Anticipated or postponed organic fertilizer application in relation to nitrogen fertilizer in sugarcane crop reduces N_2O emissions

Késia Silva Lourenço[a,b,], Raffaella Rossetto[c], André Cesar Vitti[c], Zaqueu Fernando Montezano[a], Johnny Rodrigues Soares[d], Rafael de Melo Sousa [a], Janaina Braga do Carmo[e], Eiko Eurya Kuramae[b]; Heitor Cantarella[a]*

[a] Soils and Environmental Resources Center, Agronomic Institute of Campinas (IAC), Av. Barão de Itapura 1481, 13020-902 Campinas, SP, Brazil.

[b] Microbial Ecology Department, Netherlands Institute of Ecology (NIOO), Droevendaalsesteeg 10, 6708 PB, Wageningen, The Netherlands.

[c] Polo Regional Centro Sul, APTA, Piracicaba, SP, Brazil.

[d] School of Agricultural Engineering (FEAGRI), Campinas University (UNICAMP), Av. Cândido Rondon, 501, 13083-875, Campinas, Brazil.

[e] Department of Environmental Sciences, Federal University of São Carlos (UFSCar), Rod. João Leme dos Santos Km 110, 18052-780 Sorocaba, SP, Brazil.

Corresponding author: Heitor Cantarella, tel. +55 19 21370766, e-mail: cantarella@iac.sp.gov.br

Running title: Vinasse applied before or after inorganic N mitigate N_2O emissions

Key-words**:** bioethanol; greenhouse gases; sugarcane residues; N emission factor; vinasse; nitrogen.

ABSTRACT

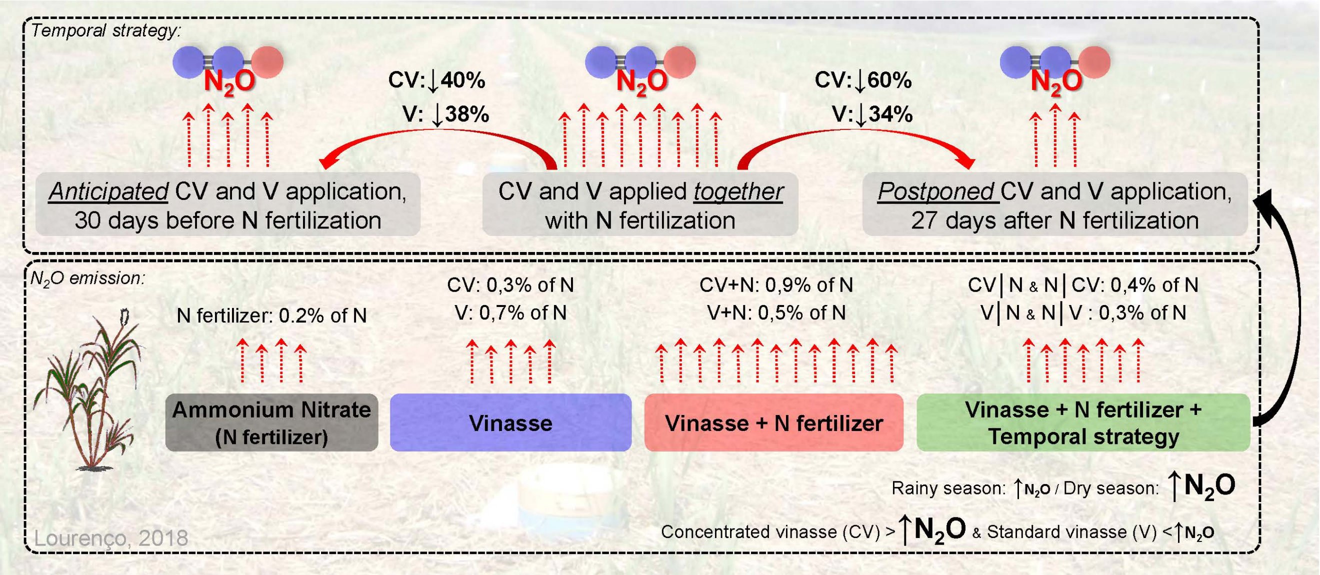
Vinasse is a major byproduct of the sugarcane biofuel industry which is recycled as fertilizer. However, there is evidence that the application of vinasse with mineral nitrogen (N) fertilizers enhances the emission of greenhouse gases (GHGs). Therefore, strategies are needed to decrease the environmental impacts of both inputs. We carried out three sugarcane field experiments by applying N fertilizer (ammonium nitrate) and vinasses (concentrated-CV and standard-V) in different combinations (vinasses with N fertilizer and vinasses one month before or after mineral N fertilization). The gases N_2O, CO_2, and CH_4 were measured for three seasons in fields harvested at the beginning (dry) and end (rainy) of the harvest season. Sugarcane fields were a sink rather than a source of CH_4, while total CO_2 emitted was similar between seasons and treatments. The effect of mineral fertilization and vinasses (CV and V) on N_2O emissions was highly affected by rain events. The N_2O-N fertilizer emission factor (EF) varied from 0.07% to 0.51%, whereas the average EF of V and CV were 0.66% and 0.34% respectively. Based on the average of the three experiments, the application of CV with N fertilizer caused higher N_2O emission (EF = 0.94% of applied N) than N fertilizer solely (EF = 0.23%). The increase in N_2O emission for V plus N (EF = 0.47%) was observed in two out of three experiments. Vinasse (CV and V) combined with N fertilizer increased 2.9-fold N_2O emissions; however, the strategy of anticipating or postponing vinasse application by one month with respect to mineral N reduced the N_2O emissions by 51 % for CV (EF = 0.60% of applied N), but not for V (EF = 0.36% of applied N). Therefore, to avoid boosting of N_2O emissions, we suggest applying vinasses (CV and V) in a different time of mineral N fertilization.

Strategies to mitigate the nitrous oxide emissions from nitrogen fertilizer applied with organic fertilizers in sugarcane

Highlights

- N_2O emission in well-drained soil with sugarcane is low, 0.2% of N fertilizer.
- Vinasse applied with N fertilizer increases N_2O emissions.
- Anticipated or postponed vinasse in relation to N fertilizer reduces N_2O emissions.
- Sugarcane fields were sinks rather than sources of CH_4.

Graphical Abstracts



1. INTRODUCTION

Vinasse is a byproduct generated by the sugarcane biofuel industry in large quantities: 10 to 15 liters of vinasse per liter of ethanol (Wilkie et al., 2000). Up to 360 billion liters of vinasse are produced every year in Brazil (CONAB, 2017). In 2016/2017 approximately 659 million tons of sugarcane were harvested in Brazil and 53% was used for ethanol production (28 billion liters) (CONAB, 2017). Vinasse is a rich source of potassium (K) (2 g L^{-1}), carbon (10-20 g C L^{-1}) and nitrogen (0.4 g N L^{-1}) and is usually directly applied on sugarcane fields as fertilizer in rates varying from 50 to 200 m^3 ha^{-1} (Christofoletti et al., 2013; Elia-Neto and Nakahodo, 1995; Fuess and Garcia, 2014; Fuess et al., 2017; Macedo et al., 2008; Rodrigues Reis and Hu, 2017). However, the cost of transportation and the logistics for applying such large volumes of vinasse is a limitation, especially if fields are located more than 30 km of the ethanol plant (Mutton et al., 2014). In addition, environmental regulations (CETESB, 2014) restrict the volumes of vinasse that can be applied depending on the soil characteristics. Therefore, the concentration of vinasse by evaporation is an alternative strategy to reduce the volume without loss of nutrients and reduce the transportation costs (Christofoletti et al., 2013). Concentrated vinasse is applied in the plant row similarly to mineral fertilizer allowing higher amounts of nutrients close to the plants. However, application of vinasse plus mineral N fertilizer usually increases the N_2O emissions in sugarcane fields.

In Brazil, N_2O is the most important GHG (N_2O, CO_2, and CH_4) emitted from sugarcane soils (Cerri et al., 2009). Recent studies showed that the N_2O emission factor from mineral fertilizer applied to sugarcane fields range from 0.2 to 1% (Filoso et al., 2015), lower than the IPCC default value (1%) (IPCC, 2013) or those previously reported by Lisboa et al. (2011) in sugarcane fields. However, when vinasse was applied with N fertilizer, the emission increased up to 3% of added N (Carmo et al., 2013), which may compromise the sustainability of Brazilian ethanol production. Similar results were obtained by Pitombo et al. (2015), who found that the proportion of N emitted as N_2O was 2.4% when vinasse and N were applied in the soil. The stimulation of N_2O emission by vinasse is related to the addition of easily mineralized organic carbon and probably depends on local conditions because other authors reported less intense effects of vinasse. For instance, Siqueira Neto et al. (2016) found that vinasse did not increase N_2O emissions when combined with mineral N fertilizer, N_2O emissions were 0.52% and 0.59% of applied N and vinasse plus N fertilizer, respectively. Paredes et al. (2014) also examined the effect of vinasse and fertilizer application in a field experiment. The N_2O emission for mineral N was 0.2% but reached 0.6 and 0.7% when N was applied two days prior to or after vinasse. When vinasse was applied with a delay of 3 or 15 days with respect to mineral N fertilizer, 0.77% and 0.78% of the applied N was lost as N_2O (Paredes et al., 2015) as opposed to 0.58% of N applied as mineral fertilizer. The results of N_2O emissions in the literature are quite variable; however, often the application of vinasse with mineral N in the same area increases N_2O emissions. The anticipated or postponed vinasse application by 2, 3 or 15 days was not enough to decrease the negative impact of vinasse-related to N_2O emissions and the emissions were higher than those of mineral N (Paredes et al., 2015; Paredes et al., 2014). It seems that these are short time intervals to lessen the effect of vinasse. Proper management of the vinasse and mineral N fertilizer are needed to reduce GHG emission and consequently the negative impacts of N_2O emission in ethanol production.

