

# Soil N<sub>2</sub>O fluxes in integrated production systems, continuous pasture and Cerrado

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**Abstract** The objective of this study was to evaluate N<sub>2</sub>O fluxes from integrated crop-livestock (ICL) and integrated crop-livestock forest (ICLF) systems, continuous pasture and native Cerrado. The experiment was conducted at Embrapa Cerrados, Planaltina-DF, in a Red Oxisol, between February 2012 and April 2014, following the transition of crop to livestock, which began in March 2012, with the sowing of *Brachiaria brizantha* cv. Piatã, intercropped with sorghum. The experimental design was a randomized block with three replications. The treatments were: cultivated area intercropped with rows of *Eucalyptus*, spaced 2 × 2 m between plants and 22 m between rows (ICLF); and an area cultivated without tree species (ICL), and also two adjacent reference areas: native Cerrado and continuous pasture. N<sub>2</sub>O productions were characterized by fluxes below 20 μg N m<sup>-2</sup> h<sup>-1</sup>. The ICL system had

the highest cumulative flux with 2.84 kg N ha<sup>-1</sup>, while the ICLF system obtained cumulative fluxes of 2.05 kg N ha<sup>-1</sup>. The native Cerrado showed a negative balance, with -0.05 kg N ha<sup>-1</sup>. The dry season was mostly characterized by low N<sub>2</sub>O fluxes ranging between 10 μg N m<sup>-2</sup> h<sup>-1</sup> and negative values, whereas higher N<sub>2</sub>O fluxes were observed after precipitation events, especially those following a long drought period. The water filled pore space was the factor that best explained N<sub>2</sub>O fluxes, but higher fluxes were observed after the application of nitrogen fertilizer. There was a positive correlation between microbial biomass carbon and N<sub>2</sub>O fluxes in the ICL and ICLF systems.

**Keywords** Integrated crop-livestock-forest systems · Available nitrogen · Nitrous oxide · Greenhouse gas · Soil microbial biomass

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## Introduction

Among the greenhouse gases (GHG), N<sub>2</sub>O is considered a very active gas in the process of global warming due to its high ability to absorb infrared radiation and because it is a stable gas in the atmosphere, contributing to approximately 6% of the radiative potential of GHG, and has a half-life of 120 years (WMO 2012). An increase of 0.2–0.3% in N<sub>2</sub>O atmospheric concentration would contribute to about 5% in global

greenhouse warming (Mosier et al. 2004). The increase of  $N_2O$  has been attributed to large amounts of nitrogen (N) fertilizers applied in agriculture, the conversion of forest areas to agriculture, use of fire and the intensification of livestock (Bustamante et al. 2012; Smith et al. 2010).

Currently in Brazil, 64% of the total  $N_2O$  emissions come from the land use change and the agricultural sectors (MCTI 2014). However, the agricultural sector is responsible for about 87.2% of this total of  $N_2O$  emissions, which is attributed mainly to animal excreta and management of agricultural soils (Signor and Cerri 2013). The Brazilian government has made considerable efforts to reduce the impact of the different sectors of its economy on climate change in the medium and long-term, through public policies such as the Low Carbon Emission Agriculture Program (ABC-Program) (Brasil 2015). The ABC-Program intends to adopt further measures that are consistent with the goal of holding the increase in global average temperature to less than 2 °C above pre-industrial levels in the agriculture sector, strengthen the efforts for sustainable agriculture development and sustainable intensification, including the restoration of an additional 15 million hectares of degraded pasturelands and the enhancement of 5 million hectares of integrated crop-livestock-forest systems (ICLF) by 2030.

The adoption of ICLF systems may be important to reduce GHG emissions and also provide environmental and economic benefits, such as soil and water conservation, carbon sequestration, wood production, and improved animal welfare, which can increase milk and beef production (Cordeiro et al. 2015). However, it is necessary to evaluate the effects of trees in ICLF on  $N_2O$  emissions, since all the components of the ICLF compete for environmental resources i.e. water, light and nutrients (Franchini et al. 2014). According to Lorenz and Lal (2014), some agroforestry systems in particular, have received increased attention regarding their net carbon (C) sequestration effect by their ability to capture atmospheric  $CO_2$  and store carbon in plants and soil.

The best understanding of  $N_2O$  fluxes in ICLF systems can help to predict the possible impact that the increasing ICLF area in Brazil may cause. The global  $N_2O$  emission from crop residue has been estimated at 0.4 Tg N per year, using the IPCC default emission factor of 1.25% of applied residue N emitted as  $N_2O$

(Mosier et al. 1998). However, this default emission factor is based on relatively few experimental studies (Novoa and Tejeda 2006). Thus, information is needed as to the likely impact of crop residues on soil  $N_2O$  emission in ICLF systems. Some studies have been conducted in Cerrado soils to assess  $N_2O$  fluxes under agricultural use (Martins et al. 2015; Metay et al. 2011; Lessa et al. 2014), but there are few that evaluate the emission of this gas in integrated crop-livestock-forest or agroforestry systems. Although agroforestry systems have shown to play an important role in the mitigation of GHG in the atmosphere (IPCC 2000; Verchot et al. 2008), there are no reports on the effects of using eucalyptus trees in ICLF systems on the  $N_2O$  soil fluxes in the Cerrado region of Brazil.

The objective of this study was to evaluate the  $N_2O$  fluxes from integrated crop-livestock (ICL) and integrated crop-livestock forest (ICLF) systems, continuous pasture and native Cerrado. It was hypothesized that the inclusion of tree component in crop-livestock systems can reduce  $N_2O$  emissions by affecting soil attributes (co-variables) in comparison to the absence of trees, having as reference areas native Cerrado and continuous pasture.

## Materials and methods

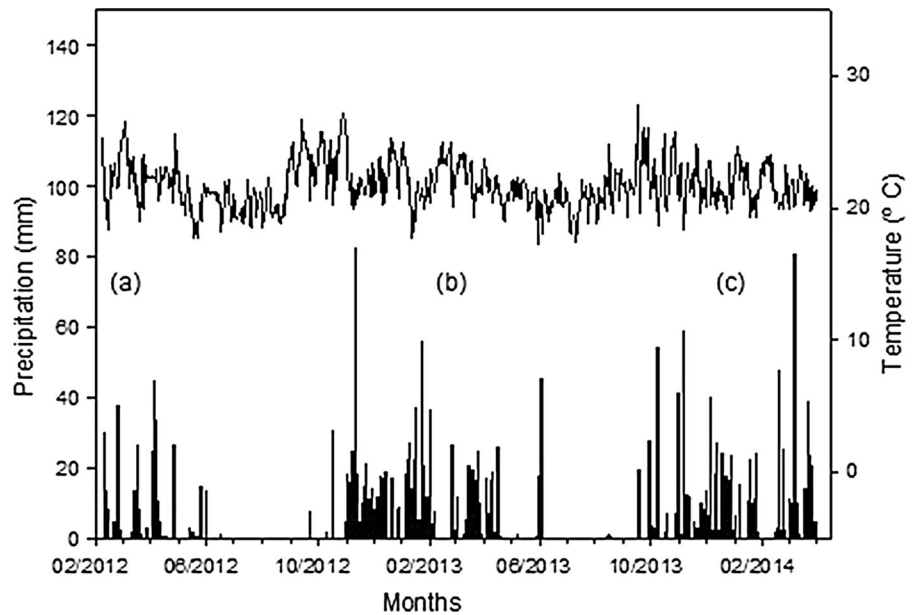
### Experimental site and weather conditions

