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Research Article

CH_4 and N_2O fluxes from planted forests and native Cerrado ecosystems in Brazil

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ABSTRACT: Forest soils are N_2O sources and commonly act as CH_4 sinks. This study evaluated the dynamics of the CH_4 and N_2O fluxes of soils under *Eucalyptus* plantations and native Cerrado vegetation, as well as possible interactions between environmental factors and fluxes. The study was carried out in the Distrito Federal, Brazil, during 26 months, in three areas: in two stands of the hybrid *Eucalyptus urophylla* × *Eucalyptus grandis*, planted in 2011 (E1), and in 2009 (E2) and native Cerrado vegetation (CE). Measurements to determine the fluxes in a closed static chamber were carried out from Oct 2013 to Nov 2015. Soil and climate factors were monitored. During the study period, the mean CH_4 fluxes were –22.48, –8.38 and –1.31 μg CH_4 m^{-2} h^{-1} and the mean N_2O fluxes 5.45, 4.85 and 3.85 μg N_2O m^{-2} h^{-1} from E1, E2 and CE, respectively. Seasonality affected plantations in the studied sites. Cumulative CH_4 influxes were calculated (year-1: –1.86 to -0.63 kg ha^{-1} yr^{-1}; year-2: –1.85 to –1.34 kg ha^{-1} yr^{-1}). Cumulative N_2O fluxes in the three sites were ≤ 0.85 kg ha^{-1} yr^{-1}. The change in land use from Cerrado to *Eucalyptus* plantations did not significantly changed regarding greenhouse gases (GHG), compared to the native vegetation. Flux rates of both gases (N_2O and CH_4) were low. Temporal variations in GHG fluxes and different ages of the stands did not cause significant differences in cumulative annual fluxes.

Keywords: *Eucalyptus*, greenhouse gases, forest stand age, savanna

Introduction

Changes in land use have led tropical soils to estimated emissions of 0.2 Gt C yr^{-1}, accounting for 10-30 % of total C emissions from deforestation (Houghton, 1999; Achard et al., 2004). In 2010, GHG emissions were estimated at 49 × 10^9 Mg CO_2eq, of which 21 to 24 % were generated by agriculture, forestry, and other land uses (Tubiello et al., 2013; IPCC, 2014). In the context of a low-carbon economy, forest stands can be an option to reduce pressure on the native vegetation and mitigate the effects of climate change (Bonan, 2008). In forest stands, *Eucalyptus* is the second most commonly planted genus, due to its high adaptability, fast growth, and economic value (Iglesias-Trabado et al., 2009). Moreover, nutrients released in forest litter may enhance stabilization mechanisms of soil organic carbon (SOC) (Huang et al., 2011).

Land-use changes alter chemical, physical, and biological properties of the soil, modifying the GHG fluxes (Kim and Kirschbaum, 2015). In addition, forest type (Masaka et al., 2014) and soil management practices play an important role (Kim et al., 2016), as well as water-filled pore space (Santos et al., 2016; Smith, 2017), soil O_2 contents, pH, plants, and N input (Hickman et al., 2015; Carvalho et al., 2017). Moreover, long-term N inputs may favor soil C stocks, mainly in litter with a high lignin content (Grandy and Neff, 2008). Microbial communities can be altered by climate warming, N fertilization, pH and the C:N ratio (Högberg et al., 2007; Lucas et al., 2007). In particular, metabolic responses of the microbial community to N enrichment are complex and highly variable (Michel and Matzner, 2003).

Native forests and commercial *Eucalyptus* stands of different ages have been little studied about the potential emission or consumption of GHG, but they are main drives for GHG fluxes in the Cerrado region. This study investigated GHG emissions with a two-year GHG monitoring program in natural and planted forests. The objectives were: (a) evaluate CH_4 and N_2O fluxes of soils under planted *Eucalyptus* forests of different ages (established in 2009 and 2011) and managements, and under native Cerrado vegetation, (b) describe fluxes and their interactions with seasonality and environmental variables, and (c) determine cumulative N_2O and CH_4 fluxes in soils of *Eucalyptus* forests of different ages and in native Cerrado vegetation.

Materials and Methods

Description of the study site

The three studied sites were located in the central region of Brazil (Central Plateau) in Paranoá, Distrito Federal (Figure 1). The native forest consisted of an area of 3.5 ha of native Cerrado vegetation (CE) (Latitude 15°53′45.51″ S, Longitude 47° 38′40.69″ W; Altitude 930 m a.s.l.) and belonged to the phytophysiognomic formation "cerradão", a woodland savanna with short semideciduous forest, 10 to 15 m tall, of medium density (FAO, 2001).

The planted forest consisted of, respectively, 12 and 19 ha, each with a *Eucalyptus urophylla* × *Eucalyptus grandis* hybrid stand: E1- clone EAC 1528 (Latitude 15°53′06.44″ S, Longitude 47°39′37.10″ W; 948 m a.s.l.) and E2 - clone GG100 (Latitude 15°53′48.24″ S, Longitude 47°38′37.22″ W; 946 m a.s.l.).

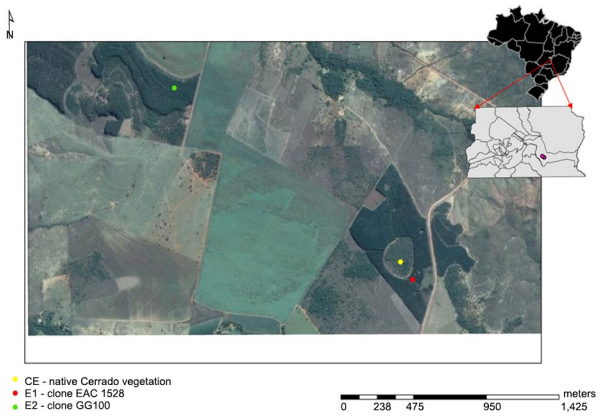

Figure 1 – Location of the study site, rural center Quebrada dos Neres, Paranoá, Distrito Federal, Brazil. Source: IBGE (2009).

