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Nitrous oxide (N_2O) emissions in Savanah agrosystems

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Abstract

In Brazil, 87% of N_2O released into the atmosphere comes from agriculture, emphasizing the importance of assessing emissions in agricultural systems. The aim of this study was to evaluate N_2O fluxes and emissions in agroecosystems and to identify how physical and chemical attributes of soil may affect the emissions. The study was carried out in the northeastern savannah (Cerrado), in an area under current agricultural expansion, in the municipality of Bom Jesus, State of Piauí. The treatments were composed of grain cultivation systems under no-tillage: exclusive soybean with biological nitrogen fixation (FBN), exclusive corn and corn intercropped with brachiaria. An adjacent area under native Cerrado was evaluated as reference ecosystem. N_2O fluxes were monitored using manual static chambers between February 18 and April 22, 2017, covering the period from planting until the beginning of the harvest. Corn cultivation systems presented the highest N_2O fluxes and the highest total emissions. Nitrogen fertilization significantly contributed to soil N_2O fluxes as opposed to FBN. The soybean system and the native Cerrado had the lowest N_2O emissions. Substantial amounts of N_2O may be emitted during plant residue decomposition, however, it was not evaluated in this study. The concentrations of NH_4^+ and NO_3^-available in the soil were different among the cropping systems, presenting a positive correlation with N_2O fluxes.

Keywords: greenhouse gases, nitrogen fertilization, cropping systems, biological N fixation.

Abbreviations: N_2O_ Nitrous oxide; GHG_ greenhouse gases; CO_2_ carbon dioxide; N_ nitrogen; C_N_ carbon and nitrogen ratio; BNF_ biological nitrogen fixation; NO_3^-_ nitrate, NH_4^+_ ammonium; θg_ gravimetric moisture; OC_ organic carbon; WFPS_ water-filled pore space; P_ Phosphorus; K_ potassium; Bd_ Bulk density; TP_ total porosity; Mi_ microporosity; Ma_ macroporosity; AT_ air temperature; ST_ soil temperature; pH_ hydrogen potential; TP_ total porosity; Al^{3+}_ aluminun; Ca^{2+}_ calcium; Cu_ copper; Fe^{3+}_ iron; H+Al_ hydrogen+aluminun; K^+_ potassium; AS_ aluminum saturation; Mg^{2+}_ magnesium; Mn_ manganese; OM_ organic matter; P_ phosphorus; pH_ hydrogen potential; S_ sulfur; SB_ sum of bases; CEC_ cation exchange capacity; BS_ base saturation; Zn^{2+}_ zinc.

Introduction

Brazil is considered a major emitter of greenhouse gases (GHG) by the United Nations Food and Agriculture Organization (FAO, 2014). According to the latest Brazilian climate change inventory (MCTI, 2017), which refers to the period from 1990 to 2015, Brazil emitted 614.7 Gg of N_2O, divided into five sectors. From this total, 7.7% comes from the energy sector, 0.3% from industrial processes, 83.1% from agriculture, 7.7% from land use change and forestry, and 1.3% from waste treatment (domestic effluent). Ninety six (96.1) % of the emissions of the agriculture sector originate from agricultural soils, mainly coming from grazing animals, use of synthetic fertilizers, application of animal waste and vinasse (byproduct of the sugarcane industry), agricultural waste and organic soils (MCTI, 2017). Thus, the management of agricultural soils is crucial to control N_2O emissions.

Cerrado is a neo-tropical savannah covering central Brazil and partially the southeastern and northeastern regions. In the last 20 years, the northeastern Cerrado has shown great expansion in the production of grain crops. The last agricultural frontier in the country called MATOPIBA is situated here, an area of over 73 million hectares between the states of Maranhão, Tocantins, Piauí and Bahia, with significant production of soybean, corn and cotton (Pereira et al., 2018). Therefore, the conversion of natural environments of this biome into agricultural systems may contribute to the increase of N_2O emissions to the atmosphere.