The study was conducted on the experimental station of Embrapa Cerrados, located in Planaltina, DF (15°35'30''S, 14°42'30''W and altitude of 1007 m) from February 2012 to April 2014. The climate is seasonal and corresponds to the rainy Aw-tropical type (Köppen), with an average annual rainfall of 1383 mm, concentrated in the months from October to March, and an average annual temperature of 27.7 °C (Silva et al. 2014). Figure 1 shows the average air temperature and daily precipitation during the period from February 2012 to April 2014 in the experimental area. The soil is characterized as an Oxisol, with clayey texture (Embrapa 2014).

### History, treatment and management of the experimental area

The treatments consisted of four areas with different land use: integrated crop-livestock-forest system

**Fig. 1** Mean temperature and daily precipitation during the period from February 2012 to April 2014. The accumulated precipitation, number of rainy events and the average precipitation of single events during the rainy seasons were as follow: *a* (02/2012–05/2012)—355.60 mm, 35 rainy events, 10.16 mm rainy events<sup>-1</sup>; *b* (10/2012–05/2013)—1147.50 mm, 111 rainy events, 10.34 mm rainy events<sup>-1</sup>; *c* (10/2013–04/2014)—1037.70 mm, 100 rainy events, 10.38 mm rainy events<sup>-1</sup>, respectively



(ICLF) with two rows of *Eucalyptus urograndis* (spaced 2 m between rows and trees) and spaces between the alleys of 22 m; a cultivated area at full sunlight in absence of tree species (ICL); and two adjacent areas used as references: native Cerrado and continuous pasture (CP). The ICL and ICLF areas consisted of experimental plots with 1.2 ha in a complete randomized block design with three replications. The reference sites consisted of homogeneous adjacent areas and therefore three sampling points were considered to determine N<sub>2</sub>O fluxes and soil co-variables.

The ICL and ICLF areas were implemented in January 2009 with the planting of an annual crop with no-tillage. The chemical characteristics of the soil, prior to the setup of the experimental plots are shown in Table 1. The cropping history from the ICL and ICLF from 2009 to 2014 is presented in Table 2. Previously, between 2007 and 2009, the area was planted with *Brachiaria brizantha* cv. Marandu; *Brachiaria brizantha* cv. Marandu intercropped with *Stylosanthes guianensis*; and *B. brizantha* intercropped with *Leucaena leucocephala* (Lam.) de Wit (*Fabaceae*). In March 2012, after the soybean harvest, seeds of *B. brizantha* cv. BRS Piatã were broadcast (8 kg ha<sup>-1</sup> of viable seeds) immediately before sowing the sorghum to establish a pasture in the off-season period. After harvesting sorghum (July 2012), the pasture of *B. brizantha* was left to establish until

January 2013, when the livestock (cattle) were introduced. The pasture was left to establish from July 2012 to January 2013 because of its intercropping period with sorghum that retarded its development due to competition for nutrients and light. Also, the dry season of approximately six months (April to September—Fig. 1), did not help to produce enough quality forage for the animals. Therefore, a rainy period is recommended to establish enough and good quality forage for the animals (Cordeiro et al. 2015). The ICL and ICLF pastures were managed under continuous grazing with variable stocking rates. The pastures were grazed according to forage availability (7–10 kg of forage per 100 kg of animal weight) and the mean carrying capacities from April 2013 to April 2014 were 2.5 and 1.44 AU (=450 kg live weight) for ICL and ICLF, respectively. The quantity of forage biomass removed from grazing in the period from January 2013 to March 2014 (420 days) was estimated based on dry matter intake (DMI) with the grazing pressure set at 10% (Mandarino et al. 2015) and corresponded to 10,836 and 6086 kg ha<sup>-1</sup> in ICL and ICLF, respectively. Nitrogen fertilizer was applied to ICL and ICLF pastures in March 12, 2013 (200 kg ha<sup>-1</sup> of urea) and in February 24, 2014 (130 kg ha<sup>-1</sup> of urea).

The CP was established in November 2007 with *Brachiaria brizantha* cv. Piatã (6 kg ha<sup>-1</sup> of viable seeds). The CP was also grazed according to forage

**Table 1** Soil chemical and physical analysis at layers 0–20 and 20–40 cm, before establishing the ICL and ICLF experiment, EMBRAPA Cerrados, Planaltina - DF, Brazil

Layers (cm)	pH (H <sub>2</sub> O)	P (mg L <sup>-1</sup> )	K (mg L <sup>-1</sup> )	Al (cmolc dm <sup>-3</sup> )	Ca (cmolc dm <sup>-3</sup> )	Mg (cmolc dm <sup>-3</sup> )	H + Al (cmolc dm <sup>-3</sup> )	CEC (cmolc dm <sup>-3</sup> )	V (%)	Organic matter (%)	Sand (g kg <sup>-1</sup> )	Silt (g kg <sup>-1</sup> )	Clay (g kg <sup>-1</sup> )
0–20	5.55	0.48	67.24	0.14	1.45	0.86	4.41	6.9	35.7	2.86	262	144	594
20–40	5.47	0.64	33.44	0.23	0.96	0.57	4.19	5.81	27.37	2.32	261	119	620

The soil was analysed following Embrapa (1997) CEC total cation exchange capacity, V base saturation

availability (7–10 kg of forage per 100 kg of animal weight) and the mean carrying capacity from April 2013 to April 2014 was 2.1 AU per ha. The quantity of forage biomass removed by grazing (January 2013 to March 2014) corresponded to 6749 kg ha<sup>-1</sup>.

The native Cerrado was described as a xeromorphic forest type known as *cerradão* and typically dominated by woody species (Ribeiro and Walter 1998). The *cerradão* annual litterfall production has been reported to range from 5.4 to 7.8 t ha<sup>-1</sup> and to be highly seasonal and negatively correlated with relative humidity and air temperature (Cianciaruso et al. 2006; Peres et al. 1983).