Tree seedlings were planted in E1 in Dec 2011, at 3.5 m × 1.7 m spacing in a site previously covered with native Cerrado vegetation. Soil tillage consisted of disking and subsoiling in the planting row to a depth of 1.2 m.

The tree seedlings in E2 were planted in Dec 2009 (3.5 m × 1.7 m). The site had previously been used for cropping soybean (2003 to 2005), sorghum (2006), and soybean again (2007-2009). Soil tillage consisted of heavy disk harrowing (width 1.5 m) and opening furrows in the center of the row to a depth of 25 cm.

In the *Eucalyptus* plantations (E1 and E2), soil acidity was corrected by incorporating dolomitic limestone (2.5 t ha^{-1}) to a depth of 20 cm and with 700 kg ha^{-1} agricultural gypsum applied on the soil surface two months later. Fertilization at planting consisted of NPK (5-25-15), corresponding to 16.52 kg N ha^{-1}. After one year, a side dressing of 60 kg K_2O ha^{-1} was applied in the form of potassium chloride, 50 kg N ha^{-1} in the form of urea, and 1 g boron per plant in the form of borax. In Jan 2014, the application of 60 kg K_2O ha^{-1} was repeated.

The soil of the experimental site was classified as clayey Oxisol (Typic Haplustox) (Soil Survey Staff, 2006). The soil chemical properties and density (0-5 and 5-10 cm depth) are listed in Table 1. Table 2 shows the litter quality in E1, E2 and CE. The C:N ratio in the litter ranged from 66:1 to 75:1 and the lignin content, from 99.5 to 162.0

The regional climate is Aw (rainy tropical), according to Köppen-Geiger classification (Cardoso et al., 2014), with two well-defined seasons: dry season (May–Sept) and rainy season (Oct–Apr). The mean annual precipitation observed in the last 40 years was 1345.8 mm (Silva et al., 2017b). The precipitation in year-1 and year-2 was 1210.9 and 1305.6 mm, respectively, and > 94 % of the rain was concentrated in the rainy season. The average air temperature was 21.4 and 22.2 °C in year 1 and 2, respectively (Figure 2).

Table 1 – Soil chemical properties in two layers (0-5 and 5-10 cm) in *Eucalyptus* stands (E1, clone EAC 1528 planted in 2011 and E2, clone GG100 planted in 2009); and CE (native Cerrado vegetation), Distrito Federal, Brazil.

Variables		E1	E2	CE	E1	E2	CE
Property	Unit of measurement	Soil depth 0-5 cm			Soil depth 5-10 cm		
Organic matter	dag kg^{-1}	3.3	3.0	3.8	2.9	2.6	3.2
pH	(H_2O)	5.0	5.4	5.1	4.9	5.3	5.1
P*	mg dm^{-3}	1.5	5.3	1.8	1.4	3.5	1.2
H+Al	mg dm^{-3}	8.2	7.0	9.5	8.3	7.1	8.6
SB	mg dm^{-3}	1.8	4.3	1.3	1.6	2.6	0.8
CEC	mg dm^{-3}	10.0	11.3	10.8	9.8	9.7	9.5
V	%	17.7	38.0	11.8	15.8	26.7	8.7
B	mg kg^{-1}	0.5	1.1	0.5	0.5	0.9	0.5
Zn	mg L^{-1}	0.3	0.6	0.6	0.3	1.3	0.4
Soil density	g cm^{-3}	0.98	0.96	0.97	0.99	0.96	0.98

P* = phosphor Mehlich-1; H+Al = potential acidity; SB = sum of bases; CEC = cationic exchange capacity; V = base saturation; B = boron; Zn = zinc.

Table 2 – Litter quality in *Eucalyptus* stands (E1, clone EAC 1528 planted in 2011 and E2, clone GG100 planted in 2009); and CE (native Cerrado vegetation), Distrito Federal, Brazil.

Area	Ls	K	C	N	L	C:N
	Mg ha^{-1}	g kg^{-1}				
E1	10.8	0.0012	463.4	6.15	99.5	75:1
E2	13.7	0.0009	465.7	7.04	114.6	66:1
CE	7.6	0.0021	460.0	6.53	162.0	70:1

Ls = litter stock; K = decomposition constant; C = carbon; N = nitrogen L = lignin.

Measurements of CH_4 and N_2O fluxes

The CH_4 and N_2O fluxes were measured from Oct 2013 to Nov 2015, distinguished in year-1 (Oct 2013 to Sept 2014) and year-2 (Oct 2014 to Nov 2015). The years were analyzed separately to identify the behavior of GHG fluxes and contributions of the soil-climate variables.

The closed-chamber method was used for measurements (Alves et al., 2012), with a sampling frequency of three times per month (Zanatta et al., 2014), since the sites with fully established *Eucalyptus* trees were not fertilized with synthetic N. Three 30 m × 30 m plots were randomly delimited in each site. To ensure representativeness of the system, four closed chambers were installed per plot, two in the *Eucalyptus* rows and two in-between rows, spaced 10 m. The chambers in the rows did not receive N fertilization after the first experimental year. In CE, four static gas chambers were placed randomly in each plot, resulting in 36 chambers installed in the sites. Each closed chamber consisted of a metal base (0.38 m × 0.58 m) inserted into the soil, and an upper part of PVC (height 9.5 cm), coated with a thermal aluminum blanket, which, together with the metal base, sealed the space covered by the chamber, where the gases accumulated for later collection and determination.