N_2O is emitted in smaller amounts than other GHGs (IPCC, 1995), however, it has a global warming potential 310 (100 years) times higher than CO_2. It has important environmental effects due to its persistence in the atmosphere, high radiative forcing and inefficient management for its mitigation (Gillete et al., 2017). N_2O is emitted through soil nitrification and denitrification processes (Cardenas et al., 2019), where soil moisture and microbial processes are key factors (Wu et al., 2017). The main factors that can influence N_2O fluxes are edaphoclimatic (porous soil space filled with water and mineral N), anthropic interference (especially physical soil disruption) and nitrogen fertilization (Corrêa et al., 2016). On average, 1% N applied as fertilizer is lost as N_2O (IPCC, 2007). However, this value has been observed with variations generally related to the management of the agricultural system and the soil itself. Campanha et al. (2019) observed that there are higher N_2O emissions in soils under conventional tillage than in no-tillage systems.

Thus, studies detailing the contribution of the main cultivation systems in grain producing regions such as the northeastern Cerrado are important to assess the emission potential of the main agroecosystems in the region and to encourage practices that promote the reduction of N_2O emissions. Such information is also relevant to feed national GHG emissions reports and mathematical models with predicted climate change scenarios.

Therefore, the aim of this study was to evaluate the dynamics of N_2O fluxes in grain production systems in the northeastern Cerrado and to identify the relationship of these fluxes with the physical and chemical soil attributes.

Results and Discussion

N_2O fluxes

The highest average N_2O fluxes throughout the crop cycle occurred in the exclusive corn and corn intercropped with brachiaria, with mean values of 622.77 and 490.09 μg N-N_2O m^{-2} h^{-1}, respectively (Fig.1). The exclusive soybean presented an average flux of 122.53μg N-N_2O m^{-2} h^{-1}. The native Cerrado, as the reference ecosystem, presented an average flux of 31.02μg N-N_2O m^{-2} h^{-1}, which is lower than in the agricultural systems evaluated in the study.

The high emission values in the agricultural systems with corn is a result of mineral N fertilization. This increase in fluxes is explained by the rapid action of the urease enzyme on urea used as a source of N, which increases available soil ammonium for denitrification under anaerobic conditions (Carvalho et al., 2006). The lower fluxes in soybean can be explained by the supply of N via biological N fixation that completely replaced the mineral N.

In a laboratory study under controlled conditions, lentil and pea inoculation with *Rhizobium* sp. (strain R. l*eguminosarum*, 99A1 or RGP2) did not increase denitrification and N_2O emissions (Zhong et al., 2009). However, the decomposition of the residues of N fixing species, that normally have low C:N ratio, N_2O emissions may increase. Schwenke et al. (2015) observed that N_2Oemissions during legume growth were low. However, during the postharvest period, where crop residues were deposited on the soil, emissions increased. Thus, the hypothesis that the release of N from decomposing legume residues contributes to N_2O emissions rather than the biological N_2 fixation process is reinforced (Jensen et al., 2012; Schwenke et al., 2015).

The emission of N_2O related to the addition of cover crop residues correlated with the C:N ratio of the residues and with the N concentration in the plant tissue (Sant'Anna et al., 2018). Thus, higher emission was observed when the C:N ratio was low and the N levels in the plant tissues were high. On the other hand, the addition of *Arachis pintoi* crop residues resulted in lower N_2O emissions (0.34 kg N_2O-N h^{-1}season^{-1}) compared to organic poultry manure (0.68 kg N_2O-N ha^{-1} season^{-1}) applied between coffee lines (Rose et al., 2019).

In systems with corn, both exclusive and with intercropping, there were peaks of N_2O fluxes between March 4 and 8 due to the application of N fertilizer on March 4, 2017. Initially, the soybean cultivation system presented higher fluxes than 200 μg N-N_2O m^{-2} h^{-1}, followed by a decrease and stabilization of fluxes. The native Cerrado maintained low and constant fluxes throughout the study period.