### N<sub>2</sub>O fluxes

Soil N<sub>2</sub>O fluxes were measured using the technique of static chambers (Gomes et al. 2009; Mosier et al. 2006). The sampling period was from February 2012 to April 2014, representing the period of harvesting of soybean and the intercropping of sorghum with *Brachiaria*. Three static chambers were placed in each plot, totaling 18 chambers in the ICL and ICLF. For each reference area (native Cerrado and continuous pasture) three chambers were installed. Each chamber consisted of a rectangular hollow metal frame (38 cm wide, 58 cm long, 6 cm in height) that was inserted 5 cm into the soil and a top polyethylene tray that was coupled to the base during gas sampling. The chambers were equipped with a layer of sponge and of aluminium foil to avoid heat insulation. In order to ensure that the system was sealed during sampling, the metal base contained a trough that was filled with soft rubber and the tray was coupled with rubber bands stretched over the top and clipped with both ends to the metal base. The top of the tray contained a triple Luer valve for fastening the sampling syringes, thus allowing the removal of the gases at the time of sampling. The samples were collected in 60 mL polypropylene syringes and immediately transferred to 20 mL glass pre-evacuated vials (–80 kPa). The glass vials were closed with 2 mm butyl rubber septa, sealed with aluminum tops.

At the ICLF plots, the chambers were placed parallel to the rows of sorghum, two of which remained at a distance of three meters from the rows of *E. urograndis* and the other one in the middle of the plot. At the ICL plots, the chambers were placed according to the same alignment of the chambers

**Table 2** Management events at ICL and ICLF from 2007 to 2014, Embrapa Cerrados, Planaltina—DF

Plant species	Planting date	Planting and topdressing fertilization
<i>Brachiaria brizantha</i> cv. Marandu, <i>Stylosanthes guianensis</i> ; <i>Leucaena leucocephala</i>	Nov/2007	2.0 t ha <sup>-1</sup> and 800 kg ha <sup>-1</sup> of limestone and gypsum
<i>Eucalyptus urograndis</i> (ICLF)	Jan/2009	150 g NPK 0-20-20 per hole
<i>Sorghum bicolor</i> BRS 310	Mar/2009	350 kg ha <sup>-1</sup> NPK 8-20-15 and micronutrients
<i>Glycine max</i> Baliza (RR)	Dec/2009	400 kg ha <sup>-1</sup> NPK 0-20-20
<i>Glycine max</i> BRS 850	Nov/2010	400 kg ha <sup>-1</sup> NPK 0-20-20
<i>Glycine max</i> Favorita	Oct/2011	1500 kg ha <sup>-1</sup> limestone and 400 kg ha <sup>-1</sup> NPK 0-20-20
<i>Sorghum bicolor</i> in consortium with <i>B. brizantha</i> cv. Piatã	Mar/2012	350 kg ha <sup>-1</sup> NPK 08-20-15
<i>B. brizantha</i> cv. Piatã	Mar/2013	200 kg ha <sup>-1</sup> urea
<i>B. brizantha</i> cv. Piatã	Feb/2014	130 kg ha <sup>-1</sup> urea

placed in the ICLF plots. The chambers were frequently moved (every three months) to avoid soil compaction around them, keeping the same distance between the chambers.

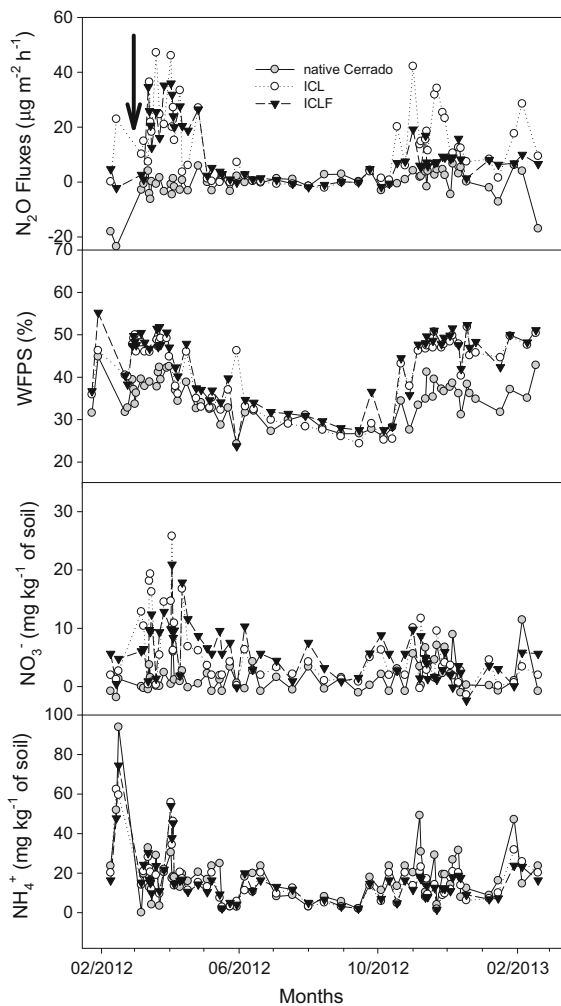
Air samples from the chambers were collected at time 0, immediately after the closure of the chambers, and after 15 and 30 min of closure, always in the morning, between 08:00 and 11:00 h (Alves et al. 2012). Gas sampling frequency was carried out, on average, three to four consecutive days a week during the rainy season, weekly during the short period of drought, and biweekly during the dry season. In the rainy season, between October and April, samples were collected on the three following days after raining events, and on the five days following N fertilizer applications. In addition to the gas sampling, air temperatures were also measured inside the chambers, as well as soil temperatures using portable digital thermometers (Incoterms, 9791.16.1.00).

The analysis of N<sub>2</sub>O concentrations were performed at the Laboratory of Gas Chromatography of the Embrapa Cerrados, using a gas chromatograph (ThermoTraceGC) equipped with a Porapak Q packed column and an electron capture detector. The result of the analysis of each sample was obtained by integrating the area under the curve representing the variation of the gas concentration. The standards used were 200, 600, 1000 and 1500 ppb of N<sub>2</sub>O. The N<sub>2</sub>O fluxes (FN<sub>2</sub>O) consisted of the difference between the concentrations of each sample of treatment and the concentration of air samples. Initially, the linear regression was made between the concentrations of

the standards and their respective areas, finding a factor of transformation of the reading of the samples. After conversion, the fluxes were calculated by the equation:  $FN_2O = \delta C / \delta t (V/A) M/V_m$ ; where  $\delta C / \delta t$  is the change of N<sub>2</sub>O concentration in the chamber during the incubation period; V and A are respectively the volume of the chamber and the ground area covered by the camera; M is the molecular weight of N<sub>2</sub>O and V<sub>m</sub> is a molecular volume in the sample temperature. The N<sub>2</sub>O flux was calculated from the slope of the curve obtained with N<sub>2</sub>O concentrations at 0, 15 and 30 min (Livingston and Hutchinson 1995), being expressed as  $\mu\text{g N-N}_2\text{O m}^{-2} \text{h}^{-1}$ . The correction proposed by Conen and Smith (2000) to compensate the effect of the chamber on the N<sub>2</sub>O concentration in the soil was used.