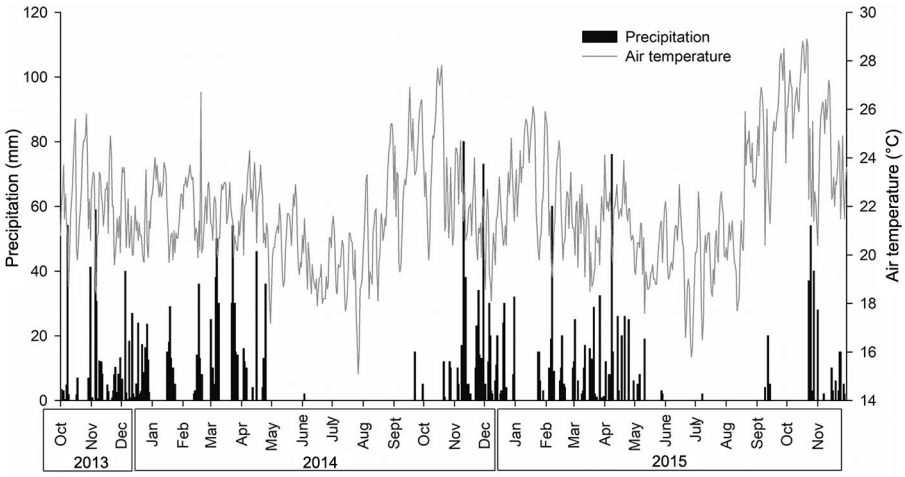

Figure 2 – Precipitation (mm) and air temperature (ºC) from Oct 2013 to Nov 2015, Paranoá, Distrito Federal, Brazil.

A hole was drilled in the center of the top part of each chamber and connected to a rubber hose and a three-way valve, by which the gas outlet could be controlled at sampling. Digital thermometers were installed to monitor the air temperature within the chambers.

The soil temperature was measured at 5 cm deep with a digital thermometer at the sampling times. The gas samples were captured between 09h00 a.m. and 11h00 a.m., following the recommendation of Alves et al. (2012), to best represent the daily average flux. The air trapped in the chambers was sampled at 0, 15, and 30 min after closing the device. A 60-mL polypropylene syringe was used, coupled with a three-way valve, in which 30 mL gas samples were collected and transferred to vials. In addition, one sample of atmospheric gas per plot was taken as reference to analyze gas samples. Before and after sampling, the vials were transported in ice-cooled thermal boxes and stored in a refrigerated environment at 16 °C for measurements.

The CH_4 and N_2O concentrations were determined with a gas chromatograph (Trace 1310 GC ultra) equipped with a Porapak Q column at 65 °C, an electron capture detector (ECD) and a flame ionization detector (FID). The following standards were used: 200, 600, 1000 and 1500 ppb N_2O; and 1000, 5000, 10000 and 50000 ppb CH_4. The calculated detection limit was 51 ppb for N_2O and 145 ppb for CH_4 and the calculated quantification limit 154 ppb for N_2O and 484 ppb for CH_4. The CH_4 and N_2O fluxes were measured by the linear variation in gas concentration in relation to the incubation time in closed chambers, and calculated by Equation (1), as proposed by Bayer et al. (2015):

$$Flux = \delta C/\delta t\ (V/A)\ m/Vm \quad (1)$$

where in the flux (μg m^{-2} h^{-1}); δC/δt is the change in gas concentration (nmol N_2O and CH_4 h^{-1}) in the chamber in the incubation interval (*t*); *V* and *A* are, respectively, the chamber volume (m^3) and the soil site covered by the chamber (m^2); *m* is the molecular weight of N_2O or CH_4 (μg), and *Vm* is the molar volume at the sampling temperature.

The fluxes were calculated for the sampling times 0, 15, and 30 min, expressed in μg N_2O m^{-2} h^{-1} and μg CH_4 m^{-2} h^{-1}. The average daily N_2O and CH_4 fluxes were calculated from the average value of the four chambers installed per plot. To determine the cumulative fluxes, the area under the curve was integrated, based on the daily N_2O and CH_4 soil fluxes (Santos et al., 2016). The amount of equivalent carbon (C eq) required to mitigate the cumulative annual fluxes of CH_4 and N_2O was calculated by Equations 2 and 3, respectively.

$$CH_4 = (CAI^* 16/12) * GWP * F \quad (2)$$

where: CAI is the cumulative annual influx (kg CH_4 ha^{-1} yr^{-1}); GWP is the global warming potential of CH_4 (25 kg CO_2, IPCC (2007)); F is the factor 0.273 (used for the conversion from CO_2 to C);

$$N_2O = (CAF^* 44/28) * GWP * F \quad (3)$$

where: CAF is the cumulative annual flux (kg N_2O ha^{-1} yr^{-1}); GWP is the global warming potential of N_2O (298 kg CO_2, IPCC (2007)); F is the factor 0.273(used for conversion from CO_2 to C).

Environmental factors

For each gas sampling, soil samples from the 0-5 cm layer were also collected to determine mineral N in the forms of nitrate (NO_3^-) and ammonium (NH_4^+), at eight points near the chambers, forming a composite sample. From each soil sample, a sub-sample was taken to determine gravimetric soil moisture. For the extraction of NO_3^- and NH_4^+, we used 50 mL of a solution of 2 mol L^{-1} KCl , according to the methodology of Bremner and Mulvaney (1982). The solution was

analyzed by spectrophotometry with a system of flux injection analysis (FIA) (Hambridge, 2007a, b) to determine NO_3^- and NH_4^+ concentrations.

Soil particle density was determined by the ring and volumetric flask methods, respectively (Embrapa, 1997). Soil moisture was calculated by oven-drying a soil sub-sample of known weight at 105 °C for 48 h. From these variables, the water-filled pore space (WFPS in %) was calculated for each gas sampling date and determined by the equation:

$$WFPS = (\text{gravimetric moisture} \times BD) / [1 - (BD/PD)] \times 100 \quad (4)$$

where: the gravimetric moisture is expressed in %, BD is the bulk density (g cm^{-3}) and PD is the particle density (2.65 g cm^{-3}).