Increased N_2O emissions after mineral N fertilization, were also observed by Carvalho et al. (2017), in Oxisol, also in the Cerrado region. They observed that 50% of all N_2O emission occurred after the application of N fertilizer.

N_2O fluxes of around 136 μg N-N_2O m^{-2} h^{-1} in native Cerrado were reported by Meurer et al. (2016), while Carvalho et al. (2017) observed consumption (influx) of N_2O (-0.05 kg N ha^{-1}). The authors explained the N_2O influx by the low N (NH_4^+ and NO_3^-) levels and high soil porosity under native Cerrado. High soil porosity is associated with rapid water drainage that creates unfavorable conditions for the production of this gas (Carvalho et al., 2014). Figueiredo et al. (2018) also observed lower N_2O emissions under native Cerrado compared to corn and soybean cultivation under conventional tillage and no-tillage. Certain characteristics of the Cerrado ecosystem, such as high C:N ratio (~ 60) and the predominance of ammonium (NH_4^+) over nitrate (NO_3^-) are factors that contribute to maintaining low levels of N (Figueiredo et al. 2018; Bustamante et al. al., 2012).

Among the factors that most influence N_2O fluxes are the availability of N in inorganic forms (NH_4^+ and NO_3^-), which increases soon after the nitrogen fertilizer application (Rochette et al., 2014), and soil moisture which is controlled by water regime, texture, soil structure (Sotta et al., 2008), and also anthropogenic interference, especially soil revolving (Corrêa et al., 2016).

Denitrification, one of the most important processes for the emission of N_2O from soils is conditioned to anaerobic environment (Signor and Cerri, 2013). Martins et al. (2015) observed, in Oxisols of the Cerrado, emission of N_2O only 36 days after the application of N due to the occurrence of precipitation, which highlights the importance of concomitantly humid conditions to the presence of N for N_2O release. Lessa et al. (2014) had similar observations in a Cerrado Oxisol (530 g kg^{-1} clay) under pasture, where the measurable N_2O fluxes after the deposition of bovine urine in the dry season occurred only at the beginning of the rainy season.

Total N_2O emission

The highest total N_2O emission, calculated for the whole experimental period occurred in the exclusive corn (1.57 kg N-N_2O ha^{-1}) and the lowest in the native Cerrado (0.11 kg N-N_2O ha^{-1}) (Fig.2). In the system with exclusive soybean and corn intercropped with brachiaria, lower emissions were

Table 1. Soil chemical attributes in the N_2O emission monitoring period in agroecosystems in the northeastern Cerrado, Bom Jesus, Piauí State, Brazil.

Systems	NH_4^+	NO_3^-	OC	pH
	--------------mg kg^{-1}	----------------	g kg^{-1}	
Soybean	16.8b	10.4b	1.48b	6.00a
Corn	25.6a	24.1a	1.45b	5.77a
Corn + Brachiaria	23.3a	23.1a	1.45b	5.93a
Native Cerrado	16.8b	10.1b	1.82a	4.63b

Different letters in the same column indicate significant differences between treatments by the SNK test with p<0,05. NH_4^+: Ammonium; NO_3^-: nitrate; OC: organic carbon; pH: hydrogen potential.

