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Nitrous Oxide Emissions from a Long-Term Integrated Crop–Livestock System with Two Levels of P and K Fertilization

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Abstract: Nitrous oxide (N_2O) emissions resulting from nitrogen (N) fertilization have been documented. However, no data on the effects of other nutrients, such as phosphate (P) and potassium (K), on N_2O emissions in integrated crop–livestock systems are available so far. In the 2015/2016 and 2016/2017 growing seasons, we measured N_2O emissions from a long-term system, established in 1991 in the Cerrado biome (a tropical savanna ecoregion in Brazil), fertilized with two P and K levels. The studied no-tillage farming systems consisted of continuous crops fertilized with half of the recommended P and K rates (CC-F1), continuous crops at the recommended P and K rates (CC-F2), an integrated crop–livestock system with half of the recommended P and K rates (ICL-F1), and an integrated crop–livestock at the recommended P and K rates (ICL-F2). The cumulative N_2O emissions (603 days) and soil chemical properties were analyzed as a 2 × 2 factorial design (long-term agricultural systems x fertilization). The cumulative N_2O emissions from CC-F2 and ICL-F1 were 2.74 and 1.12 kg N ha^{-1}, respectively. The yield-scaled N_2O emissions from soybean were 55.5% lower from ICL-F1 than from CC-F2 in the 2015/2016 growing season. For off-season sorghum, the mean yield-scaled N_2O emissions were 216 mg N_2O m^{-2} kg^{-1} (in a range from 79.83 to 363.52 mg N_2O m^{-2} kg^{-1}, for ICL-F2 and CC-F1, respectively). The absence of pasture and the presence of soybean and sorghum promoted the highest cumulative N_2O emissions, favored by the recommended rate in relation to half of the P and K. In the total evaluation period (603 days), the presence of grazed land in the years prior to this study and land fertilized with half the recommended P and K rates in an integrated crop–livestock system reduced the resulting cumulative N_2O emissions by 59%. Thus, we conclude that crop–livestock systems can be beneficial in reducing P and K applications and also in mitigating N_2O emissions in comparison with continuous cropping systems fertilized with the full recommended P and K rates. In view of the global fertilizer crisis, this aspect is extremely relevant for agriculture in Brazil and around the world.

Keywords: sustainable agriculture; greenhouse gas emissions; low carbon agriculture

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1. Introduction

Brazil is one of the largest food producers in the world and accounts for 12% of the global agricultural production [1]. The Cerrado biome, with more than 2 million km^2 of savanna-like vegetation, is the most important agricultural region of the country. In the last four decades, nearly one million km^2, or 50% of the total Cerrado area, has been converted into agricultural land [2]. The rapid agricultural expansion in the Cerrado has led to substantial changes in the biogeochemical cycles [3], particularly in the N and P dynamics, and increased greenhouse gases (GHG) emissions [4].

On a 100-year timescale of GHGs, the global warming potential (GWP) of nitrous oxide (N_2O) is 6%, which is 265–298 times greater than that of carbon dioxide (CO_2), and its lifetime in the atmosphere is 121 years. [5,6]. The agriculture and livestock sector accounts for 87% of the national N_2O emissions [7]. In view of the significant contribution of agriculture to N_2O emissions, it is imperative to find new solutions for sustainable food production [8]. Although the N_2O emissions from soils under native Cerrado vegetation are low [3,9], the agricultural systems with high nitrogen input can significantly increase the N_2O emissions to the atmosphere [10].

Global N_2O emissions from agricultural residues have been estimated at 0.4 Tg N per year, based on the standard emission factor of 1.25% N_2O, defined by the Intergovernmental Panel on Climate Change (IPCC) [11]. These N_2O emissions are mainly the result of the N fertilization and crop residue management of agricultural soils [12]. Nitrous oxide emissions result from microbial nitrification and denitrification and are influenced by the substrate and N content, and the edaphoclimatic conditions (O_2, water-filled pore space (WFPS), soil pH, and temperature), which are modified, in turn, by a range of agricultural management practices (e.g., soil tillage). Nitrous oxide fluxes are intensified mainly by the increased use of fertilizers and mainly by the increased nitrogen-based fertilizer applications (e.g., urea and ammonium sulfate) [13,14].

In view of the international commitments of the Brazilian government related to GHG reductions in the agricultural sector, efforts are underway to make agriculture practices more sustainable, e.g., by using integrated crop–livestock (ICL) systems that increase crop yields and reduce environmental impacts [15]. A greater diversification of plant species and the capacity to accumulate soil carbon (C) [16,17] and mitigate GHG, in particular N_2O, has been reported in these ICL systems, which are based on pasture–crop rotation [18,19]. In addition, the soil N_2O emissions from integrated systems are lower than from livestock grazing systems, possibly due to the lower water-filled pore space (WFPS) in the soils under integrated systems, which supposedly have a lower number of anaerobic microsites for denitrification [19].

Studies have shown that nitrogen (N) dynamics are strongly affected by the soil phosphorus (P) content, especially at high nitrogen availability [20,21]; consequently, P can influence N_2O emissions. Phosphorus availability is also influenced by crop residues left on or worked into the soil in crop–livestock systems based on pasture–crop rotations [22–24].

In agricultural systems, research has shown that the residual available P from previous crop fertilizations was more efficiently used by soybean than the P fertilizer applied in the furrow at sowing [25]. This result shows that we should not only consider fertilizing the cash crop (e.g., soybean) but also part of what is needed for the subsequent pasture cycle (e.g., *Urochloa decumbens*) to improve the bioavailability of P in pasture–crop rotations. Nitrogen fertilization in agricultural systems has been shown to strongly increase N_2O emissions [10]. However, little information is available about the interaction of N_2O fluxes with nutrients, such as P and K. Studies have reported that the P application reduces N_2O emissions because it stimulates N plant uptake [26,27]. These results were confirmed by the reduced N_2O emissions of *Acacia mangium* in response to a P application [28]. However, other authors have observed that higher P rates in tropical forests did not restrict the N soil levels, the N transformation rates, the soil NO_3^- levels, or the N_2O fluxes [29,30].

Studies on the effects of K fertilizer application on N_2O emissions are even more scarce. An incubation trial showed that K application to acidic agricultural soil can increase the N_2O emissions between 1.6 and 10.8 times [31]. These increases in N_2O emissions were related to an intensified activity of denitrifying and acid-resistant nitrifying microorganisms, caused by higher K^+ concentrations and lower soil pH.

The sustainability of the system was improved by means of the diversification of the crop residues in integrated systems based on pasture–crop rotations [16], which should increase N use efficiency and would explain the lower N_2O emissions from ICL systems [18]. However, the effects of ICL systems with pasture–crop rotation and P and K fertilization on N_2O emissions are not fully documented.