#### Soil sampling and analysis

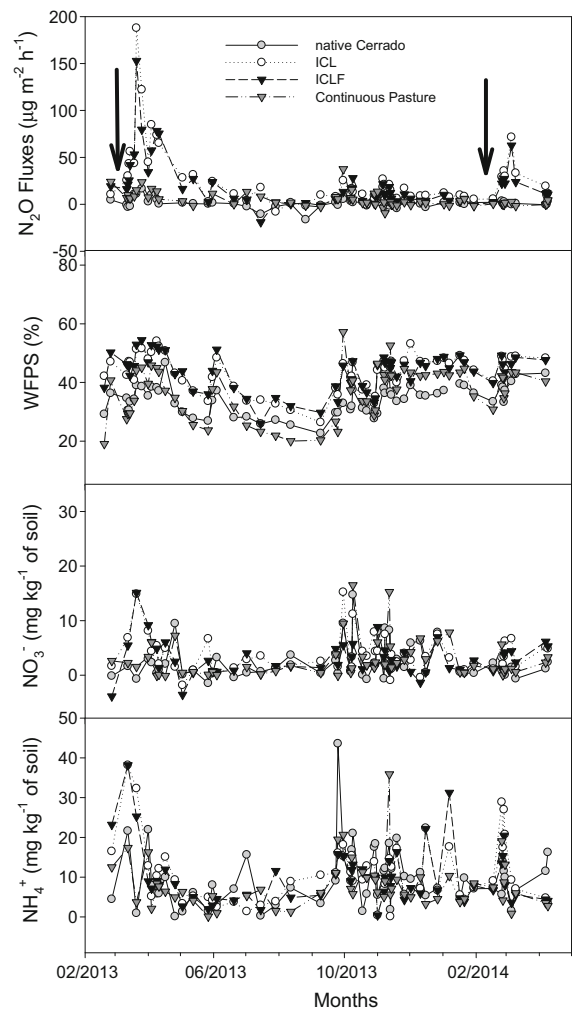
Soil samples were also collected at each gas sampling to determine the gravimetric water content and the concentration of mineral forms of N in the soil (N-NO<sub>3</sub><sup>-</sup> and N-NH<sub>4</sub><sup>+</sup>). Soil samples, composed of three sub-samples were collected at each plot at depths of 0–5 and 5–10 cm, with the use of a Dutch auger. The samples were subsequently placed in metal cans, for the determination of soil moisture, and another portion was kept wrapped in a Styrofoam box with ice and kept in the freezer until the moment of analysis. The gravimetric soil water content was determined after drying soil samples at 105 °C for 48 h. Based on these results and the bulk density, the percentage of WFPS



**Fig. 2** Average daily fluxes of  $N_2O$ , water-filled pore space (WFPS), soil nitrate ( $N\text{-NO}_3^-$ ) and ammonium ( $N\text{-NH}_4^+$ ) during the period from February 2012 to February 2013. The arrow indicates the application of  $30 \text{ kg N ha}^{-1}$  in March/2012

was calculated, using the following formula:  $\text{WFPS} (\%) = (\text{gravimetric moisture} (\%) \times \text{bulk density}) / \text{total soil porosity} \times 100$ ; where:  $\text{total soil porosity} = [1 - (\text{bulk density} / 2.65)]$ , with  $2.65 \text{ [g cm}^{-3}\text{]}$  the assumed density of the soil particles. Nitrate ( $N\text{-NO}_3^-$ ) and ammonium ( $N\text{-NH}_4^+$ ) were analyzed following Embrapa (1997).

In order to evaluate carbon (C) and N in the microbial biomass, soil samples were collected in triplicate at 0–10 cm depth in two periods: September/2013 and February/2014 in each land use. Microbial biomass N (MBN) was determined with the method of chloroform fumigation-extraction, described by



**Fig. 3** Average daily fluxes of  $N_2O$ , water filled pore space (WFPS), soil nitrate ( $N\text{-NO}_3^-$ ) and ammonium ( $N\text{-NH}_4^+$ ) during the period from February 2013 to April 2014. The first arrow from left to right indicate the application of  $90 \text{ kg N ha}^{-1}$  in March/2013, and the second arrow the application of  $60 \text{ kg N ha}^{-1}$  in February/2014

Brookes et al. (1985) and Vance et al. (1987), using a correction factor of 0.54 (Wardle 1994). Microbial biomass carbon (MBC) was determined according to Vance et al. (1987), using a correction factor of 0.38 (Wardle 1994). Total organic carbon (TOC) and total N (TN) were analyzed according to Embrapa (1997). Basal respiration (BR) was also estimated by measuring  $\text{CO}_2$  released from pre-incubated soil samples for a period of seven days (Alef and Nannipieri 1995). The carbon microbial quotient (qMic) was calculated by

dividing the MBC by TOC and was expressed in percentage (%).

### Statistical analysis

A descriptive analysis of N<sub>2</sub>O fluxes and of soil and climate covariables was performed. Emissions were estimated by plotting the average values of N<sub>2</sub>O fluxes and the time scale on a chart and calculating the resulting area under the curve by integration, using the Sigmaplot<sup>®</sup> Version 10 software (Systat Software Inc., Chicago, USA, 2007). Emissions of N<sub>2</sub>O were divided into 3 seasons, each one comprising different month intervals of the sampled years: dry season, which considered the intervals from May to September 2012 and May to September 2013; rainy season, when there was no application of N fertilizer, comprising the periods of October 2012–February 2013 and October 2013–February 2014; and the rainy season, in which there was fertilizer application, corresponding to the periods of February–May 2012, February–May 2013, and February–April 2014.

The resulting data were subjected to analysis of variance and averages were compared using the nonparametric Kruskal–Wallis test ( $p < 0.05$ ) that allowed the comparison of the treatments distributed in randomized blocks (ICL and ICLF) and references (CP and native Cerrado) that have no experimental design. Pearson's correlation and multiple linear regressions to relate emissions of N<sub>2</sub>O and microbiological soil properties were used. Aiming at an exploratory analysis of all soil variables and possible relations with soil nitrous oxide fluxes, a principal component analysis (PCA) was performed in a matrix composed of 24 lines (four land use, two sampling periods and three replications) and 11 columns (soil attributes: nitrous oxide fluxes—N<sub>2</sub>O; microbial biomass carbon—MBC; microbial biomass N—MBN; basal respiration—BR; organic carbon—OC; total N—TN; carbon microbial quotient—qMic; soil temperature—T<sub>soil</sub>; nitrate—NO<sub>3</sub><sup>-</sup> and ammonium—NH<sub>4</sub><sup>+</sup> contents; water filled pore space—WFPS) to identify the variables with greater weight on the linear combination of the first two principal components. To facilitate the interpretation of results, in addition to the correlation circle between the eigenvectors of the variables, a discriminant analysis, based on the Mahalanobis distance or dissimilarity was applied to compare the mathematical distances between samples

from land use. This type of analysis uses a permutation test, which calculates the total inertia interclass for each random distribution of individuals and, by association with a statistical probability, maximizes the discriminating power of the analysis. This step was performed using ADE-4 software (Thioulouse et al. 1997).

