emissions at different scales, mainly in crop systems. Integrated cropping systems show potential for diversification of agricultural production in a small area and for reduced N_2O losses to the atmosphere.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2021.107684.

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