We believe that using half of the recommended P and K rates in the ICL systems reduces plant residue production and, consequently, N mineralization and N_2O emissions. Furthermore, we hypothesized that ICL systems fertilized with half of the recommended

P and K rates can mitigate N_2O emissions in comparison with continuous crops at the recommended P and K rates. To test this hypothesis, N_2O emissions, edaphic and climatic co-variables, soil chemical properties, the amount of crop residues, grain yield, and yield-scaled N_2O emissions were evaluated in a long-term, integrated crop–livestock system fertilized with two of the P and K rates, in the Cerrado biome, from 1991 to 2013.

2. Materials and Methods

2.1. Site Description

The study was conducted in an experimental area of the Brazilian Agricultural Research Corporation Embrapa Cerrados (15°39′ S; 47°44′ W and 1200 m above mean sea level) in Planaltina, Federal District, in the Cerrado biome (Figure S1). The rainy season in the region lasts from October to April, with a long-term mean annual rainfall of ~1400 mm (in the last 30 years). Rainfall, air temperature, and relative humidity data of the experimental site for the period from 1978 to 2017 were obtained from the main weather station of Embrapa Cerrados (Figure S2). The soil is classified as Oxisol [32], with 611 g kg^{-1} clay, 80 g kg^{-1} silt, and 309 g kg^{-1} sand. The mineral composition of the diagnostic horizon (Bw) is 500 g kg^{-1} gibbsite, 180 g kg^{-1} goethite, 140 g kg^{-1} kaolinite, 70 g kg^{-1} hematite, 100 g kg^{-1} quartz, and 10 g kg^{-1} of other minerals. Soil sampling for soil fertility analysis of the 0–10 10–20, and 20–30 cm layers was carried out in January 2016 (Table S1).

2.2. Experiment Design and Management Systems

The long-term experiment based on crop–pasture rotations was established in 1991, on 40 m × 50 m plots (2000 m^2) in a 2 × 2 factorial design. The factors were represented by the interaction between the agricultural production systems and the soil fertility levels. The treatments consisted of (Table S2): continuous crops at half of the recommended P and K rates (CC-F1), continuous crops with the recommended P and K rates (CC-F2), an integrated crop–livestock system with half of the recommended P and K rates (ICL-F1), and an integrated crop–livestock system at the recommended P and K rates (ICL-F2). In addition, an adjacent native Cerrado plot (20,000 m^2), characterized as typical savanna, was used as a reference area for N_2O emissions.

Information about the agricultural practices (e.g., cropping sequences and fertilizer application) used in the experiment from 1991 to 2013 is shown in Tables S2 and S3. During the first four years (from 1991 to 1995), the soil was limed and tilled with a disc and moldboard plow to establish the no-tillage system. Irrespective of the system, fertilization was according to the cash crop only, according to regional technical recommendations. In the integrated crop–livestock systems (ICL), the only nutrient supply for pasture consisted of residual fertilizer applied to the previous cash crop. No N fertilizer was applied to the pasture or soybean. In this study, in the growing seasons of 2015/2016 and 2016/2017, there was no grazing; however, in the years preceding this study, cattle were left to graze the pasture–crop rotation plots (ICL-F1 and ICL-F2) [18]. The ICL-F1 and ICL-F2 plots were grazed according to the fodder availability and to maintain the forage supply of 8–10 kg per 100 kg of animal weight.

Soybean cv. BRS 8180 RR was sown on 15 November 2015, and the cycle lasted 126 days. The crop was harvested on 27 March 2016. *Panicum maximum* (cv. BRS Tamani) was planted in succession, on the same day that the soybean was harvested.

In the 2016/2017 growing season, soybean cv. NS 7200 RR was planted on 4 November 2016. On the day of soybean harvest (24 February 2017) *Sorghum bicolor* AG 1080 was planted and intercropped with *Panicum maximum* (cv. BRS Tamani) in the ICL-F1 and ICL-F2. On the CC-F1 and CC-F2 plots, *Sorghum bicolor* was intercropped with a mix of species (*Eleusine coracana*, *Brachiaria brizantha* Cv. Paiaguá, *Cajanus cajan* IAPAR 43, *Crotalaria spectabilis*, and *Raphanus sativus*). Sorghum was fertilized with 90 Kg ha^{-1} of NPK 4:30:12 (3.6 kg N ha^{-1}, 27 kg ha^{-1} P_2O_5/11.8 kg ha^{-1} P, and 10.8 kg ha^{-1} K_2O/8.96 kg ha^{-1} K) and soybean (for both growing seasons) with 400 kg ha^{-1} of NPK 0:20:20 (80 kg ha^{-1} of P_2O_5/34.9 kg ha^{-1} P and 80 kg ha^{-1} of K_2O/66.4 kg ha^{-1} K). The soybean seeds were inoculated with *Bradyrhizobium japonicum* (1×10^9 CFU g^{-1} of inoculant) at 200 g per 50 kg seeds for both growing seasons.

2.3. Nitrous Oxide Sampling and Analysis

Two static chambers were placed on each plot of four treatments, totaling 16 chambers to measure the N_2O emissions from November 2015 to July 2017 [33]. The chambers were placed parallel to the rows, one close to the row and the other in between two rows, covering the entire mid-row surface. After the growth of the plants to a 20 cm height, all chambers were placed between rows. Each chamber consisted of a rectangular hollow metal frame (38 cm wide, 58 cm long, and 6 cm in height) inserted 5 cm into the soil, and a top polyethylene tray was coupled to the base during gas sampling. To ensure the airtightness of the system during sampling, the metal base held a trough filled with soft rubber, and the tray was fixed with rubber bands stretched over the top and clipped with both ends to the metal base. A triple Luer valve was installed in the top part of the tray to fasten syringes, thus allowing gas removal at sampling. The samples were collected in 60 mL polypropylene syringes and immediately transferred to 20 mL pre-evacuated glass vials (−80 kPa). Moreover, the air temperature was measured at each sampling time, and the soil and chamber temperatures were measured with digital thermometers during gas sampling at a soil depth of 5 cm. In addition, four static chambers were placed in an adjacent native Cerrado used as a reference area for N_2O emissions. Air samples were collected between 8:30 am and 10:30 am, as pre-established in a previous study, at 0, 15, and 30 min after closing the chamber [33]. Measurements of N_2O fluxes were performed for two years (2015/2016 and 2016/2017), on a total of 603 days with 78 samplings, between November 2015 and July 2017. Emissions were measured for up to five consecutive days after sowing and N fertilization. Frequent N_2O flux measurements were also made up to 2–3 consecutive days after tilling and harvesting and during rainy periods. During the dry season, air sampling was performed every 15 days.