## Results and discussion

### Temporal variation of N<sub>2</sub>O fluxes in the soil

The temporal variation of N<sub>2</sub>O fluxes in the study areas during the two years of monitoring was mostly characterized by values below 20 µg N m<sup>-2</sup> h<sup>-1</sup>, combined with periods of higher fluxes during the rainy season (Figs. 2, 3). Also, the temporal variation of N<sub>2</sub>O fluxes in the ICL and ICLF varied from the first and the second year of evaluation; the highest N<sub>2</sub>O flux observed in the first year (March/2012) was approximately 50 µg N m<sup>-2</sup> h<sup>-1</sup> (Fig. 2), whereas the highest flux in the second year (March/2013) was higher than 150 µg N m<sup>-2</sup> h<sup>-1</sup> (Fig. 3). These peaks were observed after the application of N fertilizer and in the rainy season. In the first year, the amount applied was 30 kg N ha<sup>-1</sup> compared to 90 kg N ha<sup>-1</sup> applied in the second year, which may explain the higher peak in the second year. However, during the dry season (from June to October) and in both years of evaluation, fluxes of N<sub>2</sub>O from ICL, ICLF and native Cerrado were mostly less than 10 µg N m<sup>-2</sup> h<sup>-1</sup> and often near zero.

Due to low natural fertility of the Cerrado Oxisols, the N application resulted in high response in N<sub>2</sub>O fluxes, with over 50% of fluxes observed during the experiment being obtained in the period of application of fertilizers. Hickman et al. (2014) in an experiment testing different doses of N fertilizers in an African savanna, noted that over 60% of fluxes occurred in the three weeks following the application of fertilizer. However, such fluxes were only noticed after a rainfall event, when soil moisture increased. Generally, the response to N occurs from the first to the second week after its application and generally disappears within two months (Signor et al. 2013).

The areas under native Cerrado and continuous pasture (CP) showed lower N<sub>2</sub>O fluxes, with 51 and 35% of these fluxes below 1 µg N m<sup>-2</sup> h<sup>-1</sup>,

**Table 3** Cumulative N<sub>2</sub>O fluxes from soil in integrated systems (ICL and ICLF), continuous pasture and native Cerrado

System	Periods						
	02–05/12 (rainy w/N) (kg N ha <sup>-1</sup> )	05–10/12 (dry) (kg N ha <sup>-1</sup> )	10/12–02/13 (rainy wo/N) (kg N ha <sup>-1</sup> )	02–05/13 (w/N) (kg N ha <sup>-1</sup> )	05–10/13 (dry) (kg N ha <sup>-1</sup> )	10/13–02/14 (rainy wo/N) (kg N ha <sup>-1</sup> )	02–04/14 (rainy w/N) (kg N ha <sup>-1</sup> )
Cerrado	-0.12 aB	0.02 aA	0.00 aB	0.07 aB	-0.07 aA	0.04 aA	0.01 aA
CP				0.02 aB	0.18 aA	0.19 aA	0.03 aA
ICL	0.38 bcA	0.03 cA	0.47 bA	0.99 aA	0.35 bcA	0.25 bcA	0.37 bcA
ICLF	0.32 bA	0.02 bA	0.26 bAB	0.84 aA	0.19 bA	0.15 bA	0.27 bA

Means followed by the same small case letters in the row and same capital letters in the column, did not differ from each other by Kruskal–Wallis, test ( $p < 0.05$ )

*Cerrado* native Cerrado, *CP* continuous pasture, *ICL* integrated crop-livestock, *ICLF* integrated crop-livestock forest, *w/N* with the application of N, *wo/N* without the application of N

respectively. In the ICL and ICLF, these lower N<sub>2</sub>O fluxes represented only 13 and 15%, respectively. These results were also observed in other studies in the Cerrado region with values between -0.6 and 0.5 µg N m<sup>-2</sup> h<sup>-1</sup> (Carvalho et al. 2006; Cruvinel et al. 2011; Metay et al. 2007).

These lower values can be attributed to the physical and chemical characteristics of the Cerrado Oxisols (Carvalho et al. 2006; Cruvinel et al. 2011; Metay et al. 2007), which are generally well-drained and with relative low production of N–NO<sub>3</sub><sup>-</sup> that rarely exceeds the amount demanded by microorganisms and plant roots (Nardoto and Bustamante 2003). Well-drained soils (with high drainable porosity) tend to have few anaerobic sites, limiting the N<sub>2</sub>O production from denitrification (Baggs and Philippot 2010).

In our study, N<sub>2</sub>O influx was mainly observed in the native Cerrado during the dry season (soil WFPS < 40%) associated with nitrate levels below 10 mg kg<sup>-1</sup> (Figs. 2, 3). The influx of N<sub>2</sub>O under native vegetation may be related to low soil pH found under Cerrado vegetation in Brazil (Lopes and Cox 1977), since nitrification tends to decrease with increasing soil acidity (Hickman et al. 2014). Cuhel et al. (2010) observed that the activity of denitrification (with N<sub>2</sub>O production) at acid pH was three times lower than that observed at alkaline pH.

Grover et al. (2012), in an experiment in the Australian savanna, obtained negative N<sub>2</sub>O fluxes in a native forest and a low productive pasture during the dry season. These negative values were associated with the low N content (Chapuis-Lardy et al. 2007) and humidity of the soils, when sufficient air can

diffuse through soil macro and micropores, allowing the microorganisms to use N<sub>2</sub>O as a source of N (Rosenkranz et al. 2006). Carvalho et al. (2014) mention that the low fluxes of N<sub>2</sub>O soil under the Cerrado region are associated with low mineral N levels and rapid drainage of water in the soil, not offering favorable conditions for high N<sub>2</sub>O fluxes. Therefore, the low N<sub>2</sub>O fluxes observed in our study are likely due to high soil porosity, dry condition and low N content.

#### Cumulative flux of N<sub>2</sub>O in the soil

Soil cumulative N<sub>2</sub>O fluxes from the native Cerrado and CP did not change among the evaluated periods (Table 3). However, ICL and ICLF cumulative fluxes varied between the two years of evaluation. The highest cumulative fluxes were found during the rainy season and with the application of N fertilizer in 2013 (02–05/2013) in the ICL and ICLF, representing more than 50% of the total N<sub>2</sub>O cumulative flux from these systems. Hickman et al. (2014) also observed that 60% of the total N<sub>2</sub>O fluxes occurred up to three weeks after the application of N fertilizer and may last up to two months (Signor et al. 2013). There was no variation in the cumulative fluxes between the areas of native Cerrado and CP throughout the study period (Table 3). Such systems did not receive N fertilizer or any other fertilizer during our study.