The N_2O emission of vinasse applied to soil is usually higher than the IPCC default value of the N_2O emitted by organic fertilizers (1% of the N applied in the field) (IPCC, 2013). In a pot experiment, the emissions varied from 11.5 to 14.9% of N applied as vinasse (Paredes et al., 2014) while in field conditions, the values ranged from 0.44% to 4.59% of the vinasse N (Carmo et al., 2013; da Silva et al., 2017; Oliveira et al., 2013; Paredes et al., 2014; Siqueira Neto et al., 2016). To our knowledge, up to date, there is only one study reporting values of N_2O emission of CV (Pitombo et al., 2015). The authors found that the proportion of N emitted as N_2O was 1.16% when CV was applied to the soil. A better understanding of GHGs emissions from vinasses [concentrated (CV) and standard vinasse (V)] will help to determine effective management strategies to reduce the N_2O emissions during sugarcane production.

Vinasse and mineral fertilizer are common inputs for sugarcane production in Brazil (Christofoletti et al., 2013; Otto et al., 2016). Therefore, there is an urgent need for management options to mitigate N_2O emissions in sugarcane production when they are used. Sugarcane harvest period in Central-Southern region of Brazil is between April and November, which covers three seasons, fall to spring. Thus, to assess the GHG emission it is necessary to take into account the environmental conditions at the time of vinasse and mineral N application (da Silva et al., 2017). It is expected that in the rainy season the N_2O emissions will be higher than in the dry season because of the conditions for N_2O emissions by denitrification. Furthermore, the separation of vinasse and fertilizer application by a few weeks may lessen the conditions that lead to high N_2O emissions. The objective of this study was to evaluate the N_2O losses in the sugarcane ratoon cycle after CV and V applications before, after or along with mineral fertilization focusing on the timing of applications and on different seasons (winter-drought/spring-rain) of fertilizers application.

2. MATERIAL AND METHODS

2.1. Experimental set up

The study comprised three field experiments with sugarcane (variety RB86-7515) at APTA - Paulista Agency for Agribusiness Technology in Piracicaba, SP (22°41′S; 47°33′W). This region is responsible for more than 50% of the sugarcane production in Brazil. The experiments were conducted in different seasons: rainy season, 2013/2014 cycle (R1), dry season, 2014/2015 cycle (D1) and rainy season, 2014/2015 cycle (R2) with sugarcane of third, fourth and second ratoon stage, respectively. The soils at R1 and D1 are classified as a Ferralsol, and at R2 as Rhodic Nitisol (FAO, 2015). The chemical (Van Raij et al., 2001) and physical properties (Camargo et al., 1986) of the 0- to 20-cm and 20- to 40-cm layer are shown in Table 1.

The experiments were in a randomized block design with ten treatments and four blocks (40 plots). The treatments comprised of a combination of CV and V with or without mineral N fertilizers; the vinasses and the mineral N were applied with different time intervals. The vinasses were applied before (30 days) – anticipated (V_a | N and CV_a | N), together (V+N and CV+N) or after mineral fertilizer (27 days) – postponed (N | V_p and N | CV_p). All experiments had control treatments without N or vinasses (CV or V). The experiment in the first rainy season (R1) and dry season (D1) had similar treatments; vinasses were applied 30 days before nitrogen fertilization. However, because of missed plots, the R1 experiment had only treatments with CV applied on the same day of N fertilizer (without temporal strategy). The treatments are shown in Table 2. In R1 there were 32 plots (8 treatments x 4 blocks) in 3,840 m^2 of total experimental area. Each plot comprised five 16-m-long rows planted with sugarcane, spaced at 1.5 m. In D1 and R2 each plot contained four 8-m and five 10-m-long rows planted with sugarcane, spaced at 1.5 m, respectively (1,920 m^2 and 3.000 m^2).

The fields where the experiments were set up had the previous ratoon cycles mechanically harvested in September 2013 (R1), May 2014 (D1) and September 2014 (R2). The amounts of straw left on soil were 12, 16 and 9 t ha^{-1} dry mass in R1, D2, and R2, respectively (Table S1). In R1, standard vinasse (V) was applied on November 13, 2013, to 0.5-m tall sugarcane plants, the second application of vinasses (V and CV) and mineral N fertilizer was on December 13, 2013 (plant 1.5-m-tall). In D1, vinasses (CV and V) were applied on July 15, 2014 (plants 0.4-m-tall) and the second application of vinasse (CV and V) and mineral N fertilizer was on August 15, 2014 (plant 0.6-m-tall). In R2, the mineral fertilizer and first vinasse application were on October 14, 2014 (sugarcane 0.40 m tall) and the second application of vinasse was on November 10, 2014 (plants 0.80-m-tall). The N fertilizer source was ammonium nitrate, applied at a rate of 100 kg N ha^{-1} as recommend for sugarcane in Brazil (Van Raij et al., 1996). Phosphorus was applied to all plots as single superphosphate (45 kg P_2O_5 ha^{-1}). Potassium, as KCl, was applied to the plots that did not receive vinasse, in rates equivalent to K added as vinasse (290, 345, and 320 kg K_2O ha^{-1} in R1, D1, and R2, respectively). Standard vinasse was sprayed over the entire experimental plot at a rate of 100 m^3 ha^{-1}, using a motorized pump fit with a flow regulator. This is the average rate of vinasse in sugarcane plantations in the State of São Paulo. Concentrated vinasse was banded 20-cm from the sugarcane row, at a rate of 17.2 m^3 ha^{-1} for all experiments, approximately 5.8 times less volume than V, based on its K content (average of sugar mill). Mineral fertilizers (N, P, and K) were also band-applied as it is usually performed in commercial areas. The chemical properties of the vinasses are shown in Table 3.

2.2. Greenhouse gases analysis

Fluxes of N_2O, CO_2, and CH_4 were measured using PVC static chambers, 20 cm in height x 30 cm in diameter, according to the method used by Varner et al. (2003).

The chambers were inserted 5 cm into soil and 10 cm from the sugarcane rows. The chamber cap had two openings, each fitted with a valve, one for gas sampling and the other for internal and external pressure equilibrium. All inputs (CV, V, and N) were weighted for the GHG chamber in amounts proportional to the field area where they were applied in order for the chambers to reflect actual field conditions. After closing the chambers, 60 ml gas samples were collected at time points 1, 15, and 30 min using syringes. The samples were transferred and stored in pre-evacuated exetainers vials (12 mL) and analyzed in a gas chromatograph (model GC-2014, Shimadzu Co.) with an electron capture detector for N_2O determination and a flame ionization detector for CO_2 and CH_4 determinations. GHG fluxes were calculated by linear interpolation of the three sampling times (Soares et al., 2015; Soares et al., 2016).

Extensive GHG gases measurements were carried out in all experiments. In the R1, the GHG measurements were conducted for 317 days from November 2013 to September 2014 when the sugarcane was harvested. In the D1 experiment, the GHGs evaluation period was 381 days, from July 2014 to July 2015. For the third experiment, R2, gas samples were collected during a period of 290 days after fertilizer application, from October 2014 to July 2015. Gas samples were collected in the mornings, starting five days before fertilizer and vinasse application in order to check whether emission was stable; when the treatments were applied, the gases were sampled every day during the first week; three times per week for the first 4 months and weekly or biweekly, subsequently.

Cumulative emissions were calculated by linear interpolation between adjacent sampling dates (Allen et al., 2010). We first tested GHG fluxes for normality and subsequently transformed the data using the Box-Cox transformation method (Statistica, version 10). Total cumulative emission per chamber (fertilized bands parallel to the crop line) were compared by orthogonal contrasts using SISVAR statistical software. For treatments with V, which were applied over the whole field, cumulative emissions on a hectare basis were also calculated. The fertilized bands accounted for 16% of the total experimental area and the space between fertilized bands (inter-row) for 84%. Proper controls (plots with no mineral fertilizer and vinasse, or plots with vinasse without fertilizer) were used to calculate the inputs of N_2O-N emission factors (EF).

The N_2O-N emission factors (%) were calculated using emission from the chambers since the amounts of vinasse (CV or V) and mineral N fertilizer placed in the chambers were known, the EF is computed with the following equation (1):

$$EF = \frac{N_2O\text{-}N_{treat} - N_2O\text{-}N_{control}}{N_{applied}\ (CV\ or\ V + fert)}\ x\ 100$$

(1)

where N_2O–N_{treat} (mg N m^{-2}) and N_2O–$N_{control}$ (mg N m^{-2}) are the cumulative emissions of the fertilized and unfertilized chambers, respectively, and N applied is the amount of N added to the chamber as ammonium nitrate and/or vinasse (CV or V).