The N_2O concentration was analyzed by gas chromatography (Thermo Scientific Model Trace 1310, Milan, Italy) with a Porapak Q column, 32 columns, and an electron detector. The gas chromatograph was calibrated for N_2O at four levels (concentrations of 200, 600, 1000, and 1500 ppb N_2O). The estimated detection limit was 51 ppb, and the estimated limit of quantification was 154 ppb. The fluxes of N_2O (FN_2O) were measured by the linear variation of gas concentration in relation to the incubation time in the sampling chambers and calculated by the following equation: $FN_2O = (\delta C/\delta t) \times (V/A) \times (M/Vm)$, where $\delta C/\delta t$ is the change in N_2O concentration in the chamber during the incubation interval; V and A are the chamber volume and the covered soil area, respectively; M is the molecular weight of N_2O, and Vm is the molecular volume at each sample temperature. The molecular air volume was corrected to the temperature inside the chamber (T) at the moment of sampling, multiplying it by a factor of 22.4*[273/(273 + T)]. The cumulative emissions per unit grain yield (yield-scaled N_2O emissions) were calculated as the ratio between the total cumulative flux (cumulative FN_2O, kg N_2O ha^{-1}) and the mean yield of the system (P_{mean}, kg grain ha^{-1}) [34].

2.4. Edaphic, Climatic Co-Variables, Chemical Attributes, and Amount of Crop Residues

During the entire N_2O flow sampling period, soil samples were collected at 0–10 cm depth to determine soil nitrate, ammonium, and gravimetric moisture in each of the 78 N_2O flux samples. Two soil samples composed of eight subsamples were collected with a Dutch auger from beside the chambers. The water content of the soil samples was determined by the gravimetric method after drying the soil samples at 105 °C for 48 h. Based on gravimetric moisture and soil density, the percentage of WFPS was calculated by the formula $WFPS = (\Theta \times (BD/WD) \times 100)/(1 - (PD/BD))$, where WFPS is the water-filled pore space (%); Θ—gravimetric water content (g g^{-1}); BD—bulk density (g cm^{-3}); WD—water density (1.0 g cm^{-3}); and PD—particle density (2.65 g cm^{-3}). Bulk density was calculated according to [35].

After extraction with KCl 1 mol L^{-1}, the NH_4^+ and NO_3^- were analyzed colorimetrically with a Lachat Quikchem FIA (Lachat Instruments, 5600 Lindburg Drive, Loveland CO 80,539 USA). In addition, the mean air temperature and daily precipitation were provided by the weather station of Embrapa Cerrados, installed near the experimental area.

The soil chemical attributes (Al; Ca; H + Al; pH; K; P; and SOM) of each plot were measured in the soil samples (from the layers of 0.00–0.10, 0.10–0.20, and 0.20–0.30 m depths) at the soybean flowering stage (Table 1) in January 2016. Ten soil samples were taken between the rows to form one composite soil sample per plot and analyzed according to the following methodologies: pH (H_2O) at a soil solution ratio of 1:1; Al^{3+}, Ca^{2+}, and Mg^{2+} were extracted by KCl 1 mol L^{-1}; K and P were extracted by the Mehlich I method; cation exchange capacity at pH 7.0; and organic matter (OM) was determined according to [36].

Table 1. Chemical attributes of the soil in the treatments with different managements and fertilization.

Treatments	Al cmolc. dm^{-3}	Ca cmolc. dm^{-3}	pH (H_2O)	K g kg^{-1}	P g kg^{-1}	OM g kg^{-1}
			0–10 cm			
CC-F1	0.04 A	4.06 B	5.65 A	111.25 B	3.82 A	4.06 AB
CC-F2	0.02 A	5.49 A	5.51 AB	139.00 B	7.82 A	4.59 A
ICL-F1	0.03 A	3.56 B	5.35 B	108.00 B	12.31 A	3.78 B
ICL-F2	0.03 A	5.37 A	5.55 A	169.75 A	13.87 A	3.59 B
SE	0.018	0.34	0.06	12.85	3.87	0.25
			10–20 cm			
CC-F1	0.62 A	0.60 B	5.41 B	49.25 B	8.67 A	2.20 B
CC-F2	0.20 B	1.56 AB	5.62 AB	78.75 A	8.88 A	2.34 B
ICL-F1	0.29 B	1.08 B	5.66 AB	53.00 B	2.59 A	2.60 AB
ICL-F2	0.14 B	2.29 A	5.67 A	55.50 B	3.33 A	3.13 A
SE	0.06	0.40	0.08	5.91	2.20	0.26
			20–30 cm			
CC-F1	0.21 B	0.37 B	5.23 B	31.25 AB	2.25 A	2.23 A
CC-F2	0.40 A	0.75 B	5.33 AB	46.75 A	2.77 A	2.25 A
ICL-F1	0.30 AB	0.60 B	5.50 AB	28.50 B	0.82 B	2.21 A
ICL-F2	0.21 B	1.14 AB	5.61 A	38.00 AB	1.07 B	2.35 A
SE	0.04	0.25	0.10	5.52	0.28	0.23

Integrated crop–livestock with half of the recommended P and K rates (ICL-F1); integrated crop–livestock with the recommended P and K rates (ICL-F2); continuous crop with half of the recommended P and K rates (CC-F1); continuous crop with the recommended P and K rates (CC-F2). Means followed by the same letters for each soil attribute do not differ from each other, by the Tukey–Kramer test ($p < 0.05$). SE = standard error. OM—Organic matter.

2.5. Statistical Analyses

Total data were checked for normality of the residuals by the Lillieforts test and the homogeneity of variances by the Hartley, Cochran, and Bartlett tests. Daily N_2O flows were analyzed as repeated measures in a pairwise comparison (F-value; $p < 0.05$). The assumptions of normality were verified by the Shapiro–Wilk test and homogeneity of variance by the Levene test. The covariance matrix was selected based on the Akaike information criterion [37]. Analysis of variance was applied considering the experiment in randomized blocks with two replications of chambers, and the mixed model (Proc Glimmix) was used, with a fixed effect for the treatments and random effect for the chambers. Tukey's test was applied at 5% probability to compare the treatment means.

Cumulative N_2O and environmental data were subjected to multivariate analysis (principal component analysis, PCA) to analyze the variation during the study period, resulting in a diagram of the order of variables. In addition to the correlation circle between the eigenvectors of the variables, a discriminant analysis was performed. This analysis is based on the Monte Carlo permutation, i.e., Mahalanobis' distance or dissimilarity was applied to compare the mathematical distances between the samples from the agricultural systems with different fertility levels. 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 [38].