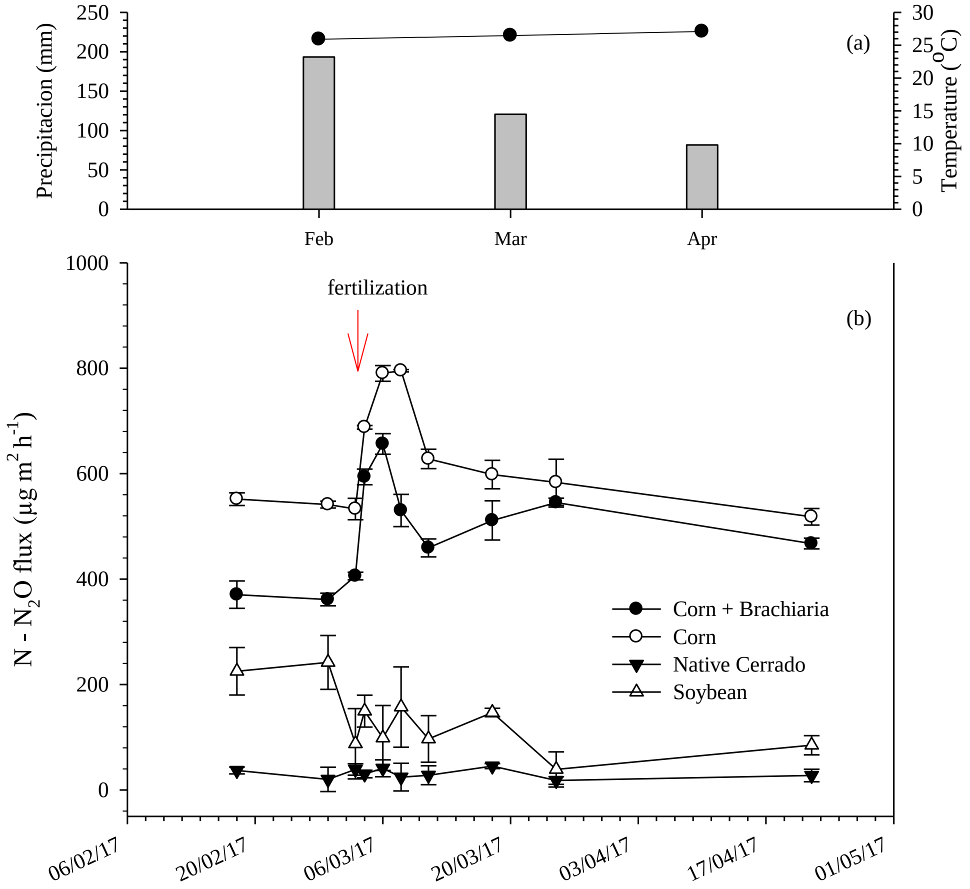

Fig 1. N_2O fluxes (a) and air temperature and precipitation (b) during the growing season of agricultural crops under no-tillage system in the northeast Cerrado, Bom Jesus, Piauí State, Brazil. Red arrows indicate the fertilization event.

Table 2. Physical attributes of the 0-10 cm soil layer and air temperature evaluated during the N_2O emission monitoring period in different agroecosystems in the northeastern Cerrado, Bom Jesus, Piauí State, Brazil.

Systems	WFPS	AT	ST	Bd	TP	Ma	Mi
	%	--------°C-------		kg dm^{-3}	------------g cm^{-3}	------------	
Soybean	70a	24.1b	24,5b	1.54a	0.36c	0.14c	0.24b
Corn	71a	25.4a	23.8c	1.45b	0.37c	0.12d	0.25a
Corn + Brachiaria	69a	25.7a	24.7ab	1.32c	0.38b	0.17a	0.21c
Native Cerrado	57b	26.3a	25.0a	1.54a	0.45a	0.21a	0.24b

Different letters in the column indicate significant differences between treatments by the SNK test with p<0,05. WFPS: soil water-filled pore space; AT: air temperature; ST: soil temperature; Bd: bulk density; TP: total porosity; Ma: macroporosity; Mi: microporosity.