2.3. Soil chemical analysis and stalk yield

Parallel to each gas sampling, the air and soil temperatures were measured and soil samples (six per plot) were collected (0-10 cm top layer) close to gas chambers for moisture content, water-filled pore space (WFPS) and concentrations of NO_3^--N and NH_4^+-N. Soil moisture was determined gravimetrically by drying the soil at 105 °C for 24 h, and all results were expressed per gram of dry soil. The concentrations of NH_4^+ (Krom, 1980) and NO_3^- (Kamphake et al., 1967) in the filtered extract were determined colorimetrically by flow injection analysis (FIAlab-2500 System) after extraction with 1 M KCl in 1:10 soil-to-solution ratio. The WFPS was calculated considering soil bulk density and porosity determined at the beginning of the experiment (Hillel, 1980).

Climatic data were obtained from a meteorological station located approximately 500 m from the experiments. Stalk yield was estimated using the number and weight of the stalks along 2 m of two sugarcane rows in each plot (6 m^2 in total), collected at randomly chosen positions.

3. RESULTS

3.1. Weather conditions and soil analysis

The climate data of the experimental period are shown in Fig. 1A, 2A and 3A. Mean air temperature varied between 13 °C and 30 °C. The cumulative rain was 654 mm, 1064 mm and 954 mm in R1 (Nov/13 to Sep/14), D1 (July/14 to Aug/15) and R2 (Oct/14 to Aug/15), respectively. These amounts of cumulative rain were lower than the historical 100 year average values for the region (R1: 1168 mm, D1: 1307 mm and R2: 1186 mm) (ESALQ, 2016). In the first three months after vinasse and N application, the cumulative rain was 276 mm, 102 mm, and 432 mm, and average WFPS in the days of soil sampling were 75%, 66%, and 69% in R1, D1, and R2, respectively. The N applied as fertilizer significantly altered soil mineral N concentration (NH_4^+-N and NO_3^--N) for approximately 40 days after fertilizer application in R1 and R2, consequently susceptible to N_2O losses (Fig. S1). In D1, the mineral N was available for almost 80 days (Fig. S1). Mineral N fertilizer was applied on top of the straw and after vinasse application. This explains the lower concentration of NH_4^+-N and NO_3^--N in the dry season.

3.2. N_2O emission peaks

The control treatments of all experiments had low N_2O emissions and the average was 0.13 mg m^{-2} d^{-1} of N (Fig. 1, 2, 3). Considering only the separate application of fertilizer and vinasse (CV and V), treatments with N fertilizer had higher N_2O

emission than control, with a similar pattern of N_2O emissions peaks in all experiments. The highest N_2O emission peaks varied from 2.9 and 7.8 mg m^{-2} d^{-1} in the N fertilizer treatment (Fig. 1D, 2D, 3D). CV treatments had higher emissions peaks of N_2O than N fertilizer and V treatments in all seasons, independently of the time application (Fig. 1, 2 and 3). The highest N_2O emission peaks from the five CV treatments were between 5.5 and 18.9 mg m^{-2} dia^{-1}. Treatments with V showed lower N_2O emissions than CV. In the rainy seasons, the highest N_2O emission peaks were 0.2 and 4.6 mg m^{-2} d^{-1} to R1 and R2, respectively (Fig. 1C, 3C). In D1 the highest N_2O emissions peak was 16.7 mg m^{-2} d^{-1} of N (Fig. 2C). The N_2O emissions peak occurred after N fertilizer and single vinasses (CV and V) application and right after rain events to all seasons.

The combined application of CV or V plus N fertilizer increased the N_2O emission peaks which were higher than those of treatments with N fertilizer or single vinasse. CV+N treatments had the highest N_2O emission peaks in all seasons: 65.6, 40.6, and 41.7 mg m^{-2} d^{-1} to R1, D1, and R2, respectively (Fig. 1D, 2D, 3D). Treatments with a 30-days interval between CV and N ($CV_a|N$) had lower N_2O emission peaks than those of CV and N applied together: 20.5 and 35.8 mg m^{-2} d^{-1} of N were emitted as N_2O to D1 and R2, respectively (Fig. 2D, 3D). The N_2O emission peaks to the treatments of V plus N fertilizer application (V+N) were 12.6, 30.8 and 18.5 mg m^{-2} d^{-1} of N to R1, D1, and R2, respectively (Fig. 1D, 2D, 3D). The anticipated or postponed V application decrease the N_2O emission peaks compared to V+N, and were 3.8, 17.7, and 11.5 mg m^{-2} d^{-1} of N to R1, D1, and R2, respectively (Fig. 1D, 2D, 3D).

3.3. Cumulative N_2O emissions

After almost one year, the cumulative N_2O-N emission of the control was similar in all seasons and was equivalent to 28, 24, and 41 mg m^{-2} to R1, D1, and R2, respectively (Table 4). However, the treatment with N fertilizer had different patterns between seasons; the total N_2O emitted was 74, 322, and 48 mg m^{-2} of N_2O–N to R1, D1, and R2, respectively, which corresponded to 0.12, 0.51, and 0.07% of total N applied (Table 4). The cumulative N_2O-N emission of the treatments with single vinasse was lower than that of N fertilizer and similar between both vinasses (CV and V) (Table 4). Concentrated vinasse emitted about 93 mg m^{-2} of N_2O–N (45 - 185 mg m^{-2} of N_2O–N) which corresponded 0.34 % of total N applied in the fertilized band. Standard vinasse, on the contrary, emitted, on the average of six different vinasses, 55 mg m^{-2} of N_2O–N (0 – 164 mg m^{-2} of N_2O–N), which represented 0.66 % of the total N applied (Table 4).

Application of mineral N fertilizer, single vinasse or combined application of vinasses plus N (all treatments – control) resulted in cumulative N_2O emission significantly higher than control in D1 and R2: almost 324 and 174 mg N m^{-2} was emitted as N_2O above the emissions of the control treatment (Table 5). Furthermore, the combined application of vinasse (CV or V) and N fertilizer had higher N_2O-N emission than single vinasses in all seasons. Treatments with combined application of vinasse plus N fertilizer emitted in average 380 mg N m^{-2} of N_2O more than single vinasses (Table 5). The cumulative N_2O-N emission from CV plus N treatment ($CV_a \mid N$, CV+N, and $CV_p \mid N$) were significantly higher than cumulative N_2O-N emission from V ($V_a \mid N$, V+N, and $V_p \mid N$) for both rainy seasons in the fertilized band with N fertilizer (Tables 4, 5). The combined application of CV plus N treatment emitted 1,225 and 176 mg N m^{-2} as N_2O more than V plus N (Tables 4, 5).

The temporal strategy of separating vinasse and N fertilizer to reduce the N_2O-N emission from treatments with combined application of vinasse (CV and V) plus mineral N fertilizer was significantly efficient only for CV (Table 5). Anticipating or postponing CV application with respect to N fertilizer reduced the N_2O-N emitted in

327 mg N m^{-2} on average for D1 and R2. In the R1, due the missed plot, there is no information about the temporal strategy for CV plus N; however, the treatment with CV applied on the same day of N fertilizer showed high losses: almost 1317 mg m^{-2} as N_2O-N, which corresponded to 1.39% of total N applied (Table 4). In the D1, after 381 days CV applied 30 days before N reduced in 39% the N_2O-N emission (302 mg m^{-2}) compare to CV applied together with N fertilizer. The N_2O-N emission represented 0.56 and 0.79% of the total N applied to $CV_a│N$ and CV+N, respectively (Tables 4, 5). In the R2, the temporal strategy reduced the N_2O-N emission in 61%; the cumulative N_2O–N emission was 580 and 227 mg m^{-2} (0.63 and 0.26% of the N applied) for CV+N and $N│CV_p$, respectively (Table 4).

Contrasting with the results observed for CV, the application of V before N ($V_a│N$) did not reduce the N_2O emission in all the seasons (Table 5). In the R1 study, treatments with V had low emission even when vinasse was applied with N in the same day (121 mg m^{-2} of N), 0.13% of the N applied was lost as N_2O. In D1 and R2 despite the lack of statistical significance, the anticipated or postponed V application with respect to time of N fertilizer application reduced in 31% the cumulative N_2O-N emissions in average. Besides, the cumulative N_2O-N emission from V+N treatments in D1 and R2 were higher than emissions in the R1 (Tables 4, 5). In D1 the N_2O emission from $V_a│N$ and V+N were approximately 0.55 and 0.71% of the total N applied respectively, whereas in R2 the N_2O emissions were 0.39 and 0.23 % of the total N applied to V+N and $V_p│N$, respectively.