3. Results

3.1. Weather Conditions

The mean daily temperature in the period of N_2O flux measurements was 23.5 °C. The total precipitation from November 2015 to July 2017 was 1827 mm (Figure S3). Of the total rainfall, 88% occurred in the rainy season and 12% in the dry season, in both years (Figure S3).

In the first year, i.e., growing season 2015/2016, precipitation reached 637.3 mm during the 156 days of the soybean cycle. In the 186 days of the off-season, the total rainfall was 184 mm. However, no rain fell after the soybean harvest; so, the seeds of the tamani grass (*Panicum maximum*) did not germinate after planting (Figure S3).

During the 2016/2017 soybean growing season, the total precipitation was 791 mm in 98 days. During the off-season, when sorghum was intercropped with tamani grass (*Panicum maximum*) (ICL-F1 and ICL-F2) and a mix of species (*Eleusine coracana*, *Brachiaria brizantha* Cv. Paiaguá, *Cajanus cajan* IAPAR 43, *Crotalaria spectabilis*, and *Raphanus sativus*) (CC-F1 and CC-F2), the total rainfall was 166 mm in 120 days (Figure S3).

3.2. Dynamics of Daily N_2O Fluxes

The daily N_2O fluxes were generally low, ranging from −5.33 to 73.51 μg N_2O m^{-2} h^{-1} in 2015/2016 and -3.27 to 77.17 μg N_2O m^{-2} h^{-1} in 2016/2017. The highest flux was observed for CC-F2 in year 2 and lowest for CC-F1 in year 1 (Figures 1A and 2A). The highest N_2O fluxes were recorded immediately after crop sowing, at the end of the soybean cycle and after sorghum N fertilization. The mean daily N_2O fluxes ranged from 16.9 to 23.2 μg N_2O m^{-2} h^{-1} for CC-F1 and CC-F2, respectively, and from 12.4 to 14.3 μg N_2O m^{-2} h^{-1} for ICL-F1 and ICL-F2, respectively. The daily N_2O fluxes were lowest from the Cerrado plot in that period (average of 6.12 μg N_2O m^{-2} h^{-1}) (Figures 1A and 2A).

The mean N_2O fluxes during soybean cultivation in 2015/2016 were 54.1 μg N_2O m^{-2} h^{-1} and 38.4 μg N_2O m^{-2} h^{-1} in CC-F2 and CC-F1, respectively, and 34.3 μg N_2O m^{-2} h^{-1} and 29.4 μg N_2O m^{-2} h^{-1} in ICL-F2 and ICL-F1, respectively (Figure 1A). The mean N_2O fluxes in soybean (2016/2017) were 14.2 μg N_2O m^{-2} h^{-1} and 8.8 μg N_2O m^{-2} h^{-1} in CC-F2 and CC-F1, respectively, and 7.8 μg N_2O m^{-2} h^{-1} and 6.8 μg N_2O m^{-2} h^{-1} for ICL-F2 and ICL-F1 (Figure 2A), respectively. For sorghum with intercropping, the mean N_2O fluxes were the highest ($p < 0.05$) from CC-F2 (25.7 μg N_2O m^{-2} h^{-1}) and CC-F1 (21.2 μg N_2O m^{-2} h^{-1}), while the fluxes from ICL-F1 and ICL-F2 F2 were 18.7 μg N_2O m^{-2} h^{-1} and 17.1 μg N_2O m^{-2} h^{-1}, respectively.

The highest N_2O flux from soybean in the 2015/2016 growing season was 73.5 μg N_2O m^{-2} h^{-1} ($p < 0.05$), which coincided with the period after soybean planting in CC-F2, rainfall (>30 mm) on the day before, and favorable edaphic conditions (mineral-N > 10 mg kg^{-1} soil, mainly as NO_3^- and WFPS > 43%) (Figure 1A–D). At the end of the soybean cycle (March 2016), the highest N_2O flux was 36.8 μg N_2O m^{-2} h^{-1} ($p < 0.05$) from CC-F2, measured three days before soybean harvest, when the soil mineral nitrogen (NH_4^+ and NO_3^-) exceeded 25 mg kg^{-1} soil and the WFPS was around 50% (Figure 1A–D).

3.3. Co-Variables, Soil Chemical Attributes, and Crop Residues

The highest mean WFPS were observed in the CC-F2 (53%) and CC-F1 (50.5%) plots (Figures 1D and 2D). The highest mineral nitrogen (NO_3^- and N-NH_4^+) contents in the soil were measured after the sowing of sorghum + intercropped species, at the end of the soybean cycle and after the nitrogen topdressing in sorghum (Figure 1B,C). During soybean cultivation in 2015/2016, the mineral-N (NO_3^- e NH_4^+) was highest at the onset of the crop senescence and after the harvest (mean of > 21.0 mg kg^{-1}). For the NH_4^+ contents, the values ($p < 0.05$) were highest in ICL-F1 (63.0 mg kg^{-1}) and CC-F2 (29.0 mg kg^{-1}). In the second growing season (2016/2017), the mineral-N (62 to 144 mg kg^{-1}) was highest during the soybean cultivation, with a predominance of NO_3^-. Of all the management treatments, ICL-F1 had the highest NO_3^- (81 mg kg^{-1}) and NH_4^+ (63 mg kg^{-1}) contents (Figure 2B,C). However, during sorghum cultivation and after N application on May 17, the mineral N values (NO_3^- and NH_4^+) increased and the WFPS in CC-F2, ICL-F1, and ICL-F2 was >51%.