The meteorological data were recorded using a datalogger (CR 1000) installed near the study site.

Calculations and statistical analyses

The environmental variables were subjected to the descriptive statistical analysis and applied to the normality test (Shapiro-Wilk), followed by analysis of variance (ANOVA). The daily CH_4 and N_2O fluxes had a non-normal distribution; therefore, the nonparametric Kruskal-Wallis test of medians was performed at 5 % probability to find possible differences between the areas and years studied by comparisons.

The CH_4 influxes and accumulated N_2O fluxes for the sampling dates were calculated by linear interpolation. To compare sites and years, the data of accumulated fluxes and equivalent carbon were subjected to analysis of variance (ANOVA) and the Tukey test ($p < 0.05$).

Another analysis evaluated the seasonality effect and the relationship between CH_4 and N_2O fluxes and the environmental variables by the Pearson's correlation, using the FactoMineR package of program R (version 3.2.2). Only significant correlations are shown in the text.

Results

Temporal variation in CH_4 and N_2O fluxes and environmental variables

Daily and seasonal CH_4 fluxes for year-1 and year-2 in each site are presented in Figure 3A. Year-1 had annual average of CH_4 fluxes of –35, –3 and –2 µg m^{-2} h^{-1}, while year-2 had –22, –8 and –1 µg m^{-2} h^{-1} in E1, E2, and CE, respectively. Regarding the seasonality effect, the average CH_4 fluxes were –32, –12 and 11 µg m^{-2} h^{-1} and –38, 9, and –20 µg m^{-2} h^{-1} for year-1, and in year-2, –20, –5 and –6 µg m^{-2} h^{-1} and –29, –17 and –7 µg m^{-2} h^{-1} in the rainy and dry seasons in E1, E2, and CE, respectively (Figure 3A). In year-1, significant differences were observed between the environments, where the average CH_4 flux in the rainy season was higher in CE than in E1 ($p = 0.0002$) and E2 ($p = 0.0196$). In the dry season, the average CH_4 fluxes were higher in E2 ($p = 0.0191$).

The mean annual N_2O fluxes were 4, 8, and 3 µg m^{-2} h^{-1} for year-1 and 5, 5, and 4 µg m^{-2} h^{-1} for year-2 in E1, E2, and CE, respectively (Figure 3B). Average N_2O fluxes in the rainy season for E1, E2, and CE were, respectively, 1.00, 4.00, and 0.07 µg m^{-2} h^{-1} (year-1) and 7, 4, and 3 µg m^{-2} h^{-1} (year-2), and in the dry season 10,

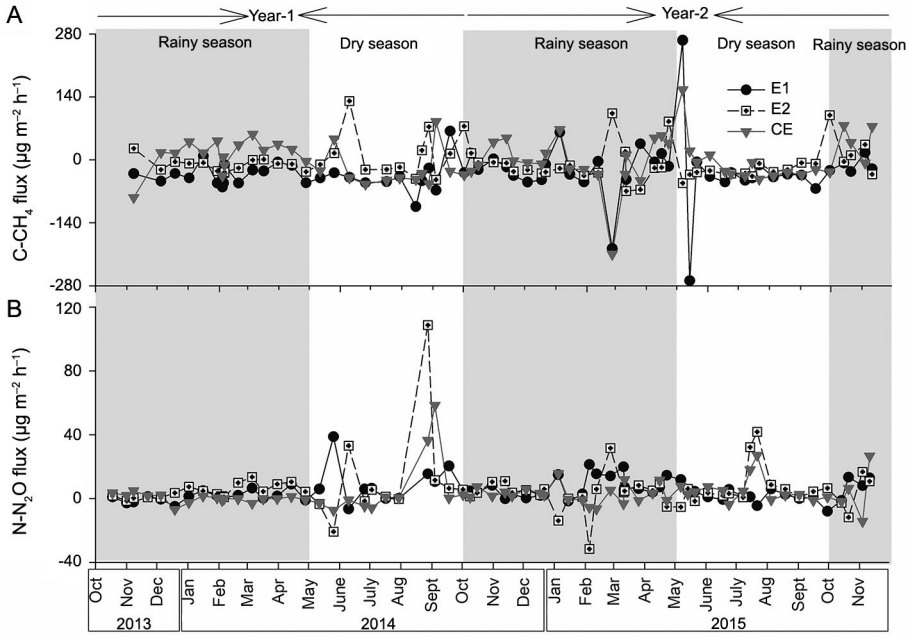

Figure 3 – (A) Soil fluxes of methane (CH_4) and (B) nitrous oxide (N_2O) in the *Eucalyptus* stands E1 (clone EAC 1528, planted in 2011) and E2 (clone GG100, planted in 2009); and in CE (native Cerrado vegetation), Paranoá, Distrito Federal, Brazil.

14, and 7 μg m^{-2} h^{-1} (year-1) and 3, 7, and 5 μg m^{-2} h^{-1} (year-2) (Figure 3B). In year-1, significant differences between N_2O fluxes were observed in the rainy season, with the highest average N_2O fluxes in E2 (p = 0.0196). However, in year-2, differences were only significant in the rainy season between the average fluxes in E1 and CE (p = 0.0273), which were higher for E1.

In all GHG evaluation, an uptake of CH_4 was observed in 44 % of the evaluations in CE, but in 19 % in the *Eucalyptus* stands. For N_2O, the average fluxes were 5, 6, and 4 μg m^{-2} h^{-1} in E1, E2, CE, respectively. In this study, the results of CH_4 and N_2O fluxes in the *Eucalyptus* rows and interrows were not shown, since the differences were not significant.