3.4. Emission Peaks and Cumulative emissions of CO_2 and CH_4

Soil emissions of CO_2-C in all seasons were similar, except soon after vinasses application (Fig. S2). Treatments with CV had higher CO_2 emission peaks than treatments with V. CO_2 peaks were 27.2 and 14.5 g m^{-2} d^{-1} of C in average for treatments with CV and V (R1, R2, and D1 in average), respectively. However, CO_2-C cumulative emissions were similar for both vinasses (CV and V), with or without N fertilizer in all seasons, except in the R1. For CH_4 there was no clear pattern among treatments (Fig. S3). Moreover, differences in cumulative CH_4 emission among treatments were not statistically significant, with or without vinasse, with or without N or between seasons. The CH_4 fluxes were of small magnitude and fluctuated between positive and negative daily rates (Fig. S3). Approximately 71, 67 and 55 % of flows were negative in R1, D1, and R2, respectively, indicating the predominance of CH_4 oxidation by aerated soils of the tropical condition. The flows ranged from -1.4 and 2.8 g C m^{-2} d^{-1} in average (R1, R2, and D1). Cumulatively, treatments with vinasse and N seemed to have a net CH_4-C sink with no clear pattern among treatments and season experiments (Fig. S3).

3.5. Stalk yield

Treatments with N and vinasse had higher stalk yield than control (Table 6). The treatments with N fertilizer, vinasses and combined application of both produced 15, 18 and 20 t ha^{-1} more than the unfertilized control (Fig. 4, Table S2). However, the application of vinasse with N did not increase stalk yield in any season and the time strategy had no effect on the sugarcane stalk yields. The stalk yields were 78, 105, and 96 t ha^{-1} in R1, D1, and R2 (average to all fertilized treatments) (Table 6).

4. DISCUSSION

Although the conditions for denitrification are less favorable in the dry season, the cumulative emissions of N_2O were higher than in the rainy seasons for all treatments. Sugarcane is a fast-growing plant, capable of accumulating between 30 and 60 t ha^{-1} of dry matter in one season (Cantarella et al., 2012; CONAB, 2017). The demand for N is high during the initial stages of ratoon growth (Franco et al., 2011; Mariano et al., 2016). If N is applied in the fast-growing stage, nutrient uptake is high, and less N will remain in the soil subject to the reactions that produce N_2O. Indeed, in the region where the studies were conducted, 75 % of the total N content accumulated by sugarcane ratoon occurs between December and March (Otto et al., 2016). This partly explains the difference in N_2O emission between the different seasons and the higher N_2O emission in D1. In the rainy season, 2013/2014 (R1), the vinasses and mineral N fertilizer were applied at the beginning of the summer (13 December) when plants were 1.5-m-tall; the average EF of all treatment was 0.29%. In R2, 2014/2015, both vinasses and N fertilizer were applied on 14 October, 30 d after harvest, in a mild but already warm spring (24 ^{o}C) (beginning of the rainy season). The N sink was not as strong as in the first experiment but, nonetheless, seem to have been significant, and the EF factor found was similar to that of the R1 (EF = 0.36% of N). However, in the dry season, vinasses and N fertilizer were applied 90 d after harvest, at the beginning of the winter when the weather conditions were dry and temperatures mild (21 ^{o}C). Sugarcane plants in dry season grew slowly and probably absorbed little N until rainfall became constant, in mid-September 2014. Therefore, inorganic N stayed long in the soil, almost 100 days. Due to low precipitation and probably slow or delayed N uptake by the sugarcane plants, significant N_2O emissions were observed throughout this period (EF = 0.76% of N) (Table 4). The emissions started 18 days after fertilizer application with the first rain.

The cumulative emission of N_2O of treatments with N fertilizer was smaller than the default value of 1% of N of IPCC (IPCC, 2013) in the three experiments, 0.23 % of the N applied was lost as N_2O (Table 4). Similar results were found in studies with sugarcane in the southeastern of Brazil (Filoso et al., 2015; Paredes et al., 2015; Paredes et al., 2014; Siqueira Neto et al., 2016). The low N_2O emissions have been attributed to the high drainage capacity of the deep and highly weathered soils grown with sugarcane in Brazil, which prevent water accumulation for long periods in the soil profile (Jantalia et al., 2008; Soares et al., 2015). Besides, N_2O emissions are lower for NO_3^--based fertilizers compared to urea, NH_4^+-based fertilizers and organic or synthetic-organic sources (Siqueira Neto et al., 2016; Snyder et al., 2009). These low N_2O emissions could be also related to weather conditions. The experiments R1 and D1 were conducted in atypically dry years in the region. The cumulative rain values were lower than the historical values (ESALQ, 2016). Consequently, the anaerobic condition required by denitrifier bacteria occur in a short period of time and soon after rain events. Besides, all experiments were conducted in sugarcane ratoons with surface application of fertilizers, without physical destruction of stumps, plowing or opening and closing of the furrows. These practices would cause the incorporation of crop residues and aeration of the soil surface layer, which favors soil organic matter mineralization, increasing background N_2O emission (Siqueira Neto et al., 2016; Soares et al., 2015).

In our experiments, we evaluated the N_2O emissions from five different concentrated vinasses and six standard vinasses. These allowed us to have a better understanding of the impact of solely applied vinasse on N_2O emissions. The N_2O emission in treatments with CV was similar to those of mineral N in all seasons, varying from 0.18% to 0.56% (0.34% in average). Conversely, the EF values for V were quite variable: from 0.00% to 1.84% (average 0.66%) of the vinasse-N. It is likely that the N in the V is less recalcitrant than that of the CV because the former does not undergo a dehydration process (Parnaudeau et al., 2008), what explains the higher EF of V than of CV. The variation of EF from CV and V may also be associated with the variable chemical composition of vinasses, especially C and N (Elia-Neto and Nakahodo, 1995; Mutton et al., 2014). Although all the vinasses came from the same mill, their composition was not uniform. The N rates of vinasses varied from 30 to 52 kg ha^{-1} and 51 to 157 kg ha^{-1} to CV and V, respectively. However, the N concentration of the different vinasses used is in the range of typical values of vinasse applied in Brazil (Mutton et al., 2014). The chemical composition variation of vinasse depends on sugarcane variety used for ethanol production, stage of plant development, soil type, etc (Christofoletti et al., 2013). In addition, vinasse composition is highly affected by the source of fermented feedstock (molasses, sugarcane juice or the combination of both) (Rodrigues Reis and Hu, 2017), which depends on whether the industry is producing more sugar or ethanol at the moment (Mutton et al., 2014).

Strategy to reduce N_2O emission

The reason to apply vinasse 30 days prior or after N fertilization was to reduce the effect of high moisture and labile organic carbon on the activity of soil microbial community when plenty of N is available in the soil. This timeframe of vinasse application and nitrogen fertilization would allow plants to take up N and/or microbes to consume the easily mineralizable organic C from vinasse, thus avoiding the synergic effect of both on N_2O emission (Carmo et al., 2013; da Silva et al., 2017; Lourenço et al., 2018; Pitombo et al., 2015). However, the strategy of anticipating or postponing the application of vinasses (CV and V) was not efficient for all conditions. The N_2O emission in sugarcane ratoon was dependent on the type of vinasse (CV or V), time of the year when nitrogen was applied in the soil (rainy or dry season) and stages of plant growth.

Both vinasses (CV and V) applied with N increased N_2O emission almost three-fold compared to that of N fertilizer (Table 4). The interaction between C and N were responsible for high N_2O emissions, independent of vinasse application time. The organic carbon can stimulate microbial growth and activity, and provide the organic carbon needed by soil denitrifiers (Cameron et al., 2013; Lourenço et al., 2018; Pitombo et al., 2015). The high emissions of CO_2 soon after vinasse application (Fig. S2) indicate that both vinasses (CV and V) increased microbial activity and respiration (Barton and Schipper, 2001). Additionally, microbial growth increases the consumption of O_2 and generates a low pressure of O_2 with anaerobic microsites necessary for nitrifier-denitrification and denitrification. Liang et al. (2015) found that N_2O emissions increased minimally with N additions, while without additional N, total N_2O emissions increased linearly with C additions. When both C and N were added together the largest increases in N_2O emissions occurred.

Especially for CV, the application of both, N fertilizer and vinasse, anticipated or postponed ($CV_a \mid N$, CV+N or $N \mid CV_p$), increased the N_2O emissions (EF: 0.94% of N) compared with mineral fertilizer alone (EF: 0.23 % of N) regardless of the season. Apparently, the time between CV and N applications was not enough to cause significant C decomposition e N mineralization from CV and/or N fertilizer uptake by plants. Parnaudeau et al. (2008) and Silva et al. (2013) evaluated the net and potential N mineralization of CV and V in a laboratory experiment. The authors found that CV released N and C at a slower rate than V. It is likely that the organic C and N present in the CV could stimulate the microbiota responsible for N_2O production for a longer time, through the period of CV decomposition (Parnaudeau et al., 2008; Silva et al., 2013). Besides, CV and N fertilizer were applied in bands, close to sugarcane rows, increasing the local concentration of both organic C and fertilizer-N compared to V. Despite the high N_2O emission, the strategy of applying CV one month prior or later of mineral N fertilizer, reduced in 50 % the total N emitted as N_2O. Thus, in order to avoid boosting N_2O emissions, CV should be separated from mineral N fertilizer

Anticipated or postponed V application related to N fertilizer tended to reduce to (35%) the cumulative N_2O-N emissions (average of three seasons) despite the lack of statistical significance. The cumulative emissions of N_2O of all treatments with V plus N fertilizer ($V_a \vert N$, V+N, $N \vert V_p$) were low (0.36 % of the N applied) compared with the results of Carmo et al. (2013) (1.82% of N) and Pitombo et al. (2015) (1.88% of N). In the study of Carmo et al. (2013) very high emissions (EF > 3) were only found when the amount of straw was also high (21 t ha^{-1} of dry matter), whereas in the present studies the amount of straw varied from 9 to 16 t ha^{-1}. Those authors also associated the high N_2O emissions with vinasse application after a period of rainfall, in which high soil water and anoxic conditions probably occurred (Carmo et al., 2013; Pitombo et al., 2015). Instead, our experiment was conducted in atypical weather conditions: rains were half of the amounts that were expected for the same period (ESALQ, 2016).