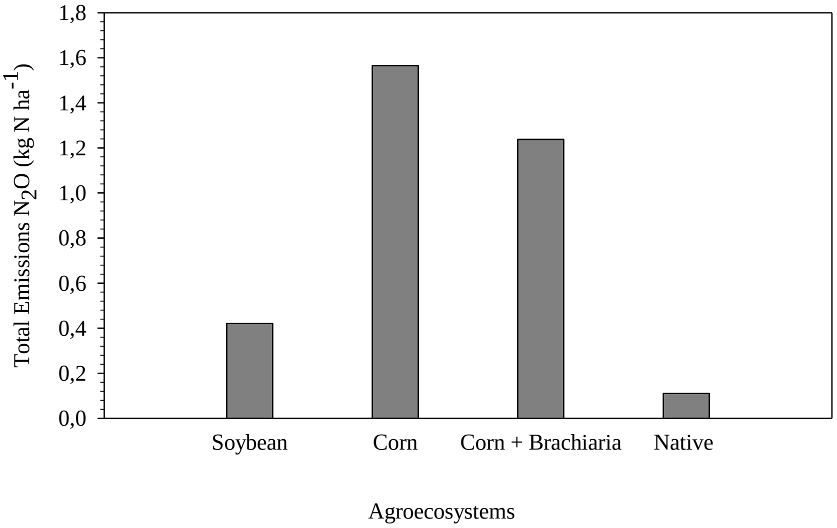

Fig 2. Total N_2O emissions in the cropping systems for the evaluated period (February to May 2017), Bom Jesus, Piauí State, Brazil.

Table 3. Correlation between soil physical and chemical attributes and nitrous oxide (N_2O) in agroecosystems in the northeastern Cerrado, Bom Jesus, Piauí State, Brazil.

	NH_4^+	NO_3^-	OC	WFPS	pH	AT	ST	Bd	TP	Ma	Mi
N_2O	0.854	0.791	-0.516	0.472	0.216	-0.002	-0.168	-0.426	-0.467	-0.573	0.129
	<0.001	<0.001	0.006	0.0286	0.1817	0.9910	0.3001	0.0061	0.0024	0.001	0.4327

OC: organic carbon; Bd: Bulk density; WFPS: soil water-filled pore space; Ma: macroporosity; Mi: microporosity; N_2O: nitrous oxide; NH_4^+: ammonium; $NO3^-$: nitrate; pH: hydrogen potential; TP: total porosity; AT: air temperature; ST: soil temperature;

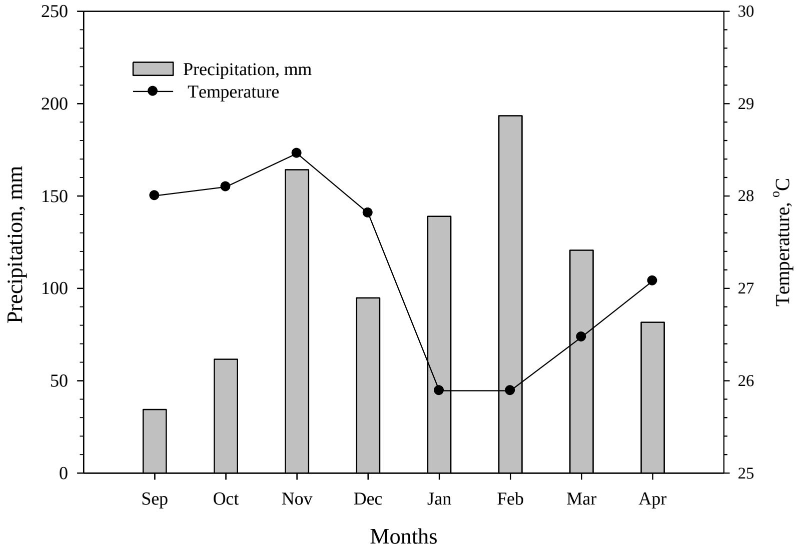

Fig 3. Precipitation and air temperature, in the 2016/2017 growing season, when the experiment was carried out.

Table 4. Soil chemical properties before the implementation of the experiment.