The environmental variables showed that the CH_4 pulse in μg m^{-2} h^{-1} (64 ± 77 in Sept 2014, 131 ± 179 in June 2014 and 85 ± 131 in Sept 2014) coincided with WFPS values of 25 % for E1, 42 % for E2, and 33 % for CE in year-1. In year-2, CH_4 pulses 266 ± 365 (May 2015), 104 ± 429 (Feb 2015), and 156 ± 292 μg m^{-2} h^{-1} (May 2015) coincided with WFPS values of 47 %, 40 %, and 68 % for E1, E2, and CE, respectively (Figures 3A and 4A). The N_2O pulses of 39 ± 72 (May 2014), 108 ± 97 (Aug 2014), and 58 ± 87 μg m^{-2} h^{-1} (Sept 2014) coincided with WFPS values of approximately 33 % in all treatments in year-1, while in year-2, the N_2O pulses 21 ± 81 (Feb 2015), 42 ± 65 (July 2015) and 27 ± 83 μg m^{-2} h^{-1} (May 2015) coincided with WFPS values of 37 %, 34 %, and 39 % for E1, E2, and CE, respectively (Figures 3A and 4A).

In year-1, the Cerrado was the only site with a correlation of 0.58 (p = 0.0018) and −0.68 (p = 0.0015) between the CH_4 flux and WFPS, respectively, in the rainy and dry seasons. Only in E2, correlations were detected between N_2O fluxes and WFPS in the rainy and dry seasons (0.63 (p = 0.0005) and −0.59 (p = 0.0041), respectively). In year-2, WFPS ranged from 19 % to 71 % in the rainy and from 30 % to 43 % in the dry season. With regard to correlations, CH_4 and WFPS in CE positively correlated (0.84; p < 0.0001). For N_2O, a correlation with WFPS of 0.62 (p = 0.0190) was observed in E1, both correlations occurred in the dry season.

In year-1, the soil temperature in all sites remained between 17 and 24 °C. The highest CH_4 and N_2O pulses occurred at soil temperatures ≥ 19 °C. With regard to correlations, CH_4 and soil temperature in CE positively correlated (0.65; p = 0.023). In year-2, the soil temperature ranged from 16 to 26 °C and was correlated with CH_4 flux in E2 (0.59; p = 0.024).

Soil mineral N dynamics

The highest soil NO_3^- and NH_4^+ concentrations were recorded in the rainy season in all sites (p < 0.0240) (Figures 4B and 4C) and did not coincide with the GHG pulse. In the rainy season of year-1, a correlation of −0.68 between CH_4 × NO_3^- (p = 0.0004) in E1 was identified of 0.49 and 0.53 between N_2O × NO_3^- (p = 0.0033), and N_2O × NH_4^+ (p = 0.0023) in CE, while in the dry season, the correlation was 0.55 between CH_4 and NO_3^- (p = 0.0065) in CE.

The ammoniacal N was predominant (Figure 4C). The NO_3^-concentrations were higher in the rainy season (p < 0.0093). The N_2O soil pulse coincided with NO_3^- concentrations < 0.50 mg kg^{-1} of soil and only in some moments, NO_3^- exceeded 2.00 mg kg^{-1} soil (Figure 4B). The seasonality effect on NH_4^+ concentrations differed only in CE, with higher soil concentrations in the dry season (p < 0.0001), coinciding with higher N_2O pulse in all studied sites. In year-2, NO_3^- and NH_4^+ soil concentrations were very close between the sites, except for E2, where concentrations were below 1 mg kg^{-1} of soil, from Feb 2015 onwards (Figures 4B and 4C). With regard to correlations, E1 was the only area in which N_2O and NO_3^- concentrations negatively correlated (−0.63; p = 0.002) during the dry season.

Cumulative CH_4 and N_2O fluxes and carbon equivalent (C eq)

In the study period, cumulative fluxes were not influenced by annual variation, age of *Eucalyptus* stands, or by the replacement of native vegetation for *Eucalyptus*. For N_2O, variations were 0.33 to 0.85 kg ha^{-1} yr^{-1} in year-1 and 0.32 to 0.43 kg ha^{-1} yr^{-1} in year-2 (Table 3). The CH_4values were negative, with cumulative influxes of −1.86 to −0.63 kg ha^{-1} yr^{-1} in year-1, and −1.85 to −1.34 kg ha^{-1} yr^{-1} in year-2 (Table 3).

The C eq ranged from 42 to 108 kg ha^{-1} in year-1 and from 40 to 54 kg ha^{-1} in year-2. However, on an annual basis, the cumulative C eq for CH_4 was negative in all sites evaluated (−6 to −17 kg ha^{-1} in year-1; −12 to −17 kg ha^{-1} in year-2) (Table 3).

The C eq ranged from 42 to 108 kg ha^{-1} in year-1 and from 40 to 54 kg ha^{-1} in year-2. However, on an annual basis, the cumulative C eq for CH_4 was negative in all sites evaluated (−6 to −17 kg ha^{-1} in year-1; −12 to −17 kg ha^{-1} in year-2) (Table 3).

Table 3 – Cumulative fluxes and Carbon equivalent (C eq) of N_2O and CH_4 in *Eucalyptus* stands (E1, clone EAC 1528 planted in 2011 and E2, clone GG100 planted in 2009); and CE (native Cerrado vegetation) in year-1 and year-2; Distrito Federal, Brazil.

Area	Year-1*			
	N_2O	C equivalent	CH_4	C equivalent
	kg ha^{-1}			
E1	0.43 (± 0.26)	55 (± 33)	−1.86 (± 0.26)	−16 (± 12)
E2	0.85 (± 0.30)	108 (± 58)	−0.98 (± 0.30)	−8 (± 8)
CE	0.33 (± 0.20)	42 (± 25)	−0.63 (± 0.20)	−5 (± 5)
N	9	9	9	9
P	0.05	0.05	0.05	0.05
	Year-2*			
E1	0.39 (± 0.18)	52 (± 23)	−1.34 (± 0.36)	−11 (± 3)
E2	0.44 (± 0.01)	58 (± 11)	−1.85 (± 1.40)	−15 (± 11)
CE	0.32 (± 0.01)	43 (± 10)	−1.55 (± 1.68)	−13 (± 14)
N	9	9	9	9
P	0.05	0.05	0.05	0.05

*Year-1 = Oct 2013 to Sept 2014; Year-2 = Oct 2014 to Sept 2015.