The combined application of both vinasses (CV and V) and N fertilizer did not affect the CH_4 emissions. These emissions were variable and predominantly negative in all seasons and treatments. This has been attributed to the high oxidized iron content and to the characteristically well-drained soils cultivated with sugarcane, which would prevent high rates of CH_4 formation even in the presence of organic matter from the vinasse and sugarcane straw (Lovley and Phillips, 1986). This limitation to CH_4 production in the conditions of this study probably favored methanotrophs pathways. Paredes et al. (2015) and Carmo et al. (2013) found similar results showing that the soils with sugarcane in the southeast region of Brazil are usually sinks of methane.

The application of mineral N fertilizer, vinasse (CV and V) and combined application of both fertilizer increased the stalk yield compared to the control treatment. However, the vinasse (CV and V) and N fertilizer combined application did not affect the stalk yield, regardless of the time of vinasse application with respect to N fertilizer

(Table 6). Despite the high dry matter production of sugarcane, the responses of this crop to N fertilization in Brazil are relatively small. In a network of field studies of sugarcane harvested without burning the straw, as in our study, the average N rate that maximized economic yield was 120 kg ha^{-1} (Rossetto et al., 2010) but in several investigations much lower responses were reported (Franco et al., 2010; Lofton and Tubaña, 2015; Reis Junior et al., 2000). The contribution of soil N from previous cycles or from biological N fixation cannot be ruled out (Boddey et al., 2001; Urquiaga et al., 1992; Urquiaga et al., 2012).

In our study, we could not use vinasse with a standardized composition in all experiments. Although both CV and V came from the same sugar mill, there was a one-year span between the first and the last vinasse application. The large volumes of vinasse needed in field experiments cannot be stored because vinasse rapidly deteriorates. Vinasse composition may vary widely along the year as described earlier (Elia-Neto and Nakahodo, 1995; Mutton et al., 2014). Thus, the composition of the eleven vinasses used in the six application events (twice in each of the three experiments) was variable for both CV and V. Although this may have an effect on GHGs emissions associated with the interaction of vinasse, N fertilizer, and time of application, it was suitable to evaluate N_2O emissions in sugarcane in real field conditions. Moreover, to our knowledge, this is the first study that evaluates both CV and V, with or without nitrogen fertilizer, in three different seasons. In addition, in our work, we were able to calculate the much-needed emission factors for vinasse. In average the EF for CV in our study was 0.34% of N, varying from 0.18% to 0.56% of N, five-fold smaller than the only observation of N_2O emission for CV (EF = 1.61% of N) found in the literature in a field experiment in the same region as ours (Pitombo et al., 2015). We also expanded the information on V: in average the EF for V in the literature is 1.80% of N (EF = 0.44 to

4.59% of N; n = 10) (Carmo et al., 2013; da Silva et al., 2017; Oliveira et al., 2013; Paredes et al., 2015; Paredes et al., 2014; Pitombo et al., 2015; Siqueira Neto et al., 2016), whereas the average EF observed from 6 different V applied in our experiments was 0.66% of N with values ranging from 0.00% to 1.84% of N.

Mineral N fertilizer and CV are applied in bands, close to the sugarcane plants, accounting for approximately 16% of the total field area, considering the current sugarcane management practices, whereas V is applied over the whole field. As our purpose was to compare N_2O emissions from different vinasses with or without N fertilizer we used emission values from the chambers (in mg N_2O-N m^{-2}) for statistical analyses (Table 5) since we carefully measured the amounts of N applied and of the N_2O emitted and no transformation factors or summative of background emissions were needed. Furthermore, as most of the N_2O is evolved from the fertilized areas, the experimental error is reduced by taking into account data obtained directly from chambers, which represents the fertilized bands. For V, the GHGs emissions calculated per chamber or on a hectare basis may be different but as the background or the non-fertilized parts of the field have low emissions compared to those that received N inputs, the N_2O EF for V+N did not differ from those calculated in the fertilized (band) area (Table 4).

In the present study, we conducted intense year-long measurements of N_2O emissions in three different seasons to compare emissions from a conventional N fertilizer (ammonium nitrate) and CV and V. Our results indicated a smaller N_2O emission factor for the conventional fertilizer than most results reported in the literature for sugarcane, suggesting that N_2O emissions from highly permeable soils with sugarcane in Brazil may be lower than IPCC values (1%) (Filoso et al., 2015; Jantalia et al., 2008; Morais et al., 2013; Soares et al., 2015). Nitrous oxide emissions increase with N fertilizer and vinasse application in the same area, especially with CV. Despite the generally low N_2O emissions from CV+N and V+N treatments in our study in most seasons, the cumulative emissions from CV+N were 17.7, 2.4 and 12.1 times higher than those of mineral N fertilizer for R1, D1, and R2, respectively. The corresponding values for V+N were 1.6, 1.6 and 5.7. We also demonstrated that the time strategy to reduce N_2O emissions - the gap between vinasse and N application in 30 days - worked, reducing the N_2O emissions by 50% for CV plus N.

The benefit of reducing N_2O emission by CV and mineral N fertilizer in time can also apply if both inputs are separated is space, for instance, applying vinasse and N fertilizer in bands in the opposite side of the sugarcane row. However, CV is costlier than V because of the energy spent to remove the water. Therefore, the different solutions pointed out in our study must be balanced against other factors such as logistic costs and associated emissions of GHG. Nonetheless, the data here obtained in three field experiments regarding emission factors for N fertilizer, vinasses, and their interactions are valuable to guide informed decisions and to model the GHG emissions of sugarcane cultivation. Such information is especially useful now in view of recent legislation passed in Brazil – Renovabio Program – that establishes independently audited mechanisms of financial compensation for bioenergy with low GHG emissions (MME, 2017), as part of the commitment of the Brazilian government in the Paris Agreement to reduce GHG emissions. Individual bioenergy producers will be rewarded by cleaner bioenergy and, therefore, will have incentives to adopt practices that reduce GHG emissions.

5. CONCLUSIONS

Sugarcane fields were sinks rather than a source of CH_4 and total carbon emitted as CO_2 was similar between seasons and treatments. The N_2O emission in sugarcane ratoon was dependent of the type of vinasse (CV or V), time of the year when nitrogen was applied in the soil (rainy or dry season) and stages of plant growth. Based on the average of the three experiments, the application of CV with N fertilizer caused higher N_2O emission (EF = 0.94% of applied N) than N fertilizer solely (EF = 0.23%). The increase in N_2O emission for V plus N (EF = 0.47%) was observed in two out of three experiments. The strategy of anticipating or delaying vinasse application by about one month related to mineral N reduce the N_2O emission in 50% for CV but not for V. Nevertheless, we recommend not to apply CV or V plus N fertilizer together to avoid boosting N_2O emissions.

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7. AUTHOR CONTRIBUTIONS

K.S.L., H.C., J.B.C, and E.E.K. designed research; R.R. and A.C.V helped with the experimental area and sugarcane management practices; K.S.L., R.M.S, Z.F.M. and

J.R.S. conducted the experiment; K.S.L. performed the statistical analyses; K.S.L. and H.C. wrote the paper. All authors reviewed the manuscript.

ADDITIONAL INFORMATION

The authors declare no conflict of interest.

Figures and Tables

Strategies to mitigate the nitrous oxide emissions from nitrogen fertilizer applied with organic fertilizers in sugarcane

Késia Silva Lourenço[a,b,], Raffaella Rossetto[c], André Cesar Vitti[c], Zaqueu Fernando Montezano[a], Johnny Rodrigues Soares[d], Rafael de Melo Sousa [a], Janaina Braga do Carmo[e], Eiko Eurya Kuramae[b]; Heitor Cantarella[a]*

[a] Soils and Environmental Resources Center, Agronomic Institute of Campinas (IAC), Av. Barão de Itapura 1481, 13020-902 Campinas, SP, Brazil.

[b] Microbial Ecology Department, Netherlands Institute of Ecology (NIOO), Droevendaalsesteeg 10, 6708 PB, Wageningen, The Netherlands.

[c] Center-South Regional Pole, Paulista Agency for Agribusiness Technology (APTA), Rod. SP 127 km 30, CEP13400-970, Piracicaba, Brazil .

[d] School of Agricultural Engineering (FEAGRI), Campinas University (UNICAMP), Av. Cândido Rondon, 501, 13083-875, Campinas, Brazil.