Depth	pH	H+Al	Al^{3+}	Ca^{2+}	Mg^{2+}	K^+	SB	CEC	P	$S\text{-}SO_4^{2-}$
cm	H_2O	--------------------$cmol_cdm^{-3}$-----------------------							----- mg dm^{-3} ----	
0-20	5.5	5.7	0.1	2.0	0.8	0.5	3.2	9.0	56.5	16.5
20-40	5.2	4.2	0.2	1.0	0.3	0.3	1.7	5.8	19.4	17.1
40-60	4.8	3.6	0.2	0.5	0.1	0.2	0.8	4.4	9.8	22.7
--------Micronutrients------------										
Depth	Cu	Fe^{3+}	Mn	Zn^{2+}	BS	AS	OM	Clay	Silt	Sand
cm	-----------mg dm^{-3}-----------				---- % ---		------------- gkg^{-1}-----------			
0-20	0.30	54.41	5.06	5.92	36	3	20	223	5	772
20-40	-	-	-	-	29	10	12	234	20	746
40-60	-	-	-	-	18	20	8	268	6	725

Al^{3+}: aluminun; Ca^{2+}: calcium; Cu: copper; Fe^{3+}: iron; H+Al: hydrogen+aluminun; K^+: potassium; AS: aluminum saturation; Mg^{2+}: magnesium; Mn: manganese; OM: organic matter; P: phosphorus; pH: hydrogen potential; S: sulfur; SB: sum of bases; CEC: cátion exchange capacity; BS: base saturation; Zn^{2+}: zinc.

observed compared to the exclusive corn (0.42 and 1.24 kg $N-N_2O$ ha^{-1}, respectively). The sources of N affect total emissions and flux dynamics observed over the evaluation period, so the lower emission in soybean cultivation is due to the supply of N via BNF, as previously discussed.

Comparing the two systems with corn, the lower total emission occurred in the corn intercropped with brachiaria, likely due to the simultaneous development of brachiaria with corn, resulting in higher N uptake, although no differences were observed between these treatments for the amount of NH_4^+ and NO_3^- in the soil (Table 1). Therefore, further studies on N_2O emissions in intercropped systems are needed.

Relationship of N_2O fluxes and soil attributes

The concentration of soil mineral N was different among the evaluated cropping systems due to the application of mineral N, which promoted an increase in soil N concentrations. Those with corn and corn with brachiaria intercropping presented the highest concentrations of NH_4^+ and NO_3^-, while soybean had similar values to the native Cerrado (Table 1). In the corn systems, NH_4^+ and NO_3^- were present in equilibrated levels, while under soybeans and native Cerrado, there was a predominance of the ammoniacal form over the nitric. This may also contribute to reduce the denitrification rate and N_2O emissions (Fig.1 and 2).

Organic carbon (OC) and soil pH, there was no difference among agricultural systems. However, they differed from the native Cerrado (Table 1) which presented higher values of the OC due to the large amount of decomposing litter. Regarding pH, Cerrado soils under natural conditions are acid, which limiting to microbial activity.

There was no difference in the water-filled pore space (WFPS) among the agricultural systems, differing from the native Cerrado, which presented the lowest value for this attribute (Table 2). Soil WFPS above 60% hinders O_2 diffusion by favoring the formation of anaerobic microsites, which are favorable to denitrification (Bateman and Baggs, 2005).This explains the positive and significant correlation between N_2O and WFPS (Table 3).

The moisture retained in micropores, when in greater proportion, increases WFPS, generating reducing conditions and causing increased N_2O emissions from soils (Drury et al., 2004). The physical attributes of the soil showed variability due to the differences in soil management and the cultivation systems implemented.

According to Haney et al. (2004), soil temperature is one of the factors that most influences N_2O production in soil, as it directly affects the activity of microorganisms, interfering with the mineralization of organic matter. However, in the present work, soil temperature had correlation of very low significance with N_2O (Table 3).This is probably because the temperature range observed in this study (Table 2) did not limit the activity of soil microorganisms.

On the other hand, NO_3^-, NH_4^+ and WFPS, had significant and positive correlation with N_2O (Table 3), indicating their direct influence on N_2O emissions. Significant positive correlation of NO_3^- and NH_4^+ with N_2O for a Cerrado Oxisol conducted in integrated systems using N fertilization was also observed by Carvalho et al. (2017). In different corn systems in the Cerrado, the variables that most contributed to N_2O emissions in decreasing order of contribution were: NO_3^-, NH_4^+, WFPS and soil temperature (Campanha et al., 2019).