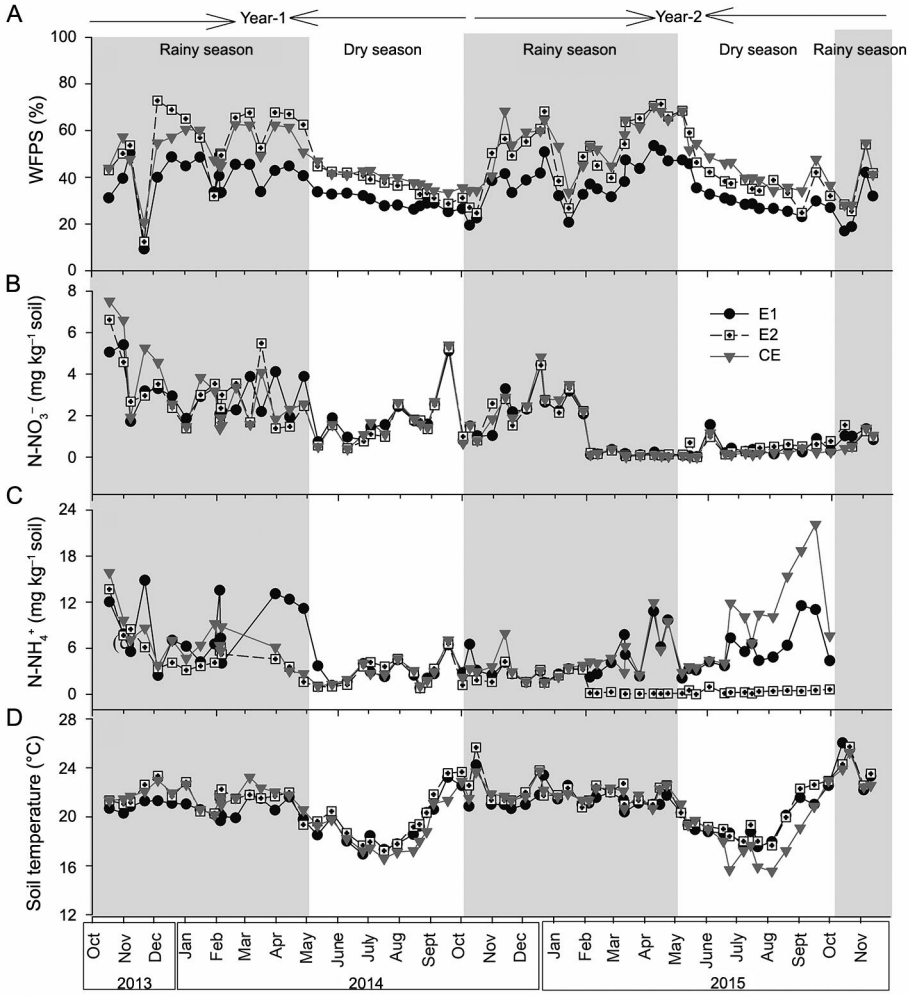

Figure 4 – (A) - Water-filled pore space (WFPS); (B) - nitrate NO_3^-; (C) - ammonium NH_4^+ and (D) - soil temperature from Oct 2013 to Nov 2015 in *Eucalyptus* stands (E1, clone EAC 1528 planted in 2011 and E2, clone GG100 planted in 2009); and CE (native Cerrado vegetation), Paranoá, Distrito Federal, Brazil.

Discussion

Temporal variability of CH_4 and N_2O fluxes and environmental variables

The CH_4 influxes were predominant in this study (81 % for E1 and E2, and 57 % for CE), reaffirming that forest soils are common sinks of this gas. The same behavior was observed in other studies (Godoi et al., 2016; Liu et al., 2017). In general, well-drained soils consume atmospheric CH_4 (Ciais et al., 2013; IPCC, 2013) and indicate CH_4 uptake from the atmosphere mediated essentially by methanotrophic bacteria due to reduced soil water content, favoring diffusion of atmospheric CH_4 into soils (Hiltbrunner et al., 2012). Other studies focused on savannas also mentioned these uptake conditions. In *Eucalyptus globulus* stands and Australian savanna vegetation, respectively, Livesley et al. (2009) recorded influxes of –7 and –16 µg m^{-2} h^{-1}. In areas under cerradão, Siqueira-Neto et al. (2011) reported negative CH_4 fluxes between –93 and –29 µg m^{-2} h^{-1}.

The CH_4 pulses in the three sites occurred in the dry season. Priano et al. (2014) found an inverse relation between CH_4 emission rate and WFPS. The results of this study also indicate that wet soils can oxidize as much CH_4 as relatively dry soils, with average WFPS values near 40 % in the study period. Previous reports also demonstrated that peaks of CH_4 soil oxidation occur at some intermediate level of soil water content (Khalil and Baggs, 2005). According to Humer and Lechner (1999), the optimum saturation degree for oxidation is between 40 and 80 % (moisture content between 25 and 50 %). Moreover, measuring potential methane (CH_4) oxidation rates in semiarid soil, Sullivan et al. (2013) observed that CH_4 oxidation rates were higher in the wet than in the dry season.

During the assessed period, N_2O fluxes in soil under native vegetation (CE) rarely exceeded 10 µg

m^{-2} h^{-1}. This is attributed to the very low amount of NO_3^- and to the predominant N form (NH_4^+) (Figures 3B and 3C) that did not induce high N_2O emissions. These results were similar to previous descriptions in studies on Oxisols under native Cerrado vegetation, which reported few measurements above 10 µg m^{-2} h^{-1} and average fluxes between 0.6 and 16 µg N_2O m^{-2} h^{-1} (Siqueira-Neto et al., 2011; Santos et al., 2016; Carvalho et al., 2017).