[e] Department of Environmental Sciences, Federal University of São Carlos (UFSCar), Rod. João Leme dos Santos Km 110, 18052-780 Sorocaba, SP, Brazil.

Corresponding author: Heitor Cantarella, tel. +55 19 21370766, e-mail: cantarella@iac.sp.gov.br

Running title: Vinasse applied before or after inorganic N mitigate N_2O emissions

***Key-words*:** bioethanol; greenhouse gases; sugarcane residues; N emission factor; vinasse; nitrogen.

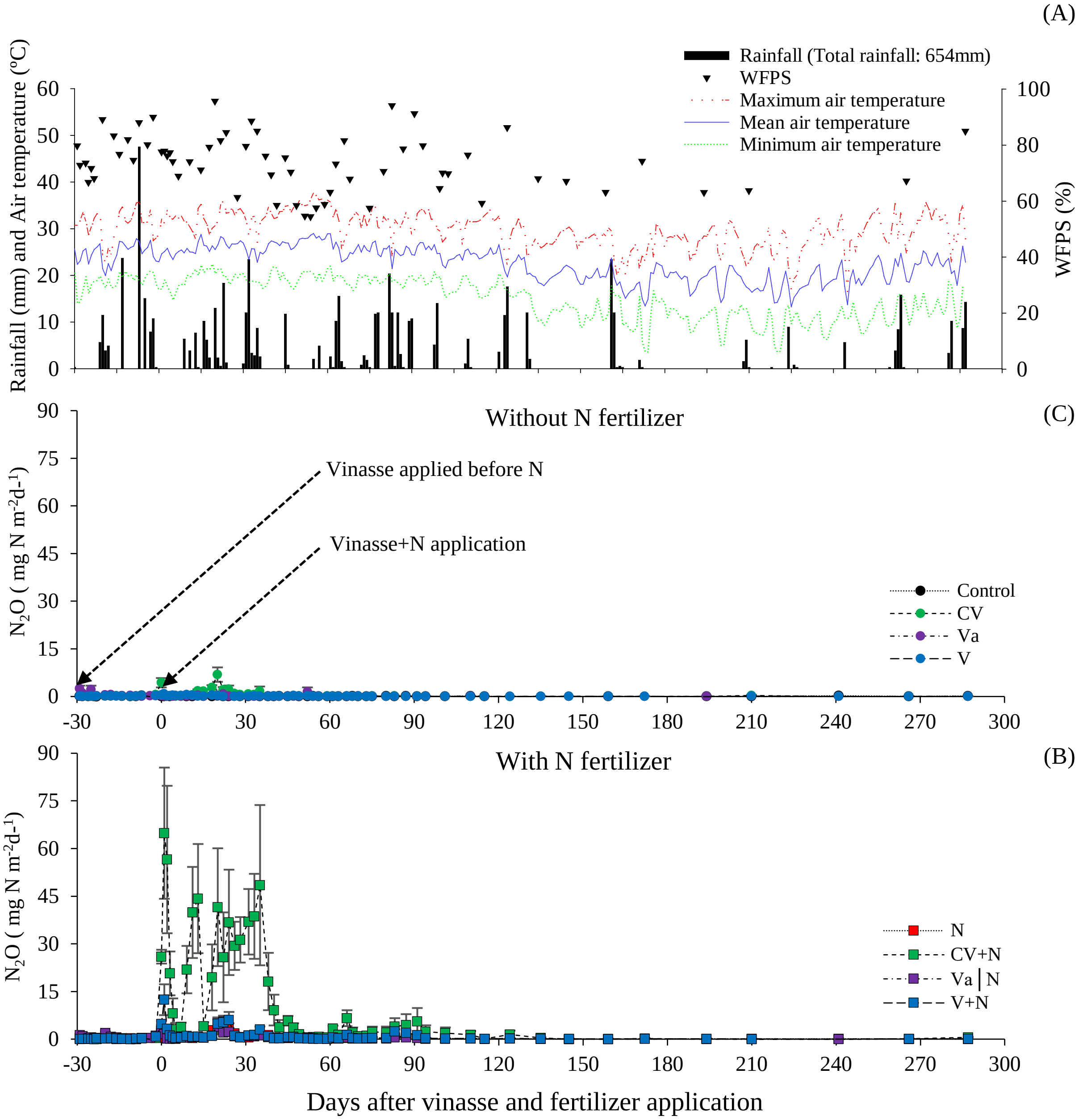

Figure 1. Rainfall, air temperature and water-filled pore space - WFPS (A), mean daily fluxes of N_2O-N without (B) or with nitrogen fertilizer (C) in the first rainy season, cycle 2013/2014 (R1). The treatments are: Control; (N) mineral fertilizer; (CV) concentrated vinasse; (V) standard vinasse; (CV+N) mineral fertilizer plus concentrated vinasse; (V+N) mineral fertilizer plus standard vinasse. V_a: Anticipated vinasse application 30 days before nitrogen fertilization. Vertical bars indicate the standard error of the mean (n = 4). Arrows indicate the time of vinasse and/or mineral fertilizer application.

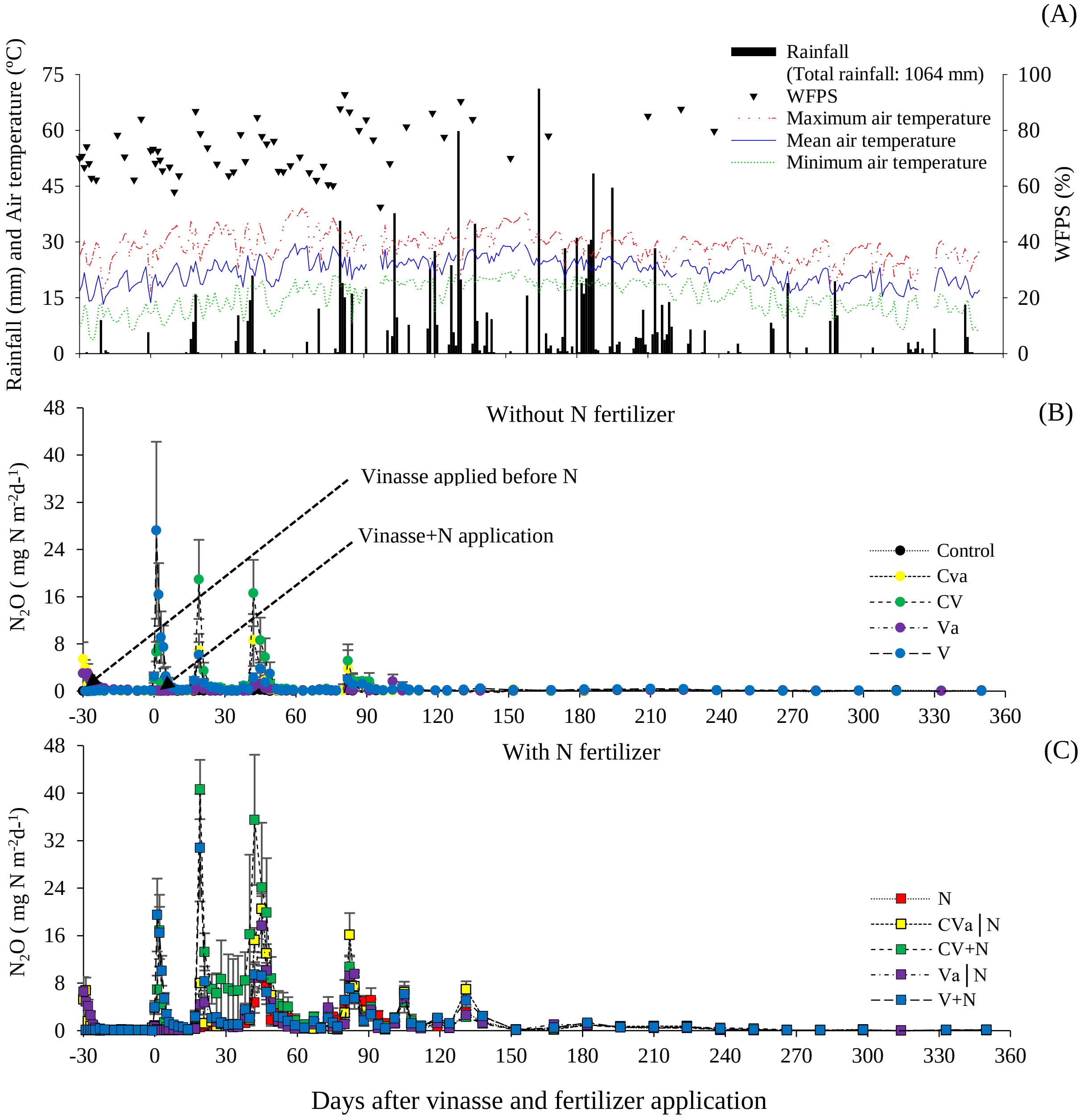

Figure 2. Rainfall, air temperature and water-filled pore space - WFPS (A), mean daily fluxes of N_2O-N without (B) or with nitrogen (C) in the dry season, cycle 2014/2015 (D1). The treatments are: Control; (N) mineral fertilizer; (CV) concentrated vinasse; (V) standard vinasse; (CV+N) mineral fertilizer plus concentrated vinasse; (V+N) mineral fertilizer plus standard vinasse. CV_a and V_a: Anticipated vinasse application 30 days before nitrogen fertilization. Vertical bars indicate the standard error of the mean (n = 4). Arrows indicate the time of vinasse and/or mineral fertilizer application.