There was a negative inverse correlation between OC with N_2O fluxes (Table 3). This negative correlation may be associated with the quality of the organic matter of the native Cerrado, which, due to the non-disturbance of the superficial soil layer, allows the accumulation of a light fraction of organic matter with low decomposition rate (Figueiredo et al., 2018).

Materials and Methods

Experiment location

The study was carried out in Bom Jesus, Piauí, (in Serra do Quilombo, location: 9º16’20”S and 44º44’56”O), Northeast Brazil. The average altitude of the experimental area is 610 m and the slope is 0.2%. The climate of the region is characterized as warm and dry tropical with well-defined dry season (Aw according to Köppen’s classification). The average annual rainfall is 1,000 mm distributed from November to March and the annual average temperature is 26ºC. Natural vegetation is characterized as remnant of sub-deciduous Cerrado, and with variations in the physiognomy in the landscape. Much of the natural vegetation has been replaced by large areas of agricultural crops, especially soybean (Pragana et al., 2012). The monthly average rainfall and air temperature in the 2016/2017 growing season is shown in Fig. 3.

In 1995, natural vegetation (Cerrado) was cleared, and rainfed rice (*Oryza sativa*) was cultivated. Rice was followed by soybean (*Glycine max*) and corn (*Zea mays*) under conventional tillage, with plowing and harrowing. In the year prior to the study (2015/2016) the area was cultivated with soybean, and after harvesting, millet (*Pennisetum glaucum*) was sown, and later cut with a mechanical crusher prior to flowering and left on the soil as straw.

The soil of the study is a typical Oxisol (Soil Survey Staff, 2014) with 200 g kg^{-1} clay. The chemical properties before the implementation of the experiment are shown in Table 4.

Experimental design and agricultural management

The experiment was carried out from December 2016 to April 2017. The treatments consisted of three agricultural crops conducted under no-tillage system with millet as cover crop and green manure, and the reference system was the natural Cerrado ecosystem. The experimental areas presented approximately 0.5 ha of the systems: exclusive soybean, exclusive corn, intercropping of corn and brachiaria and natural Cerrado, in a completely randomized experimental design, with four repetitions. In each repetition was allocated one chamber for N_2O fluxes measure.

Agricultural crops were sown on December 13, 2016 in the remaining millet straw. M8808IPRO was used as the soybean variety, with 0.5 m spacing between plants, and a population of 200,000 plants ha^{-1}.The corn variety was Syn 422 VIP3, with row spacing 0.45 m. In the corn + brachiaria intercropping, corn was sown in the same way as in the exclusive corn treatment, and 20 days after the emergence of corn, brachiaria (*Urochloa ruziziensis*) was sown between the corn rows at a density of 8 kg ha^{-1}.

In the exclusive corn and corn intercropped with brachiaria systems, 100 kg N ha^{-1} (urea) was applied on March 4, 2017. To measure N_2O fluxes, the N was applied separately inside the static chambers in a proportional amount to that applied to the whole plot. The applied amount of N was based on the recommendation by Sousa and Lobato (2004) for corn in Cerrado soils. Phosphorus (P) and K was added according to initial soil analysis (described in Table 1), also based on Sousa and Lobato (2004). In the exclusive soybean system the seeds were inoculated with *Bradyrhizobium japonicum* for biological N fixation, therefore no mineral N was applied.

Monitoring and mesuring N_2O fluxes

N_2O fluxes were monitored using static chambers (Venterea, 2010). Four chambers were allocated in each treatment equidistant at 20 meters, in a plot of 0.5 ha. The chambers were base-lid type, made of galvanized steel, with dimensions 40 x 60 x 15 cm (width, length and height, respectively). The chamber base was inserted into the ground at a depth of 0.10 m and leveled to allow water to seal in the contact rails between the base and the chamber cover during gas collection. The monitoring of the fluxes took place from 18/02/2017 to 04/22/2017, covering from the period of establishment of the crop until the beginning of the harvest in all agricultural systems. Throughout the evaluation period, gas collections were performed every seven days, except in the post-fertilization period, where it was collected every two days until the seventh day.