In year-1, the N_2O fluxes were higher in the dry than in the rainy season. Even under conditions in which fluxes are common (WFPS > 48 %, predominance of NH_4^+ and soil temperature > 21 ° C), very high fluxes were not observed in the entire dry season, but only in late Aug (08/28/2014). At this time, mineral N exceeded 5.0 mg kg^{-1} soil (lower than in the rainy season) and the soil temperature rose from 17 to 20.3 °C. From Sept onwards, the soil temperature was 23 °C. In this period, there was possibly a response in the N_2O pulses to the increase in soil temperature in E1 and CE, however low (< 59 µg m^{-2} h^{-1}).

Forest soils in the Cerrado biome usually have low N_2O fluxes, which can be attributed to the physical, chemical, and biological characteristics of the system (Martins et al., 2015a; Martins et al., 2015b; Santos et al., 2016). In general, N availability in tropical savanna ecosystems in Brazil is low, since 15 – 37 % of N is resorbed prior to leaf dehiscence (Nardoto et al., 2006) and in the Cerrado, inorganic N is available by mineralization of SOM (Catão et al., 2016). Under undisturbed conditions, N pools are at steady state and production and consumption are equal (Booth, 2005); thus, the annually mineralized inorganic N in unburned Cerrado does not exceed 15 kg ha^{-1} yr^{-1} (Nardoto et al., 2006). With these efficient cycling and use mechanisms, little N is lost by leaching or gas transformation (Bustamante et al., 2006).

Under natural conditions, available N in Cerrado soils depends to a large extent on organic sources and litter (Table 2). The equilibrium between mineralization and immobilization depends on the C: N ratio, which is high (> 65:1) in this study and on the material incorporated into the soil, which may induce N immobilization, showing that residue quality also influences N_2O emissions. According to Alluvione et al. (2010), a high C:N ratio may increase N immobilization thus reducing the occurrence of denitrification and consequently of GHG emissions.

Another important factor to be considered is the cycling efficiency. If high, low values of mineral N can be established in the soil, and the N mineralized by decomposition can be absorbed quickly by the plant root system, after nitrification and denitrification (Ugalde et al., 2007). Low rates of N_2O and CH_4 emissions were also documented in other studies on forests in Brazil, Australia, and China. Godoi et al. (2016) observed similar fluxes of CH_4 (–22.7 and –24.4 mg CH_4 m^{-2} h^{-1}) and N_2O (5.3 and 5.4 mg N_2O m^{-2} h^{-1}), for soils with Acacia and native vegetation, respectively. Werner et al. (2006) observed values near 7 mg N_2O m^{-2} h^{-1} in reforested areas in China, under tropical conditions, while Allen et al. (2009) reported a high CH_4 uptake (–1 to –50 µg CH_4 m^{-2} h^{-1}) and low N_2O fluxes (–5 to 50 µg N_2O m^{-2} h^{-1}) in subtropical Australian soils under *Eucalyptus*.

In general, when good drainage conditions, which reduce WFPS (Baggs and Philippot, 2010), are combined to a low relative NO_3^- production, the mineral N concentrations rarely exceed the N demand of microorganisms and plant roots (Martins et al., 2015b). Although several studies reported correlations between GHGs and mineral N in the soil (Siqueira-Neto et al., 2011; Santos et al., 2016; Carvalho et al., 2017), and in this study, some correlations between these N forms and gas fluxes were also observed, the gas emission pulses did not occur synchronously with the highest NO_3^- and NH_4^+ concentrations, which may have reduced the significance of relations.

In this study, the GHG fluxes were low, regardless of the season and year studied, which was mainly associated to WFPS < 50 % and NO_3^- concentrations < 8 mg kg^{-1}. In soils with high permeability, such as in the Oxisol of this study, both WFPS and texture are key factors in N_2O emission. In soils with WFPS > 60 %, denitrification tends to be the predominant process (Livesley et al., 2009; Gregorich et al., 2015), while in low WFPS soils, ammonia oxidation is favored (Bateman and Baggs, 2005). These findings show that *Eucalyptus* stands of different ages and areas of native Cerrado vegetation probably contribute to reduce N_2O emissions, where no external N sources are provided and the systems depend exclusively on internal nutrient cycling. Furthermore, based on the predominant form of mineral N (NH_4^+), it is assumed that the studied N_2O emissions may result from nitrification rather than from denitrification processes.

However, it should be considered that transformations of N (immobilization or mineralization) in an ecosystem are coupled with C transformations, especially when organic carbon molecules are converted into CO_2 by soil heterotrophic microbial populations (McGill and Cole, 1981), which can reduce the partial pressure of oxygen and favor denitrification. Significant GHG pulse emissions following the first rains after a dry season, often with a short time lag, have been reported in different seasonally dry ecosystems. The pulses are generally preceded by significant GHG emissions immediately after the soil is re-moistened, due to water-induced activation of soil microbes (Santos et al., 2016).

Studies show that soil moisture expressed by WFPS, soil temperature, and mineral N content are the main variables that control and express GHG emissions (Bayer et al., 2015). Siqueira-Neto et al. (2011) reported divergent observations for native Cerrado vegetation, where lower N_2O emissions were observed during the dry season, indicating that the seasonal variations occur due to the absence of rainfall and consequent reduction in soil moisture.

In this study, no environmental variable stood out as an environmental drive, despite variables with relevant effects on N_2O emissions in agricultural systems found by our research group (Santos et al., 2016; Carvalho et al., 2017; Sato et al., 2017). Therefore, these results indicate that the lack of consistent environmental effects may be due to both the intrinsic low GHS emission and the high spatial/temporal variability of native and cultivated forest environments in the Cerrado.