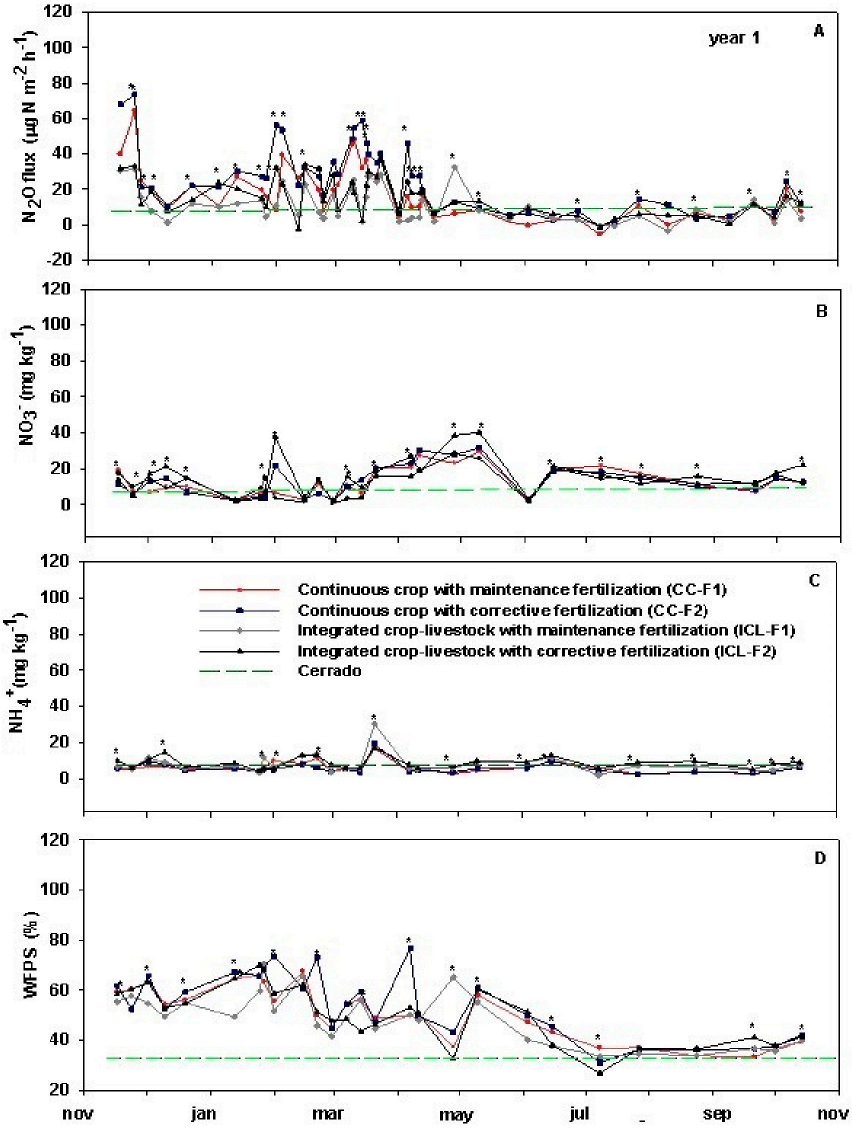

Figure 1. Daily N_2O fluxes (**A**), soil nitrate (NO_3^-) (**B**), soil ammonium (NH_4^+) (**C**), water-filled pore space (WFPS (**D**), from November 2015 to October 2016 (1st growing season), in different treatments and mean values of the Cerrado reference plot. Asterisk indicates significant differences by Tukey–Kramer test ($p < 0.05$).

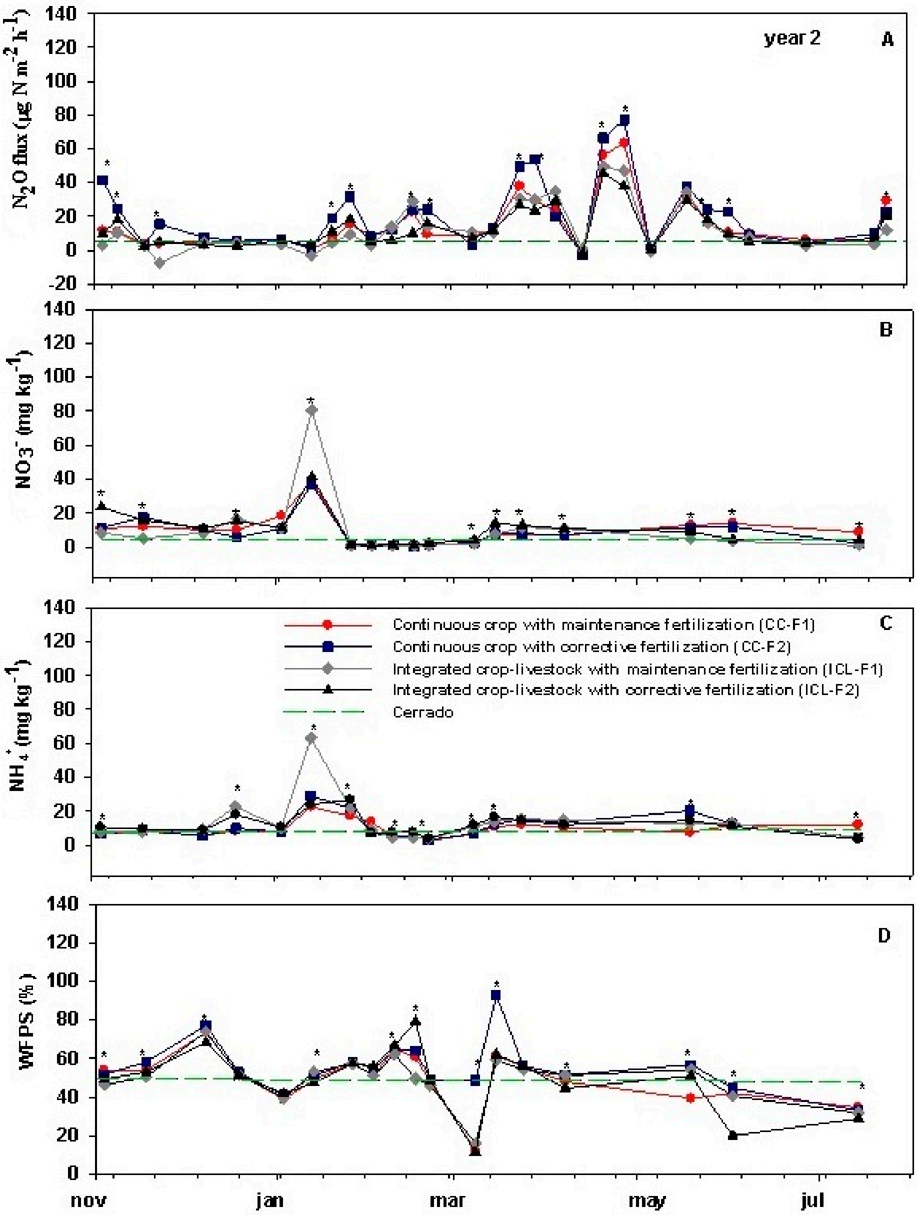

Figure 2. Daily N_2O fluxes (**A**), soil nitrate (NO_3^-) (**B**), soil ammonium (NH_4^+) (**C**), water-filled pore space (WFPS) (**D**), from November 2016 to October 2017 (2nd growing season) in different treatments and mean values for the Cerrado reference plot. Asterisk indicates significant differences by Tukey–Kramer test ($p < 0.05$).