Considering the sum of the cumulative flux of N<sub>2</sub>O during the two years of evaluation, the cumulative flux of soil N<sub>2</sub>O was higher ( $p < 0.05$ ) for ICL (2.84 kg N ha<sup>-1</sup>) compared to ICLF (2.05 kg N ha<sup>-1</sup>) and those



**Table 4** Pearson correlation coefficients representing the relationship between N<sub>2</sub>O fluxes and soil and climate variables, evaluated for stations

Variables	Total	Dry season	Rainy season w/N	Rainy season wo/N
NO <sub>3</sub> <sup>-</sup> 0–5 cm	0.203***	0.074**	0.159***	0.086***
NO <sub>3</sub> <sup>-</sup> 5–10 cm	0.226***	0.050*	0.217***	0.052*
NH <sub>4</sub> <sup>+</sup> 0–5 cm	0.144***	0.109***	0.056*	0.069***
NH <sub>4</sub> <sup>+</sup> 5–10 cm	0.058***	0.124***	-0.029 <sup>ns</sup>	0.015 <sup>ns</sup>
WFPS 0–5 cm	0.336***	0.266***	0.412***	0.212***
WFPS 5–10 cm	0.277***	0.237***	0.313***	0.145***
Rainfall	0.073***	0.184***	0.070**	-0.003 <sup>ns</sup>

<sup>ns</sup> Not significant. \*\*\*, \*\* and \* Significant at 1, 5 and 10% probability, respectively

WFPS water filled pore space, w/N with the application of nitrogen fertilizer, wo/N without the application of nitrogen fertilizer

systems presented higher N<sub>2</sub>O fluxes compared to native Cerrado (-0.05 kg N ha<sup>-1</sup>). The lower N<sub>2</sub>O fluxes in ICLF are probably due to changes in phenolic contents added by eucalyptus litter (Soumare et al. 2015), to lower soil microbial community size and enzyme activities and increased microbial physiological stress (Chen et al. 2013). In this same experimental area and period, the forage dry matter production of the Piatã grass decreased under ICLF (Santos et al. 2016).

There are few studies in Brazil that have evaluated soil N<sub>2</sub>O fluxes for a period of two years under ICL and ICLF systems. Salton et al. (2014), during a soybean cycle (December–April) in Dourados—MS found under ICL a cumulative flux of 0.486 kg N ha<sup>-1</sup>, whereas under continuous crop and no-tillage system found 0.523 kg N ha<sup>-1</sup>. Soil management, edaphoclimatic conditions and also the type of culture being cultivated can influence fluxes of N<sub>2</sub>O. Jantalia et al. (2008) found cumulative flux ranging from 0.596 to 0.984 kg N ha<sup>-1</sup> in the period of one year, in the corn-soybean-wheat rotation, in Southern Brazil.

Allen et al. (2009), in an experiment with eucalyptus reforestation with various ages and pasture grown under intensive grazing in Australia, observed that, generally, N<sub>2</sub>O fluxes are lower under the trees in relation to pasture. However, during the establishment of plants to canopy closure phase (2–3 years), the soil under the trees presented higher N<sub>2</sub>O fluxes than the adjacent pasture areas. This trend was reversed in the average rotation cycles and late (5–23 years), where the N<sub>2</sub>O fluxes under the trees were lower than the adjacent pastures. The authors associated the lower values under the trees to the plant community

composition and its interaction with the soil, as some plants can have suppressive effects on the microbial communities of the soil, and related compounds released by plants which inhibit nitrification have been identified (Niklaus et al. 2006).

In order to estimate the system productivity during the sorghum cycle and relate it to the cumulative N<sub>2</sub>O fluxes, the ratio of mg N<sub>2</sub>O m<sup>-2</sup> to grain productivity (kg m<sup>-2</sup>) was calculated (Martins et al. 2015). Although the cumulative N<sub>2</sub>O fluxes did not differ significantly between ICLF (31.33 mg N<sub>2</sub>O m<sup>-2</sup>) and ICL (29.73 mg N<sub>2</sub>O m<sup>-2</sup>) during the sorghum cycle (February–July, 2012), the cumulative N<sub>2</sub>O fluxes per unit of grain production varied significantly ( $p < 0.05$ ), with ICLF showing three times greater value (224 mg N<sub>2</sub>O m<sup>-2</sup> kg<sup>-1</sup> grains) than ICL (76 mg N<sub>2</sub>O m<sup>-2</sup> kg<sup>-1</sup> grains). This result is due to the lower sorghum yield in ICLF (1416.11 kg ha<sup>-1</sup>) in comparison to ICL (3927.5 kg ha<sup>-1</sup>), which may be explained, mainly, by the effect of the shade of the *Eucalyptus urograndis* trees (Santos et al. 2016) that suppressed nearly half of the sorghum yield. Thus, the ICL presented higher productivity, suggesting lower impact in the conversion of emission/product, around 66% less when compared to ICLF ( $p < 0.05$ ). Therefore, considering the sorghum cycle, the ICL system stands out as more efficient in the ratio of N<sub>2</sub>O fluxes/grain productivity, providing more food with less N<sub>2</sub>O fluxes.

#### Influence of covariables on N<sub>2</sub>O fluxes in the soil

The dynamics of N<sub>2</sub>O fluxes can be attributed to differences among the integrated systems, continuous

pasture and native Cerrado, due to their environmental conditions. Most of the covariables ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , WFPS and rainfall) demonstrated to be highly correlated with the  $\text{N}_2\text{O}$  fluxes, although the Pearson coefficients were low (Table 4). In addition, all correlations were positive, reinforcing the relationship and direct influence that these covariables present with  $\text{N}_2\text{O}$  fluxes. Nitrification and denitrification processes are dynamic and dependent on various edaphoclimatic factors, mainly the availability of N in the soil and soil moisture (Ussiri and Lal 2013).

#### *Water filled pore space and precipitation*

The WFPS was the variable that best explained  $\text{N}_2\text{O}$  fluxes, mainly at the 0–5 cm soil layer (Table 4). Generally, high fluxes coincide with periods after precipitation which caused an increase in WFPS (Figs. 2, 3), which was also observed by Dick et al. (2001) and Ussiri and Lal (2013). The highest  $\text{N}_2\text{O}$  emission peaks occurred in periods after rainfall and consequent elevation of the WFPS, and in periods associated with the application of N fertilizer as topdressing.

Rainfall influences  $\text{N}_2\text{O}$  fluxes by changing the soil water content. Several studies show the increase of  $\text{N}_2\text{O}$  fluxes after rainfall events (Dick et al. 2001; Grover et al. 2012; Jantalia et al. 2008; Parkin and Kaspar 2006; Ruser et al. 2006), resulting in an increased amount of WFPS. High WFPS increases denitrifying activity induced by the reduction of  $\text{O}_2$  diffusion in the soil (Dobbie et al. 1999; Ussiri and Lal 2013).

A homogeneous rainfall distribution during the rainy period was observed (Fig. 1). The accumulated precipitation, number of rainy events and the average precipitation of single events during the rainy seasons were as follows: (02/2012–05/2012)—355.60 mm, 35 rainfall events with a mean of 10.16 per event; (10/2012–05/2013)—1147.50 mm, 111 rainy events, with a mean of 10.34 per event; (10/2013–04/2014)—1037.70 mm, 100 rainy events, with a mean of 10.38 per event. Although the average precipitation of single events was approximately 10 mm, precipitation reached 80 mm and soil WFPS 61%.