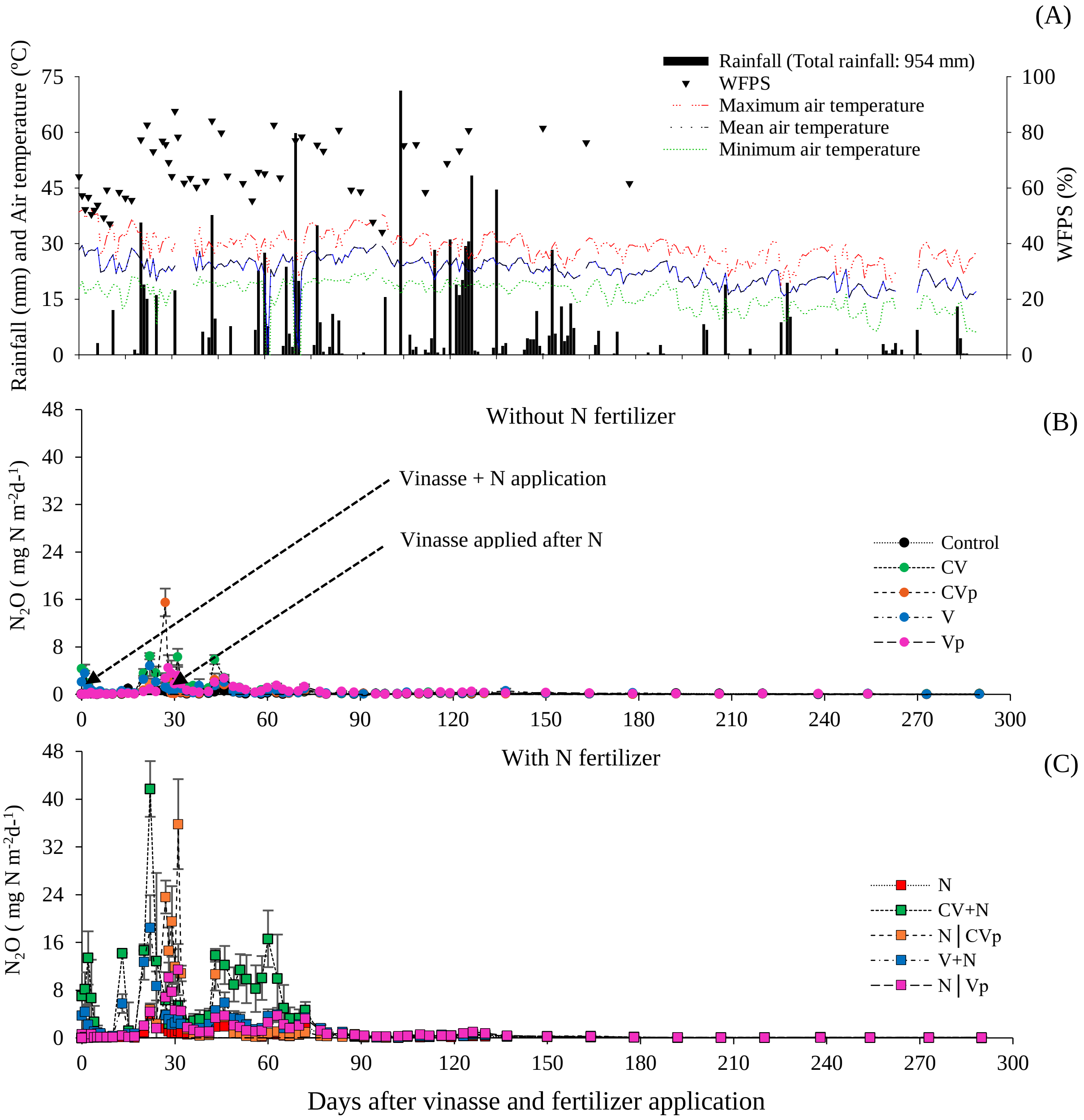

Figure 3. Rainfall, air temperature and water-filled pore space - WFPS (A), mean daily fluxes of N_2O-N without (B) or with nitrogen (C) in the second rainy season (R2), cycle 2014/2015. The treatments are: Control; (N) mineral fertilizer; (CV) concentrated vinasse; (V) standard vinasse; (CV+N) mineral fertilizer plus concentrated vinasse; (V+N) mineral fertilizer plus standard vinasse. CV_p and V_p: Postponed vinasse application 27 days after nitrogen fertilization. Vertical bars indicate the standard error of the mean (n = 4). Arrows indicate the time of vinasse and/or mineral fertilizer application.

Table 1. Properties of the soils used in the study.

Soil layer	Bulk density	pH [a]	OM [b]	P [c]	K	Ca	Mg	H+Al [d]	CEC[e]	Soil texture [f]		
										Clay	**Silt**	**Sand**
cm	g cm^{-3}		g dm^{-3}	mg dm^{-3}			mmol$_c$dm^{-3}				g kg^{-1}	
						First rainy season, 2013/2014 cycle (R1), Red Latosol						
0 – 20	1.42	5.3	23	10	0.5	45	20	31	98	619	145	236
20 - 40	1.25	4.9	19	5	0.4	27	10	38	76	668	125	207
						Dry season, 2014/2015 cycle (D1), Red Latosol						
0 – 20	1.48	5.0	21	15	0.7	17	12	35	65	631	151	218
20 - 40	1.38	4.9	15	10	0.4	12	6	36	54	703	123	174
						Second rainy season, 2014/2015 cycle (R2), Rhodic Nitisol						
0 – 20	1.45	4.5	26	9	1.1	26	14	51	92	514	124	363
20 - 40	1.35	4.2	21	5	1.1	19	8	61	89	576	108	316

[a] ($CaCl_2$; 0.0125 mol L^{-1})

[b] Organic matter.

[c] Available phosphorus, K, Ca, and Mg were extracted with ion exchange resin.

[d] Buffer solution (pH 7.0).

[e] Cation exchange capacity.

[f] Soil texture determined by the densimeter method.

Table 2. Time of application and the corresponding nitrogen rates of mineral fertilizer (N: ammonium nitrate) and vinasse (concentrated vinasse - CV and standard vinasse –V) to sugarcane ratoon. Numbers in parenthesis indicate the amount of N, in kg ha^{-1}, contained in vinasses or in the mineral fertilizer. Ammonium nitrate was always applied at 100 kg N ha^{-1}, but the amount of N in vinasse varied with the batch used.

Treatments[a]	First rainy season (R1) (2013/2014)		Dry season (D1) (2014/2015)		**Treatments**	Second rainy season (R2) (2014/2015)	
	November 2013	December 2013	July 2015	August 2015		October 2014	November 2015
Control	-	-	-	-	**Control**	-	-
CV$_a$	missed treatment		CV$_a$ (30)	-	**CV**	CV (46)	-
CV	-	CV (48)	-	CV (52)	**CV$_p$**	-	CV$_p$ (36)
V$_a$	V$_a$ (53)	-	V$_a$ (51)	-	**V**	V (74)	-
V	-	V (53)	-	V (89)	**V$_p$**	-	V$_p$ (157)
N	-	N (100)	-	N (100)	**N**	AN (100)	-
CV$_a$ | N	missed treatment		CV$_a$ (30)	N (100)	**CV+N**	CV+N (46+100)	-
CV+N	-	CV+N (48+100)	-	CV+N (52+100)	**N | CV$_p$**	N (100)	CV$_p$ (36)
V$_a$ | N	V$_a$ (53)	N (100)	V$_a$ (51)	N (100)	**V+N**	V+N (74+100)	-
V+N	-	V+N (53+100)	-	V+N (89+100)	**N | V$_p$**	N (100)	V$_p$ (157)

([a]) a: Vinasse anticipated application (30 days before N fertilization); p: Vinasse postponed application (27 days after N fertilization).

Table 3. Chemical characteristics of the vinasse applied in the experiments.