The soil chemical properties of the different management treatments are presented in Table 1. The treatments differed in relation to the soil phosphorus content ($p < 0.05$) in the 20–30 cm layer only, with higher P contents in CC-F1 and CC-F2 ($p < 0.05$). The potassium content (K) in the 0–10 cm soil layer was highest in ICL-F2 ($p < 0.05$), whereas in 10–20 and 20–30 cm the K contents were highest in CC-F2 ($p < 0.05$). The highest Ca contents were found in the surface layer (0–10 cm) of CC-F2 and ICL-F2 ($p < 0.05$). In the 10–20 cm layer, ICL-F2 and CC-F2 had higher values ($p < 0.05$) than CC-F1, while in the 20–30 cm layer ICL-F2 and CC-F2 had the highest Ca contents ($p < 0.05$) (Table 1). In the 0–10 cm layer, the organic matter (OM) content was higher in CC-F2 than in ICL-F1 and ICL-F2 ($p < 0.05$), while in the 10–20 cm layer ICL-F2 OM was higher in CC-F1 and CC-F2 ($p < 0.05$). In the 0–10 cm layer, the pH was lowest in ICL-F1 and CC-F2 ($p < 0.05$). In the 10–20 cm layer, a lower pH was found in CC-F1 ($p < 0.05$). In the 20–30 cm layer, the pH values were higher in ICL-F2 than CC-F1 ($p < 0.05$).

The amount of crop residues left on the soil surface in the 2015/2016 growing season was highest ($p < 0.05$) in ICL-F2 (Figure 3).

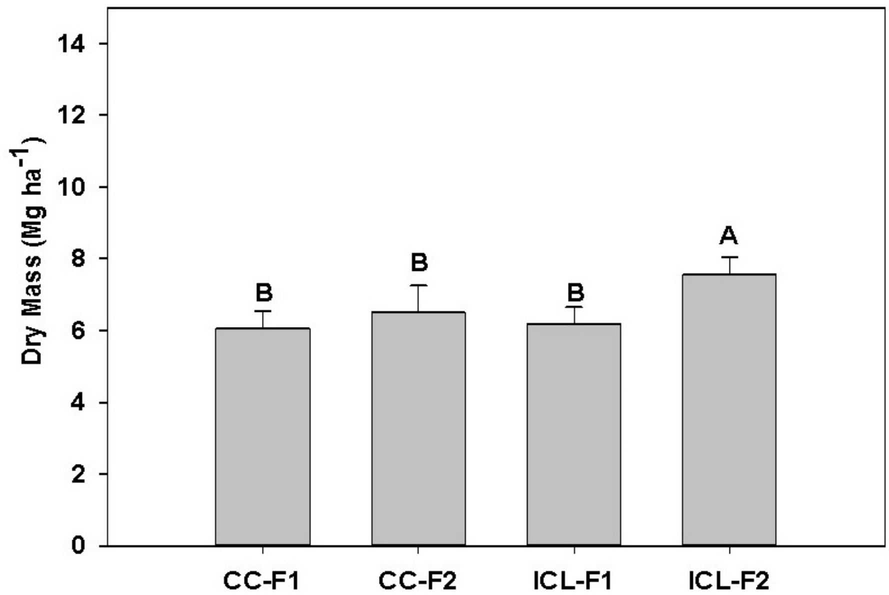

Figure 3. Production of crop residues (Mg ha^{-1}) at the end of the soybean cycle in the 2015/2016 growing season in four treatments. Integrated crop–livestock system with half of the recommended P and K rates (ICL-F1); integrated crop–livestock at the recommended P and K rates (ICL-F2); continuous crops fertilized with half of the recommended P and K rates (CC-F1); continuous crops at the recommended P and K rates (CC-F2). Means followed by the same letter do not differ by Tukey–Kramer at 5% probability.

3.4. Cumulative Emissions of N_2O, Grain yield, and Yield-Scaled N_2O Emission

In the first year, the cumulative N_2O emissions from CC-F2 were higher (1.32 kg N_2O ha^{-1}) than from ICL-F1 (0.46 kg N_2O ha^{-1}) (Table 2). However, over the total evaluated period (603 days), the N_2O emissions from CC-F2 were higher (2.74 kg N_2O ha^{-1}) than from ICL-F1 (1.12 kg N_2O ha^{-1}), representing an increase of 59% ($p < 0.05$).

Table 2. Cumulative N_2O emission (kg N_2O ha^{-1}) in year 1 and year 2.

Treatments	**Cumulative N_2O**		
	Year 1	**Year 2**	**603 Days**
CC-F1	0.83 AB	0.56 A	1.62 AB
CC-F2	1.32 A	0.74 A	2.74 A
ICL-F1	0.46 B	0.52 A	1.12 B
ICL-F2	0.68 AB	0.60 A	1.41 AB
SE	0.29	0.10	0.38

Year-1 = November 2015 to October 2016; Year-2 = November 2016 to July 2017. Integrated crop–livestock fertilized with half of the recommended P and K rates (ICL-F1); integrated crop–livestock at recommended P and K rates (ICL-F2); continuous crops fertilized with half of the recommended P and K rates (CC-F1); continuous crops at recommended P and K rates (CC-F2). Means followed by the same letter in a column do not differ by the Tukey–Kramer test at 5% probability. SE = standard error.

The cumulative N_2O emissions from soybean (17 November 15 to 23 March 16) in the off-season (1 April 16 to 14 October 16), soybean (4 November 16 to 21 February 17), and off-season sorghum with intercropping (8 August 17 to 25 July 17) are shown in Table 3. During the soybean growing season, a significant difference was observed in the 2015/2016 period when the N_2O emissions from ICL-F1 were lower (0.36 kg N_2O ha^{-1}) in N_2O ($p < 0.05$) than from CC-F2 (0.85 kg N_2O ha^{-1}) and CC-F1 (0.71 kg N_2O ha^{-1}). For ICL-F2 (0.50 kg N_2O ha^{-1}), the N_2O emissions were 41% lower ($p < 0.05$) than from CC-F2.

Table 3. Cumulative N_2O emissions (kg N_2O ha^{-1}) from soybean (17 November 15 to 23 March 16), off-season with pasture planting (1 April 16 to 14 October 16), soybean (4 November 16 to 21 February 17), and off-season sorghum intercropping with species mixture (8 August 17 to 25 July 17).