There was positive correlation between  $\text{N}_2\text{O}$  fluxes and precipitation. The diffusivity of  $\text{N}_2\text{O}$  is lower in soils that micropores predominate, such as those containing a high percentage of clay (Eickenscheidt

and Brumme 2013). Therefore, higher fluxes are only registered for a few hours or days after precipitation (Dick et al. 2001). In dry conditions, the soil serves as a sink of  $\text{N}_2\text{O}$  (Goldberg and Gebauer 2009; Grover et al. 2012; Stewart et al. 2012). Although the underlying mechanisms are not clear, one possible explanation is that  $\text{N}_2\text{O}$  diffusion from the atmosphere into the soil is favored under dry soil conditions and limited availability of inorganic N, and atmospheric  $\text{N}_2\text{O}$  is reduced to  $\text{N}_2$  by denitrifying bacteria (Dijkstra et al. 2013).

Nevertheless, the highest correlation of precipitation with  $\text{N}_2\text{O}$  fluxes in the soil occurred during the dry period, and this result may be a consequence of the “Birch effect” (Jarvis et al. 2007). This effect occurs in the drying/rewetting events, in which a long drought period is followed by rainfall events, resulting in a rapid organic matter decomposition and mineralization in soil (Birch 1964). The “Birch effect” was observed on October 18 (2012) and November 1 (2012) after rainy events of 30 and 18 mm, respectively;  $\text{N}_2\text{O}$  fluxes increased from  $2 \mu\text{g N m}^{-2} \text{h}^{-1}$  to  $20 \mu\text{g N m}^{-2} \text{h}^{-1}$  after 25 days without rain and from  $13 \mu\text{g N m}^{-2} \text{h}^{-1}$  to  $42 \mu\text{g N m}^{-2} \text{h}^{-1}$  after 13 days without rain.

In the central region of Brazil, the occurrence of rain over a long period of drought is common, which can reach up to 5–6 months, promoting an intense response in microbial activity. The addition of water quickly increases the population of microorganisms in the soil, and reactivates their metabolism (Jarvis et al. 2007) and may explain the relationship between rainfall and  $\text{N}_2\text{O}$  fluxes in the soil during the dry season. The increase of microbial activity and size can explain the  $\text{N}_2\text{O}$  peaks observed at the beginning of the rainy season in our study. The “Birch effect” promotes a peak of soil respiration, stimulating the inactive microorganisms, thus accelerating the biochemical processes in the soil (Dick et al. 2001). This process increases the available C concentration and stimulates denitrifying bacteria to use nitrate as an electron acceptor for the oxidation of C and generation of energy (Ussiri and Lal 2013). Other authors have also obtained high  $\text{N}_2\text{O}$  fluxes after soil rewetting, reaching, at certain times, higher fluxes than those obtained in response to the application of N fertilizer (Pelster et al. 2011; Ruser et al. 2006).

In this study, the WFPS showed the best correlation with the  $\text{N}_2\text{O}$  fluxes, with values ranging from 19 to

**Table 5** Linear correlation between N<sub>2</sub>O fluxes and microbiological attributes in Cerrado soil in crop-livestock integration system (ICL), integration crop-livestock-forest (ICLF), native Cerrado and continuous pasture (CP)

	MBC	MBN	BR	TOC	TN	qMic
N <sub>2</sub> O	0.453*	0.249	-0.474*	0.006	-0.218	0.390

\* Significant at 5% probability

MBC microbial biomass carbon, MBN microbial biomass nitrogen, BR basal respiration, TOC total organic carbon, TN total nitrogen, qMIC carbon microbial quotient

51% in the dry season and 29 to 55% in the rainy season. Several authors reported that WFPS is a limiting factor in N<sub>2</sub>O fluxes and below 60% the activity of denitrifying bacteria in the soil is decreased (Denmead et al. 2010; Liu et al. 2007; Pimentel et al. 2015). However, Zhang and Han (2008) observed fluxes of N<sub>2</sub>O when WFPS was below 60% in native pasture and fallow management. These authors associated these fluxes to be mainly performed by nitrifying bacteria. In our study, the WFPS was mostly below 60% and N<sub>2</sub>O fluxes and peaks were observed, thus indicating that these fluxes could be associated with nitrifying bacteria and/or to a few anaerobic sites that allow the denitrification process to happen.

#### Nitrate and ammonium in the soil

N<sub>2</sub>O fluxes can be positively correlated with the availability of inorganic N in the soil (Holtgrieve et al. 2006), as observed in this study (Table 4). The average content of N-NH<sub>4</sub><sup>+</sup> (0–93 mg N kg<sup>-1</sup>) was generally higher than the concentration N-NO<sub>3</sub><sup>-</sup> (0–25 mg N kg<sup>-1</sup>). The overall average of the N-

NH<sub>4</sub><sup>+</sup> was 73% larger compared to the N-NO<sub>3</sub><sup>-</sup> and showed more variability throughout the study (standard deviation of 12.5 mg N kg<sup>-1</sup> for N-NH<sub>4</sub><sup>+</sup> and 4.25 mg N kg<sup>-1</sup> for N-NO<sub>3</sub><sup>-</sup>). At very low levels of soil moisture, the dissolution rate of N sources becomes slower, reducing the availability of NH<sub>4</sub><sup>+</sup> solution (Cameron et al. 2013). This reduction will reflect the activity of nitrifying bacteria, especially *Nitrobacter*, resulting in the decrease of available NO<sub>3</sub><sup>-</sup> concentration (Baggs and Philippot 2010).

After the application of N as topdressing, increases in N<sub>2</sub>O fluxes in the soil of the ICL and ICLF systems were observed. The first application (90 kg N ha<sup>-1</sup>) was made on March 2013 and the second (60 kg N ha<sup>-1</sup>) on February 2014 and both resulted in increases in N<sub>2</sub>O fluxes (Fig. 2). The application also resulted in increases in soil N-NO<sub>3</sub><sup>-</sup> and N-NH<sub>4</sub><sup>+</sup> contents in both systems. Furthermore, N-NO<sub>3</sub><sup>-</sup> content and N-NH<sub>4</sub><sup>+</sup> were positively correlated with the fluxes of N<sub>2</sub>O in soil (Table 4).

The application of N fertilizers to soil can stimulate the production of N<sub>2</sub>O through biochemical processes of nitrification and denitrification under field and laboratory conditions (Baggs and Philippot 2010; Davidson et al. 2000; Fuß et al. 2011; Liu et al. 2007; Stehfest and Bouwman 2006).