Exp. [a]	**Vinasse** [b]	**Time of application**	**pH**	**Org C**	**total N**	**NH_4^+-N**	**NO_3^--N**	**P**	**K**	**C/N**
				g L^{-1}	g L^{-1}	mg L^{-1}	mg L^{-1}	g L^{-1}	g L^{-1}	
R1	CV	Dec. 13	4.0	69.7	2.80	119.8	21.2	1.00	17.3	25/1
D1	CV$_a$	Jul. 15	4.3	54.1	1.75	61.5	20.2	1.25	17.3	31/1
D1	CV	Aug. 15	4.2	65.3	3.00	100.9	23.7	0.53	21.0	22/1
R2	CV	Oct. 14	4.2	61.3	2.65	146.9	22.5	0.41	15.3	23/1
R2	CV$_p$	Nov. 10	3.8	50.6	2.05	37.9	23.4	0.80	14.3	25/1
R1	V$_a$	Nov. 13	4.7	28.2	0.53	65.8	17.6	0.08	2.9	53/1
R1	V	Dec. 13	4.1	25.7	0.53	63.4	10.8	0.17	2.6	49/1
D1	V$_a$	Jul. 15	4.8	28.8	0.51	45.7	8.8	0.11	3.5	57/1
D1	V	Aug. 15	3.9	31.4	0.89	41.6	4.1	0.23	4.7	35/1
R2	V	Oct. 14	4.2	29.6	0.74	37.7	6.8	0.10	2.1	40/1
R2	V$_p$	Nov. 10	4.7	30.3	1,57	75.9	6.6	0.25	4.8	19/1

[a] R1: First rainy season (2013/2014 cycle); D1: Dry season (2014/2015 cycle); R2: Second rainy season (2014/2015 cycle);

[b] CV: Concentrated vinasse and V: Standard vinasse. a: Anticipated vinasse application (30 days before N fertilization); p: Postponed vinasse application (27 days after N fertilization).

Table 4. Cumulative nitrous oxide emissions, N emission factor (EF) and ± mean standard error as affected by N applied as mineral N fertilizer or vinasse in three experiments with sugarcane ratoon (emission per chamber).

Treatments [a]	**Rainy season, 2013/2014 (R1)**		**Dry season, 2014/2015 (D1)**		**Treatments**	**Rainy season, 2014/2015 (R2)**	
	Cumulative N_2O-N [b]	EF [c]	Cumulative N_2O-N	EF		Cumulative N_2O-N	EF
	N_2O emission from the sugarcane fertilized band (Chamber area)						
	mg N m^{-2}	% of N applied	mg N m^{-2}	% of N applied		mg N m^{-2}	% of N applied
CV$_a$	*Missed treatment*	-	83 ±52	0.43 ±0.27	**CV**	98 ±5	0.34 ±0.02
CV	54 ±17	0.18 ±0.06	185 ±49	0.56 ±0.15	**CV$_p$**	45 ±12	0.20 ±0.05
V$_a$	4 ±9	0.07 ±0.18	44 ±12	0.86 ±0.24	**V**	58 ±17	0.79 ±0.23
V	0 ±6	0.00 ±0.12	164 ±39	1.84 ±0.44	**V$_p$**	58 ±21	0.37 ±0.14
N	74 ±11	0.12 ±0.02	322 ±61	0.51 ±0.10	**N**	48 ±3	0.07 ±0.00
CV$_a$ | N	*Missed treatment*	-	463 ±51	0.56 ±0.06	**CV+N**	580±177	0.63 ±0.19
CV+N	1317 ±316	1.39 ±0.33	765 ±304	0.79 ±0.31	**N | CV$_p$**	227 ±37	0.26 ±0.04
V$_a$ | N	61 ±12	0.09 ±0.02	376 ±82	0.55 ±0.12	**V+N**	275 ±46	0.39 ±0.06
V+N	121 ±15	0.18 ±0.02	515 ±47	0.71 ±0.06	**N | V$_p$**	180 ±13	0.23 ±0.02
	N_2O emission from sugarcane cropping systems [d]						
	g N ha^{-1}	% of N applied	g N ha^{-1}	% of N applied		g N ha^{-1}	% of N applied
V$_a$ | N	126 ±19	0.08 ±0.01	969 ±131	0.64 ±0.26	**V+N**	929 ±73	0.53 ±0.04
V+N	191±23	0.13 ±0.02	2202 ±75	1.16 ±0.04	**N | V$_p$**	772 ±21	0.30 ±0.01

[a] N: mineral fertilizer; CV: concentrated vinasse; V: standard vinasse; CV+N: mineral fertilizer plus concentrated vinasse; V+N: mineral fertilizer plus standard vinasse. a: Anticipated vinasse application (30 days before N fertilization); p: Postponed vinasse application (27 days after N fertilization).

[b] For cumulative emissions, the background values (chambers without fertilizer N or vinasse) were subtracted for this calculation. Background values were 28, 24 and 41 mg N m^{-2} for R1, D1 and R2.

[c] EF values were calculated using the total N input (from V, CV and N).

[d] Cumulative N_2O-N emissions per hectare were calculated multiplying V+N (V$_a$+N, V+N, and N+V$_p$) in mg N m^{-2} by 1.6 (1600 m^2) (16% of the total area) plus the N_2O emissions from the area between rows (84%). In this case emissions of V were multiplied by 8.4 (8400 m^2). For all other treatments (results not shown), emissions for the cropping system can be calculated by multiplying the values in mg N m^{-2} by 1.6 since the inputs were applied just in 0.25 cm-wide bands. The values of EF were the same for both band and per-hectare calculation, except for the V+N treatments.

Table 5. Statistical analysis using orthogonal contrasts for selected treatments. The net contrast values represent the differences between the amounts of N_2O emissions defined by the orthogonal contrasts parameters.

Selected contrasts ^{a}	Contrast effect^{b}	Contrast calculation	Net contrasts values $^{c, d}$		
			Rainy season 2013/14	Dry season 2014/15	Rainy season 2014/15
			-------------- mg N_2O-N m^{-2} -----------------		
I	N effect (vinasse-N or N)	(All treatments) - Control	233^{ns}	324***	174**
II	N plus vinasse effect	(All vinasse+N) - all vinasse	480***	410***	250***
III	Type of vinasse	CV - V	52^{ns}	30^{ns}	14^{ns}
IV	V: Anticipating	V_a - V	4^{ns}	-120^{ns}	-
V	Postponing	V - V_p	-	-	1^{ns}
VI	CV: Anticipating	CV_a - CV	*Missed treatment*	-101^{ns}	-
VII	Postponing	CV - CV_p	-	-	53^{ns}
VIII	Type of vinasse + N	(CV+N) - (V+N)	1226***	169^{ns}	176***
IX	V+N: Anticipating	(V_a | N) - (V+N)	-60^{ns}	-139^{ns}	-
X	Postponing	(V+N) - (N | V_p)	-	-	95^{ns}
XI	CV+N: Anticipating	(CV_a | N) - (CV+N)	*Missed treatment*	-302*	-
XII	Postponing	(CV+N) - (N | CV_p)	-	-	352***

[a] Contrasts I and II compare the overall effect of N on N_2O emission; contrasts III to XII compare the effects of type of vinasse with and without N fertilizer; contrasts within groups I and II, and III to XII are orthogonal;

[b] N: mineral fertilizer (ammonium nitrate, AN); CV: concentrated vinasse; V: standard vinasse; CV+N: concentrated vinasse plus AN; V+N: standard vinasse plus AN. a: Anticipated vinasse application (30 days before N fertilization); p: Postponed vinasse application (27 days after N fertilization).

[c] Net effect of emission for the indicated contrast. Significant difference: * $p \leq 0.10$; ** $p \leq 0.05$; *** $p \leq 0.01$; ns: non-significant.

[d] Dashes indicate treatments not included in the specific season.

Table 6. Effect of mineral N and vinasses on sugarcane stalk yields.

Treatments[a]	Number of treatments used to calculate the average stalk yields[b]		**Stalk yield (t ha^{-1})**		
			Rainy season 2013/2014	Dry season 2014/2015	Rainy season 2014/2015
Control	n = 4		63±6	87±34	76±6
N	n = 4		80±13	105±21	102±19
V	n = 8: (V_a & V) or (V & V_p)		76±10	104±22	89±19
CV	n = 8: (CV_a & CV) or (CV & CV_p)		70±11	103±21	97±13
V+N	n = 8: (V_a+N & V+N) or (V+N & V_p+N)		85±13	115±21	94±25
CV+N	n = 8: (CV_a+N & CV+N) or (CV+N & CV_p+N)		74±12	99±19	103±23
Selected contrasts	Contrast effect[c]	Contrast calculation	Rainy season 2013/2014	Dry season 2014/2015	Rainy season 2014/2015
I	N effect (vinasse or mineral N)	(All treatments) - Control	15**	18*	20**
II	Mineral N effect	N – (All vinasse+N + all vinasse)	ns	ns	ns
III	N plus vinasse effect	(All vinasse+N) - all vinasse	ns	ns	ns
IV	Type of vinasse	CV - V	ns	ns	ns
V	Type of vinasse + N	(CV+N) - (V+N)	ns	ns	ns

[a] The treatments are: Control, (N) mineral fertilizer; (CV) Concentrated Vinasse; (V) standard Vinasse; (CV+N) mineral fertilizer plus Concentrated Vinasse; (V+N) mineral fertilizer plus standard Vinasse; $_b$ and $_a$: Time of vinasse application (V and CV); $_b$: Vinasse application 30 days before N fertilization; $_a$: Vinasse application 27 days after N fertilization.

[b] Treatments with standard vinasse (V, V+N and N+V) and concentrated vinasse (CV, CV+N and N+CV) are averages of two treatments (application time) and four blocks (n = 8).

[c] Orthogonal contrast calculation. Values are the difference (t ha^{-1}) of stalk yields indicated by the contrast effect. Significant difference: * $p \leq 0.10$; ** $p \leq 0.05$; *** $p \leq 0.01$ and ns: non-significant.

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