Treatments	Cumulative N_2O			
	Soybean 2015/2016	Off-Season 2016	Soybean 2016/2017	Off-Season with Sorghum and Species Mixture 2017
CC-F1	0.71 AB	0.13 A	0.16 A	0.41 A
CC-F2	0.85 A	0.24 A	0.23 A	0.51 A
ICL-F1	0.36 C	0.09 A	0.10 A	0.42 A
ICL-F2	0.50 BC	0.18 A	0.17 A	0.43 A
SE	0.11	0.26	0.07	0.10

Integrated crop–livestock with half of the recommended P and K rates (ICL-F1); integrated crop–livestock with the recommended P and K rates (ICL-F2); continuous crops at a half of the recommended P and K rates (CC-F1); continuous crops at the recommended P and K rates (CC-F2). Means followed by the same letter in the columns do not differ by the Tukey–Kramer test at 5% probability. SE = standard error.

The soybean and sorghum yields are presented in Table 4. The yield-scaled N_2O emissions were calculated, considering both growing seasons for soybean and sorghum intercropping in the off-season of 2016/2017. The soybean yields did not differ between management systems for the growing season of 2015/2016, whereas the yield-scaled N_2O emissions from CC-F2 were higher than from ICL-F1. In the following season, the soybean yields were higher from ICL-F2 than from ICL-F1, and no differences were observed between the treatments for the yield-scaled N_2O emissions. The sorghum yields were as follows: ICL-F2 > ICL-F1 ≥ CC-F1 = CC-F2. Considering all treatments, the yield-scale N_2O emissions varied from 79.83 to 363.52 mg N_2O m^{-2} kg^{-1} grain, for ICL-F2 and CC-F1, respectively, with a mean value of 216 mg N_2O m^{-2} kg^{-1} grain. The cumulative emissions per unit yield of sorghum grains from the agricultural systems ICL-F1 and ICL-F2 were lower ($p < 0.05$) (Table 4).

Table 4. Crop productivity and yield-scaled N_2O emissions in different soil management systems.

Treatments	Productivity (kg m^{-2})	Yield-Scaled N_2O Emissions (mg N_2O m^{-2} kg^{-1} Grain)
	Soybean 2015/2016	
CC-F1	0.3644 A	203.57 AB
CC-F2	0.3487 A	240.11 A
ICL-F1	0.3468 A	106.81 B
ICL-F2	0.3342 A	154.83 AB
	Soybean 2016/2017	
CC-F1	0.4109 AB	35.82 A
CC-F2	0.4149 AB	51.45 A
ICL-F1	0.3776 B	23.75 A
ICL-F2	0.4354 A	29.94 A
	Off-season with *Sorghum* with species mixture 2017	
CC-F1	0.2869BC	363.52A
CC-F2	0.2269 C	327.21 A
ICL-F1	0.4510 B	98.34 B
ICL-F2	0.5424 A	79.83 B

Integrated crop–livestock system with half of the recommended P and K rates (ICL-F1); integrated crop–livestock at the recommended P and K rates (ICL-F2); continuous crops fertilized with half of the recommended P and K rates (CC-F1); continuous crops at the recommended P and K rates (CC-F2). Means followed by the same letter in a column do not differ by Tukey's test at 5% probability.

3.5. Principal Component Analysis

Principal component analysis was used as an indicator of the importance of environmental variables (Figure 4A) and showed that the first two principal components explained approximately 53% of the total variance: PC1 (29.47%) and PC2 (23.3%). The first axis (PC1) distinguished mainly agricultural systems and their respective fertility levels with a gradient of soil fertility (Ca, K, P, and pH) and the second (PC2) was mainly related to gradient N_2O emissions and co-variables (N_2O, OM, WFPS and NH_4^+) with positive eigenvalues and to NO_3^-, with negative eigenvalues. The discriminant analysis separated ICL from the continuous crop (CC) systems (Figure 4B).

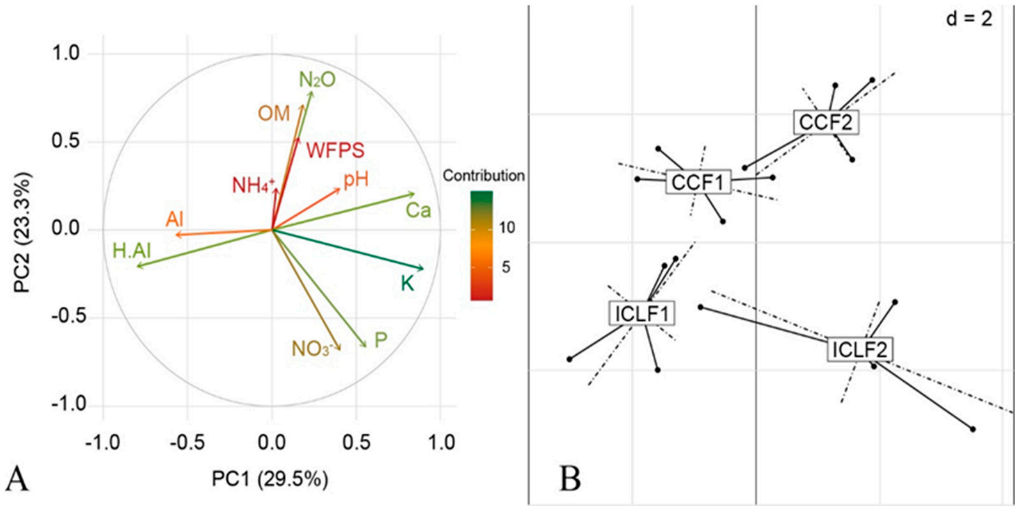

Figure 4. Principal component analysis of all soil properties in different land use systems. (**A**) Correlation circle of soil properties with principal components PC1 and PC2 projections; (**B**) ordination diagram in the PC1/PC2 plane, according to the treatments (CC-F1, CC-F2, ICL-F1, ICL-F2) and variables (N_2O—nitrous oxide fluxes, Al—aluminum, H + Al (potential acidity), pH—soil acidity; Ca: calcium, K—potassium, P—phosphorus, OM—organic matter, NO_3^-—nitrate, and NH_4^+—ammonium contents; WFPS—water-filled pore space).

4. Discussion

4.1. N_2O Emissions

The daily N_2O emissions recorded in this study were very similar to the results of several authors on investigations of the agricultural system in the Cerrado [3,9,10,18,39] and reinforce the need to intensify the installation of ICL systems based on pasture–crop rotation.

The pasture phase of the crop–livestock integration systems (ICL-F1 and ICL-F2) was preceded by cattle grazing in the years prior to this experiment (Table S2). Consequently, the forage biomass in the ICL systems was reduced by grazing, especially when associated with only half of the P and K rates, as in ICL-F1 ($p < 0.05$) (Figure 3), resulting in the lowest cumulative N_2O emissions from this system (ICL-F1). This practice of crop residue removal by grazing animals is one of the possibilities for mitigating N_2O emissions [12].