Cumulative CH_4 and N_2O fluxes

The cumulative annual CH_4 fluxes were negative for the areas and years studied (Figures 4B and 4D). The cumulative CH_4 flux from *Eucalyptus* under the conditions assessed shows the crop potential of methane mitigation. These cumulative CH_4 influxes in *Eucalyptus* plantations and native savanna vegetation are in agreement with reports of emissions from forests and savannas (Table 4) that indicate CH_4 uptake from the atmosphere, since a greater amount was consumed by the environment than the effectively produced quantity (Bustamante et al., 2012).

Studies indicate that high soil N mineral contents may stimulate CH_4 oxidation (Bodelier and Laanbroek, 2004; Liu and Greaver, 2009). Mineral N seems to be a prerequisite for CH_4 consumption, although the identification of relationships between N availability and CH_4 consumption, as well as the bacteria involved, is still a challenge. For the time being, N must be treated as a potential inhibitor and as a beneficial factor for CH_4 consumption in soils (Bodelier and Laanbroek, 2004). Ammonium can compete with CH_4 for the enzyme methane mono-oxygenase, effectively lowering CH_4 oxidation by methanotrophs in the soil (Hanson and Hanson, 1996).

The N_2O emissions were below 0.86 kg ha^{-1} yr^{-1} for all areas studied (Figures 4A and 4C). Other studies also reported cumulative N_2O fluxes below 1 kg ha^{-1} yr^{-1} (Table 4). To date, no studies have evaluated soil N_2O fluxes for a period of two years under planted *Eucalyptus* systems in Brazil. In our study, mainly during the dry season in native Cerrado, N_2O influxes and low nitrate levels were observed. The N_2O influx under native vegetation may be associated to a low N mineral content, in predominantly ammoniacal N, a rapid drainage of soil water to the subsurface layers (Martins et al., 2015b; Santos et al., 2016; Carvalho et al., 2017; Sato et al., 2017), and low soil pH (Lopes and Cox, 1977), since nitrification tends to decrease with increasing soil acidity (Hickman et al., 2014).

Table 4 – Literature review of annual or partial rates of CH_4 and N_2O fluxes in forest soils.

Country	Site	CH_4	N_2O	Reference
		— kg ha^{-1} —		
Brazil	Woodland	-	–0.50	Carvalho et al. (2017)
Brazil	Woodland	-	0.07	Silva et al. (2017a)
Brazil	Cerrado *stricto sensu*	-	0.55	Sato et al. (2017)
Brazil	Cerrado *stricto sensu*	-	0.28	Santos et al. (2016)
Brazil	Cerrado *stricto sensu*	–4.4	0.40	Carvalho et al. (2014)
Brazil	eucalyptus urograndis	-	0.7	Coutinho et al. (2010)
Africa	Savanna vegetation	-	2.33	Castaldi et al. (2013)
Australia	Native Forest (Savanna)	–1.6	0.02	Grover et al. (2012)
Australia	Native Forest (Savanna)	–1.4	0.16	Livesley et al. (2009)
Australia	*Eucalyptus globulus*	–6.8	0.15	Livesley et al. (2009)
Australia	*Pinus radiata*	–5.0	0.12	Livesley et al. (2009)

In an integrated crop-livestock-forest system, Carvalho et al. (2017) observed N_2O fluxes lower than in an integrated crop livestock system. The authors claimed that the presence of *Eucalyptus* plant residues in the system, which are rich in phenolic compounds and are acidic (Soumare et al., 2015), inhibit the enzyme and microbial activity in the soil (Chen et al., 2013).

Despite limitations of the static chambers in relation to the real conditions of free soil-atmosphere fluxes, these boxes are easy to construct and can be used under different conditions. Therefore, even low-magnitude flows can be detected and information on the spatial variability of emissions is provided, although no significant differences related to the position and no emission pattern could be observed in our study. Other drawbacks refer to variations in daily measurements that are commonly wide, the chamber measurements may be underestimated or overestimated, and the values of standard deviation are generally high. Possibly, the high spatial and temporal variability of the gas emissions was due to the quantity of variables that are interrelated and drive the flow dynamics. However, cumulative N_2O emissions from forest soils and CH_4 influxes were low (< 1.0 kg ha^{-1}), although their variability should not be left unmentioned. In evaluations at 15 positions in natural forests in Germany, Jungkunst et al. (2012) also found high variability and low spatial representativeness of the chambers, attributed to the C:N ratio, N input, and water availability by the authors. However, considering the variations observed, a random distribution of the chambers is suggested, since the measurements seem to be independent from the chamber position and distance from the *Eucalyptus* trees.

Planting *Eucalyptus* in areas of former native vegetation or previous agricultural use resulted in no marked differences between cumulative GHG fluxes. From the ecological and environmental viewpoint, greater biodiversity in areas of native vegetation stands out. This similarity in the cumulative GHG fluxes may be related to the stability observed in sites with *Eucalyptus* stands after planting.

Conclusions

The change in land use from Cerrado to *Eucalyptus* plantations did not induce significant changes in GHGs, compared to the native vegetation. The flux rates were low for both gases (N_2O and CH_4). The temporal variations in GHG fluxes and the different ages of stands caused

no significant differences between cumulative annual fluxes. Our results may be an important contribution to a better understanding of the dynamics of GHG fluxes in commercial forest plantations in the Cerrado region.

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Authors' Contributions

Conceptualization: Oliveira, A.D. **Data acquisition**: Oliveira, A.D.; Ribeiro, F.P. **Data analysis**: Oliveira, A.D. ; Ribeiro, F.P.; Zuim, D.R.; Pinheiro, L.A.; Malaquias, J.V. **Design of methodology**: Oliveira, A.D.; Ribeiro, F.P. **Writing and editing**: Oliveira, A.D.; Ribeiro, F.P.; Ferreira, E.A.B.; Gatto, A.; Pulrolnik, K.; Soares, J.P.G.; Carvalho, A.M.

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