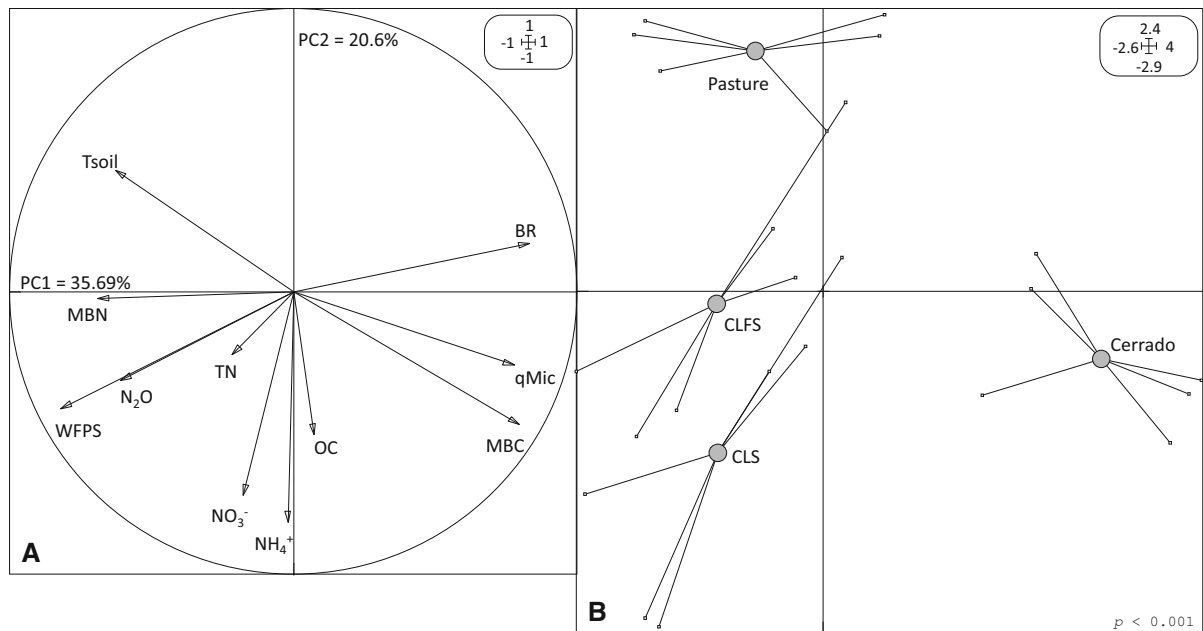
#### Correlation of soil attributes with N<sub>2</sub>O fluxes

The Pearson correlation between the cumulative fluxes of N<sub>2</sub>O and microbiological soil properties (MBC, MBN, MBC, TOC, TN and QMic) are presented in Table 5 for the ICL and ICLF treatments and the multiple linear regression between these

**Table 6** Estimated coefficients, *t* test and confidence interval of multiple linear regression between the microbiological attributes and N<sub>2</sub>O fluxes

Variables	Coefficient not standardized		Standardized coefficient Beta	<i>t</i>	Sig.	Confidence interval for B 95.0%	
	B	SD				Inferior limit	Superior limit
MBC	0.806	3.566	0.288	0.226	0.824	-6.719	8.330
MBN	-2.401	4.422	-0.119	-0.543	0.594	-11.730	6.928
BR	-9.539	4.166	-0.532	-2.290	0.035	-18.328	-0.750
TOC	-41.928	404.106	-0.082	-0.104	0.919	-894.518	810.661
TN	-227.448	174.248	-0.271	-1.305	0.209	-595.079	140.183
qMic	44.778	901.220	0.078	0.050	0.961	-1856.629	1946.186

MBC microbial biomass carbon, MBN microbial biomass nitrogen, BR basal respiration, TOC total organic carbon, TN total nitrogen, qMIC carbon microbial quotient



**Fig. 4** Principal component analysis of all soil attributes in different land use systems. **a** Correlation circle of soil attributes with principal components PC1 and PC2 projections; **b** ordination diagram in the PC1/PC2 plane, according to land use systems; *CLFS* integrated crop-livestock-forest system, *CLS* integrated crop-livestock system, *p* grouping probability by

permutation test.  $N_2O$  nitrous oxide fluxes, *MBC* microbial biomass carbon, *MBN* microbial biomass nitrogen, *BR* basal respiration, *OC* total organic carbon, *TN* total nitrogen, *qMic* carbon microbial quotient, *Tsoil* soil temperature,  $NO_3^-$  nitrate and  $NH_4^+$  ammonium contents, *WFPS* water filled pore space

factors is found in Table 6. Only *MBC* and *BR* were significantly correlated with the fluxes of  $N_2O$ . *MBC* showed a positive correlation, whereas *BR* showed a negative correlation. In the evaluation of multiple linear regression, only *BR* resulted in a significant relationship with the  $N_2O$  fluxes.

The significant correlation between *MBC* and  $N_2O$  can be associated with the quantity and quality of plant residues added to the soil (Carvalho et al. 2014). With the increase of the soil microbial biomass, the biological activity may stimulate biochemical processes such as denitrification, which is responsible for the production of  $N_2O$ . Participation of microbial biomass may be relevant to the  $N_2O$  fluxes. Stieven et al. (2014) observed lower values of soil microbial biomass in eucalypt plantations during the rainy season. The same authors describe that the shading of eucalyptus can cause these changes in ICLF system, which results in reduced plant growth of crops planted and consequently less deposition of crop residues and biological activity in the soil. Because  $N_2O$  fluxes are the result of the activity of nitrifying and denitrifying microorganisms in the soil, the reduction in biological

activity will result in a decrease in the intensity of biochemical processes, reflecting in  $N_2O$  fluxes, which will be smaller (Signor and Cerri 2013).

According to the PCA analysis of soil attributes (Fig. 4), the first two principal components explained approximately 56% of the total variance—PC1 (35.69%) and PC2 (20.6%). PC1 distinguished land uses with a gradient of *MBN*, *WFPS*,  $N_2O$  and soil temperature, with negative eigenvalues and *BR*, *qMic* and *MBC*, with positive eigenvalues. PC2 is related to a gradient of nitrate— $NO_3^-$  and ammonium— $NH_4^+$  contents and *OC*, with negative eigenvalues. Permutation tests of site coordinates, for all extracted axes, showed a significant difference ( $p < 0.001$ ) between land use systems (Fig. 4). Axis 1 separated the Cerrado with higher values of *BR*, *qMic* and *MBC* from the agricultural systems, which are related to the other variables. Axis 2 distinguished integrated systems from pasture with a tendency to high levels of C, N and  $N_2O$  fluxes in integrated systems in contrast with a higher soil temperature in continuous pasture. This result may be related to a higher N supply in integrated systems in relation to other land uses (Cardoso et al. 2016).

## Conclusions

$N_2O$  is one of the main greenhouse gases that is related to agricultural activities and integrated production systems with the use of annual crops, pasture and trees. Woody plants of economic interest, especially eucalyptus, are an alternative for diversification in agriculture and for the mitigation of greenhouse gases. Among the integrated systems evaluated during a period of two years with crop and cattle grazing phases, soil  $N_2O$  fluxes were lower under ICLF system compared to ICL. Such decrease in  $N_2O$  fluxes could have been related to eucalyptus litter that is rich in phenolic compounds leading to lower microbial biomass carbon. It should be highlighted that under ICLF the forage dry biomass and sorghum yield were lower than ICL due to the shade of the trees, thus compromising food production. The ICLF system is one of the alternatives of interest for agricultural production for the consolidation of an Economy of Low-Carbon Emission in Agriculture (Brazilian ABC Program) and can be considered an agricultural system that offers possibilities for mitigation of GHG.

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