Gas samples were taken in the morning between 7 and 9 AM, when the fluxes represented average daily fluxes, which was similar to the period determined by Alves et al. (2012). These measurements were used to estimate daily average fluxes. Samples were collected at 0, 15 and 30 minutes after the closure of each chamber. Simultaneously to gas collection, the air temperature inside the chamber and the temperature of the soil at 0.10 m depth were measured.

Samples were collected in syringes and transferred to vials sealed with butyl septum that were previously subjected to vacuum (-70hPa), performed in the field right before sampling with a hand pump.

N_2O concentration in the samples was determined by gas chromatography (Agilent, model 7890A). The chromatograph was equipped with a packed column containing Porapak Q operating at 60°C and a 63Ni electron capture detector (ECD) at 300°C. For chromatograph calibration, N_2O standards at concentrations of 350 and 1,000 ppbv were used. N_2O concentrations were calculated by linear regressions.

The N_2O flux in each chamber (F, $\mu L\ L^{-1}\ h^{-1}\ N_2O$) was calculated using the function suggested by Hutchinson and Mosier (1981) [HM function] as follows:

$$F = (C1 - C0)^2/[t \text{ x } (2 \text{ x } C1 - C2 - C0)] \text{x } \ln[(C1 - C0)/(C2 - C1)]$$

Where: F is the flux of N_2O (μL gas $L^{-1}\ h^{-1}$); C0, C1, and C2 are the gas concentrations (ppbv) sampled at 0, 15 and 30 minutes respectively; and t is the interval between those samplings (t = 15 min).

The calculated fluxes were then converted to units of mass using the universal gas law equation. The average of all chambers per treatment was considered as the mean hourly flux of treatment, expressed as μg N-N_2O $m^{-2}\ h^{-1}$ and daily fluxes were obtained by multiplying it by 24. To calculate the total emissions for the evaluation period, it was necessary to estimate the fluxes for the unmeasured days, which was done by linear interpolation.

Soil sampling and analysis

Soil samples for chemical analysis were collected on all gas-sampling days (a composite sample consisting of three sub-samples in the outer area of each chamber). Additionally, intact soil cores were collected at the fifth gas-sampling day, with three samples per chamber, collected with a cylinder of 100 cm^3.

The soil samples for chemical analysis were stored at -4ºC for further analysis, to determine nitrate (NO_3^-), ammonium (NH_4^+), gravimetric moisture (θg), soil pH (in H_2O) and organic carbon (OC). The levels of NO_3^- and NH_4^+ were determined by the Kjeldahl method (Embrapa, 1997). The soil NO_3^- and NH_4^+ concentrations were padronized for dry soil weight. Soil pH was determined in water, with solid: liquid ratio of 1: 2.5 (Embrapa, 2017). The OC was determined by the Walkley-Black method, according to Embrapa (1997).

Bulk density (Bd), total porosity (TP), microporosity (Mi) and macroporosity (Ma) were determined using the intact soil cores. Through the relationship between θg and Ds, we estimated the water-filled pore space (WFPS) as described by Paul and Clark (1996). The ratio of Ma and Mi was determined using the Richard's chambers (Embrapa, 2017).

Statistical analysis

The physical and chemical attributes of the soil were subjected to analysis of variance and SNK means test ($p < 0.05$). Correlations were calculated by Pearson's bivariate correlation.

Conclusions

The agricultural systems with corn and corn intercropped with brachiaria presented higher N_2O emissions, in this order, due to the application of mineral N fertilizer (urea). The main factors controlling N_2O emissions were the availability of mineral N and the predominance of anaerobic conditions (WFPS). Biological nitrogen fixation contributes to the reduction of N_2O emissions by substitution of mineral N.

Further studies evaluating the effect of cover crops on the dynamics of N_2O emissions in intercropped systems and the use of N-fixing plants are necessary.

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