Nitrous oxide fluxes were highest at the end of the soybean cycle due to the decomposition of nodules and crop residues [39]. Nitrogen mineralization may also be related to the higher concentrations of labile C, which is used as a microorganism substract, favoring nitrifying and denitrifying microorganisms [40].

In addition, the soybean cycle is relatively short compared to other crops such as corn, which, when grown in the first (rainy) season in the Cerrado, favors N_2O emissions after N fertilization and at harvest, when the soil is still wet because of the rain [41].

In the off-season, rainfall is scarce in the Cerrado (Figure S2), and one of the strategies used to cover the soil is to plant drought-tolerant grasses with efficient root systems to absorb the water from the deeper soil layers [42]. However, in this study, due to low rainfall (≈184 mm in six months) during the off-season of 2016, the tamani grass (*Panicum maximum* cv. BRS Tamani) did not germinate, and the cumulative emissions were rather low, except from CC-F2, probably due to the humification process as this treatment has a higher OM content (Table S1).

Although this rainfall of 184 mm is sufficient for crops, dry spells have repeatedly been observed, especially in the months of January and February, which disrupt the reproductive period of crops, causing yield reductions. Moreover, soil re-wetting after a rainless period is associated with intensified microbial activity and the presence of available N increases, and the N_2O fluxes temporally. This effect is called the "Birch effect" [43], and it contributes to the increase in N_2O emissions.

4.2. N_2O Emissions and Co-Variables

Based on the assumption that N_2O production occurs mainly by nitrification in WFPS up to 60% and by denitrification in WFPS greater than 60% [44,45], the N_2O emissions in this study are presumably mainly the result of nitrification. However, some studies in the tropics [45] and temperate climates [46] reported higher N_2O emissions at WFPS between 80–85%, while others suggest a range between 70% and 85% [19,47,48].

Some studies have already established the relationship between N_2O emissions with WFPS [19] and soil mineral N [10,49–51]. Furthermore, the soil nitrate and ammonium concentrations are influenced by factors that directly affect the soil microbial activity, such as rainfall and the agricultural system [52]. In ICL systems, the diversity of crop residues [16] can mitigate N_2O emissions by means of more efficient N cycling.

In ICL, the N_2O emissions were lower than from the continuous cultivation systems, and this study hypothesized [18] that the low soil NO_3^- levels, due to the ability of *Brachiaria* sp. roots to release biological nitrification inhibitors, block the enzymatic pathways of *Nitrosomonas* [53,54].

4.3. Agriculture Systems, Soil Fertility, and N_2O Emissions

The observations confirmed that the ICL systems and the residual effect of the K and P rates significantly altered crop residue production (Figure 3) and the soil N_2O fluxes. The higher cumulative N_2O emission from ICL and the recommended P and K rates (CC-F2) in year 1 and over 603 days may be explained by the higher phosphorus (P) content and increase in OM (Table 2).

Our results also indicate that the production of plant biomass (Figure 3) modified the concentration of nutrients and OM in the soil (Table 1) [16,55], directly reflecting the N_2O fluxes. The highest cumulative N_2O emission from CC-F2 under soybean (2015/2016) is associated with the amount of nitrogen provided by the decomposition of the N-rich soybean shoot and root biomass and the nodules and also by the sequence of phosphate and potassium fertilization that may be favoring a positive balance with the soil, representing a source of N for N_2O production [56,57]. Therefore, ICL-F1 can be considered a mitigation system to decrease 1.62 kg N_2O ha^{-1} in relation to CC-F2 over a period of 603 days.

Some studies showed that the P application reduced N_2O emissions, stimulating the N uptake by increased plant growth and nutrient uptake [26,27,55,58]. This suggestion was confirmed by the observation that the P application reduced the N_2O emissions from *Acacia mangium* [28]. The phosphorus application directly stimulated denitrification and these studies of P cycling associated with N inputs into ecosystems will become increasingly important because P is a non-renewable resource [57,59].

The results of a North American study [60] also suggested that ICL systems, including pastures or predominantly grass cover plants, are more effective in mitigating N_2O fluxes, confirming the results of our study.

4.4. Relationship of Soil Properties with N_2O emissions in PCA Analysis

The PCA analysis of the soil properties distinguished the agricultural systems (Figure 4), where the cumulative N_2O emissions in the CC-F1 and CC-F2 systems contrast with those of ICL-F1 and ICL-F2. The N_2O emissions were associated with CC-F2 system, as shown by the vector in the opposite direction to the variables of fertility and NO_3^- in the ordination diagram (Figure 4). We believe that C losses from the more labile OM fractions may have resulted in higher N_2O fluxes in the continuous crop (CC) system. In the integrated no-tillage crop–livestock (ICL) systems, these losses were lower due to the better protection of C in the micro- and macro-aggregates [18].

The PCA analysis also showed that higher OM contents are related to higher N_2O emissions. Therefore, our results indicate that soil fertility influences N_2O emissions but that it depends on the integrated agricultural system (either ICL or CC).

5. Conclusions

Our results suggest that ICL systems, which include crops and pasture, are more effective in mitigating N_2O emissions. Based on the yield-scaled N_2O emissions of sorghum intercropped with mixed species in 2017, ICL-F1 and ICL-F2 were the most efficient systems, as shown by the higher sorghum yield at both fertility levels. Thus, we conclude that crop–livestock can be more effective in mitigating N_2O emissions than continuous cropping systems with the recommended P and K rates, which, in the context of the global fertilizer crisis, is an aspect of great relevance for agriculture in Brazil and around the world.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/land11091535/s1, Table S1: characterization of fertility under continuous crops and pasture–crop systems with two levels of fertilization and Cerrado reference plot of the experimental area (0–10 and 10–20 cm), Table S2: sequence of crops used in 26 experimental years under different agricultural systems and fertilization levels., Table S3: levels of correctives and nutrients applied between 1991 and 2013 in the field experiment, Table S4: soil chemical properties in the treatments with different managements and fertilization, Figure S1: location of the study area in the Brazilian Cerrado, Figure S2: rainfall, air temperature, and relative humidity in the experimental area, from 1978 to 2017, Figure S3: schematic representation of rainfall in the experimental area in the rainy and dry seasons, between 2015 and 2017, and sampling of N_2O data in four management treatments (ICL-F1: integrated crop–livestock fertilized with half of the recommended P and K rates; ICL-F2: integrated crop–livestock with the recommended P and K rates; CC-F1: continuous crops at half of the recommended P and K rates; CC-F2: continuous crops at the recommended P and K rates), Planaltina, DF, Brazil.

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